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Handbook of Neurotoxicity

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With 244 Figures and 38 Tables

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Preface

Handbook of Neurotoxicity is a compendium intended to provide an overview on neurotoxins. In addition, processes and mechanisms attending neuronal necrosis, necroptosis, and apoptosis are discussed in detail. Also, glial perturbations, as these relate to associated neuronal dysfunction, and both in vivo and in vitro methodological approaches toward the study and use of neurotoxins are discussed.

The ***first theme*** in the *Handbook* on selective neurotoxins provides a listing of individual neurotoxins, with a description of relative selectivity of each, including cellular mechanisms of action and overall neurologic and behavioral outcome deriving from these neurotoxins' effects. The ***second section***, on dopamine-derived neurotoxins, provides highlights of neuronal mechanisms associated with the production of reactive dopamine quinones and semiquinones and reactive oxygen/nitrogen species formed by the reactive dopamine analogs. These processes and mechanisms are related to purported genesis of Parkinson's disease.

The ***third section***, on glutamate receptor agonists, is focused on the theme of neuronal excitotoxicity as an outcome of over-excitation of neurons possessing kainate receptors, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, and NMDA (N-methyl-D-aspartate) receptors. These excitotoxins are discussed in detail as to their known and putative roles in neurodegenerative and psychiatric disorders. Similarly, the roles of exogenous and endogenous glutamate receptor antagonists as neuroprotectants and as neurotoxins per se are described.

The ***fourth section*** highlights the roles of neurotrophins in neuronal development and relates their involvement in a host of neurotoxicity events in neurologic and neurodegenerative disorders. The ***last section*** of the *Handbook*, focused on diseases and disorders relevant to neurotoxins, describes processes and mechanisms associated with specific neural disorders, including effects of some of the most prominent neurotoxins in relation to human dysfunction. Additionally, impugned neurotoxicity mechanisms in neurological disorders, protective strategies for averting and delaying onset of neurologic and psychiatric disorders, and highlights of newer strategies towards therapy are also discussed.

Handbook of Neurotoxicity is intended to be useful to students, scientists, and clinicians as an initial introduction to neurotoxin-associated processes. Simultaneously, the *Handbook* is expected to be a valuable guide for those employing specific neurotoxins as there are specific details on the use of individual neurotoxins along with dosages, delineation of mechanisms of action, expected neuronal

degenerative effects, behavioral outcome, and possible duration of effect and recovery from damage.

In essence, the *Handbook* is to represent an authoritative reference resource on neurotoxins and neurotoxicity processes and mechanisms in the medical and scientific arena.

Editor-in-Chief

Richard M. Kostrzewa, Ph.D., Dr.h.c., obtained a Ph.D. (1971) in Pharmacology from the University of Pennsylvania. After positions as Research Pharmacologist at the Veterans Administration Medical Center in New Orleans, Louisiana (1971–1975); adjunct Assistant Professor in the Department of Pharmacology at Tulane Medical School (1974–1976) and Department of Psychology at the University of New Orleans (1974–1977); and Associate Professor at the Louisiana University Medical Center in New Orleans (1975–1978), Dr. Kostrzewa joined the faculty of Pharmacology at the newly established Quillen College of Medicine at East Tennessee State University in Johnson City, Tennessee (Assoc. Prof./ Professor, 1978 to present). He actively collaborated in research for 20 years with Prof. Ryszard Brus of the Medical University of Silesia in Katowice, Poland, where Dr. Kostrzewa was Visiting Professor (1997–2003) and from which he received the *Doctorate Honoris Causa* (2005). In 2001, Dr. Kostrzewa was made an Honorary Member of the Polish Pharmacological Society. His research focus has remained on selective neurotoxins, with applications in the areas of nerve regeneration, neurodegenerative disorders, and dopamine receptor supersensitivity. He has authored and edited several books and more than 200 scientific papers, including the article coauthored with Dr. David M. Jacobowitz, his former Dissertation Advisor, and designated as a "Citation Classic" in *Current Contents*: "Pharmacological Actions of 6-Hydroxydopamine." Dr. Kostrzewa was the inaugural President of the Neurotoxicity Society and served as the Society's Treasurer for ten years. He has been Editor-in-Chief of the journal *Neurotoxicity Research* from the time of its inception (1998).

Section Editors

Trevor Archer obtained an Honour's degree in Psychology and Physiology from Newcastle-upon-Tyne University in 1974, and a Ph.D. from Uppsala University, Uppsala, Sweden, in 1979. From 1978 to 1988, he was Head of CNS Drugs at Astra Läkemedel AB. He was then appointed Professor and Chair of Biological Psychology at the University of Gothenburg, Sweden. He has been Guest or Visiting Professor at: the University of Hawaii at Manoa; Örebro University; Karlstad University; Kalmar University; and Madrid University. He received an Outstanding Lifetime Achievement Award from St. Joseph's College, North Point in 2010, and Honorary Doctorship, Professorship, and Membership Awards from the Polish Academy of Science Pharmacological Society in 2013.

Gilles Guillemin is CORE Professor and Co-Director of the newly-created "MND and Neurodegenerative Diseases Research Centre" at Macquarie University. He is an Honorary Associate of the University of Sydney and the Visiting Senior Research Fellow at the Australasian Research Institute, Sydney Adventist Hospital, Wahroonga. Professor Guillemin has published over 110 papers and edited a book, *Neuroanatomie fonctionnelle: La cellule microgliale*. He has also contributed to 12 other books and presented at over 90 conferences. Dr. Guillemin is heavily involved in tryptophan research, as his group is one of the world's leading research groups working on neuroactive metabolites derived from tryptophan. He is the newly-elected President of both the International Society for Tryptophan Research (ISTRY) and the Neurotoxicity Society (NTS). His current research interest is studying the involvement of tryptophan catabolism (via the kynurenine pathway) in human neurodegenerative diseases such as amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease, Alzheimer's disease, and brain tumors.

Juan Segura-Aguilar studied chemistry with special mention in microbiology at the University of Stockholm, Sweden, and received his bachelor's degree in 1983. In 1989, he received his Ph.D. in biochemistry from the University of Stockholm. In 1999, he was awarded a stay at the University of Valencia by the European Community. From 1993 to 1998, he was at Uppsala University, Sweden, where he obtained "Docent" competence in 1994. In 1998, he moved to Chile and has been Full Professor at the University of Chile. He has been awarded several competitive research grants, including FONDECYT grants. Currently, he leads a research group

that studies the role of dopamine oxidation in the degenerative process of the nigrostriatal dopaminergic system. With Prof. Richard Kostrzewa, he founded and organized the Neurotoxicity Society, and has also organized several international NTS meetings, both regular and satellite.

Xin-Fu Zhou received his undergraduate medical degree from Third Military Medical University in China in 1977, M.Sc. in neurology from Beijing PLA Postgraduate College in 1982, and Ph.D. in biochemistry from the University of Melbourne in 1990. He was awarded an Australian Postdoctoral Fellowship by the Australian Research Council (ARC) in 1991 and completed his postdoctoral research at Flinders University. Since 1997, he has been awarded four times Research Fellowships and named Senior Research Fellow by the National Health and Medical Research Council (NHMRC). He has been awarded numerous competitive research grants by ARC and NHMRC. His main research interest has been in neurotrophins and their receptors in neural development and in neurological disorders. Recently, he is interested in functions of proneurotrophins and their receptors in neurological diseases such as Alzheimer's disease, spinal cord injury, and depression. Currently, he is NHMRC Senior Research Fellow and Research Chair in Neuroscience at University of South Australia.

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Part I

Neurotoxins

Survey of Selective Neurotoxins

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Contents

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Abstract

There has been an awareness of nerve poisons from ancient times. At the dawn of the twentieth century, the actions and mechanisms of these poisons were uncovered by modern physiological and biochemical experimentation. However, the era of selective neurotoxins began with the pioneering studies of R. Levi-Montalcini through her studies of the neurotrophin "nerve growth factor" (NGF), a protein promoting growth and development of sensory and sympathetic noradrenergic nerves. An antibody to NGF, namely, anti-NGF – developed in the 1950s in a collaboration with S. Cohen – was shown to produce an "immunosympathectomy" and virtual lifelong sympathetic denervation. These Nobel Laureates thus developed and characterized the first identifiable selective neurotoxin. Other selective neurotoxins were soon discovered, and the compendium of selective neurotoxins continues to grow, so that today there are numerous selective neurotoxins, with the potential to destroy or produce dysfunction of a variety of phenotypic nerves.

Selective neurotoxins are of value because of their ability to selectively destroy or disable a common group of nerves possessing (1) a particular neural transporter, (2) a unique set of enzymes or vesicular transporter, (3) a specific type of receptor or (4) membranous protein, or (5) other uniqueness.

The era of selective neurotoxins has developed to such an extent that the very definition of a "selective" neurotoxin has warped. For example, (1) *N*-methyl-D-aspartate receptor (NMDA-R) antagonists, considered to be neuroprotectants by virtue of their prevention of excitotoxicity from glutamate receptor agonists, actually lead to the demise of populations of neurons with NMDA receptors, when administered during ontogenetic development. The mere lack of natural excitation of this nerve population, consequent to NMDA-R block, sends a message that these nerves are redundant – and an apoptotic cascade is set in motion to eliminate these nerves. (2) The rodenticide rotenone, a global cytotoxin that acts mainly to inhibit complex I in the respiratory transport chain, is now used in low dose over a period of weeks to months to produce relatively selective destruction of substantia nigra dopaminergic nerves and promote alpha-synuclein deposition in brain to thus model Parkinson's disease. Similarly, (3) glial toxins, affecting oligodendrocytes or other satellite cells, can lead to the damage or dysfunction of identifiable groups of neurons. Consequently, these toxins might also be considered as "selective neurotoxins," despite the fact that the targeted cell is nonneuronal. Likewise, (4) the dopamine D_2-receptor agonist quinpirole, administered daily for a week or more, leads to development of D_2-receptor supersensitivity – exaggerated responses to the D_2-receptor agonist, an effect persisting lifelong. Thus, neuroprotectants can become "selective" neurotoxins; nonspecific cytotoxins can become classified as "selective" neurotoxins; and receptor agonists, under defined dosing conditions, can supersensitize and thus be classified as "selective" neurotoxins. More examples will be uncovered as the area of selective neurotoxins expands.

The description and characterization of selective neurotoxins, with unmasking of their mechanisms of action, have led to a level of understanding of neuronal activity and reactivity that could not be understood by conventional

physiological observations. This chapter will be useful as an introduction to the scope of the field of selective neurotoxins and provide insight for in-depth analysis in later chapters with full descriptions of selective neurotoxins.

Keywords

3-Nitropropionic acid • 5,6-Dihydroxytryptamine • 5,7-Dihydroxytryptamine • 6-Hydroxydopa • 6-Hydroxydopamine • AF64A • Amphetamine • AMPA • AOAA • BMAA • BOAA • Botulinum neurotoxin • Capsaicin • Cocaine • Domoic acid • DSP-4 • Excitatory amino acids • Glutamic acid • Haloperidol • IgG-Saporin • Kainic acid • Kynurenine • Methamphetamine • Methylenedioxymethamphetamine • MPTP • Neurotoxins • Parachloroamphetamine • Quinolinic acid • Quinpirole • Vanilloids

List of Abbreviations

3-NP	3-Nitropropionic acid
5,6-DHT	5,6-Dihydroxytryptamine
5,7-DHT	5,7-Dihydroxytryptamine
6-OHDA	6-Hydroxydopamine
6-OHDOPA	6-Hydroxydopa
AF64A	Ethylcholine aziridinium ion
AMPA	α-amino-2,3-hydroxy-5-methyl-3-oxo-isoxazole-propionic acid
AMPH	Amphetamine
AOAA	aminooxyacetic acid
BMAA	β-N-methylamino-L-alanine
BOAA	β-N-oxalylamino-L-alanine
BoNT	Botulinum neurotoxin
DSP-4	2-chloroethylamines N-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine
EAAs	excitatory amino acids
GLU	Glutamic acid
HPTP	4-(4-chlorophenyl)-1,4-(4-fluorophenyl)-4-oxobutyl-1,2,3,6-tetahydropyridine
MDMA	Methylenedioxymethamphetamine
METH	Methamphetamine
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PCA	Parachloroamphetamine
$RHPP^+$	4-(4-chlorophenyl)-1,4-(4-fluorophenyl)-4-hydroxybutyl-pyridinium (see)

1 Introduction

The renowned physiologist Walter B. Canon promoted the principle that the function of a nerve can be deduced by denoting the deficits produced when that nerve is destroyed. The implication is that the nerve normally acts in a way to promote functions that prevent the "absence deficits." This reasoning is sound, and this experimental

approach has been proven to be invaluable. However, while it is practical to section axons and while certain neuronal nuclei can be electrolytically destroyed with only minor injury to surrounding nerves, this approach is not so useful for brain and spinal cord because of the unintended and consequential destruction of neuronal nuclei and axons, particularly if attempting to surgically disrupt deep brain nuclei.

The advantage of selective neurotoxins is that these agents can target specific neuronal phenotypes while leaving surrounding tissue unaffected and while leaving virtually all other nerve types intact. It matters not that the target group of neurons is deep in the brain or dispersed in the spinal cord. In this way, the "absence deficit" can be discerned, and the normal function of this population of nerves can be deduced. This experimental approach is now commonplace.

Another arena for selective neurotoxins is in the modeling of neurological and psychiatric disorders. The neurotoxin 6-hydroxydopamine (6-OHDA), possessing high affinity for the norepinephrine (NE) transporter (NET), has been used to produce sympathectomy in experimental animals (Thoenen and Tranzer 1968) and also used to model attention deficit hyperactivity disorder (ADHD) (Shaywitz et al. 1976) and Lesch-Nyhan syndrome (Breese et al. 1984b). The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been used to model Parkinson's disease (Langston and Ballard 1984). N-methyl-D-aspartate receptor (NMDA-R) antagonists have been used to model schizophrenia (Farber et al. 1995; see Coyle 1996). More recently, repeated treatments with the dopamine (DA) D_2-receptor agonist quinpirole have likewise modeled schizophrenia (Peng et al. 1990; see Brown et al. 2012) as well as obsessive-compulsive disorder (Szechtman et al. 1998). Reversible and permanent models of tardive dyskinesia have been produced by haloperidol in intact and in 6-OHDA-lesioned rats, respectively (Waddington et al. 1983; Huang et al. 1997b). Quinolinic acid lesions have modeled Huntington's disease (Schwarcz et al. 1984; Beal et al. 1988). Neurotoxins have been used to model additional neurological and psychiatric disorders, and the list will continue to grow with time.

With anti-NGF having been developed in the 1950s and with both 6-hydroxydopamine and glutamate having been used as neurotoxins since the 1960s, the era of selective neurotoxins is 6 decades in the making. During the twenty-first century, the number of selective neurotoxins will inevitably grow, thereby expanding the potential and usefulness of these agents in increasing our understanding of the processes and mechanisms entailed in neuronal function. Additionally, selective neurotoxins will model ever more neurological and psychiatric disorders and thereby improve the template for development of better treatments for human disorders.

2 Terminology

The term "selective neurotoxin" refers to an agent which has toxic potential and relative selectivity for a neuron with:

1. A defined phenotype (e.g., dopaminergic nerve)
2. A specified receptor population which confers susceptibility to damage by agonists (e.g., glutamate-induced excitotoxicity)

3. A cellular protein that is inactivated by a particular agent (e.g., DSP-4 and noradrenergic nerves)
4. A metabolic schema that is specifically inactivated by an agent (e.g., rotenone inhibition of mitochondrial complex I, leading to degeneration of dopaminergic nerves)
5. A protein, uniquely targeted by an immunotoxin (e.g., IgG-saporin and cholinergic nerves)
6. Surface protein targeted by an agent that renders the nerve inactive (e.g., botulinum neurotoxin and cholinergic nerves)
7. Ion channels rendered inactive by an agent (e.g., tetrodotoxin and sodium channels)

Agents and antibodies directed towards neurotrophins can similarly prevent development of a population of neurons which are dependent on neurotrophic actions particularly at defined stages in ontogeny (e.g., anti-nerve growth factor [anti-NGF] and paravertebral sympathetic noradrenergic nerves).

As more elements in nerves become targets, the list above will expand with time. And with time, the definition of a neurotoxin will blur. Already recognized is the fact that NMDA-R antagonists can result in a neurotoxic effect by virtue of their neuroprotective action (Olney et al. 1991; Farber et al. 1995). Also, when administered particularly in ontogeny, NMDA-R antagonists prevent neural excitation by endogenous glutamate – thereby sending a "message" that the now quiescent population of neurons is unnecessary. This group of developing neurons dies, and the net effect of the neuroprotective antagonist is overt neurotoxicity (see Olney et al. 2002).

In another vein, repeated treatments with some agonists – notably the DA D_2-receptor agonist quinpirole – result in prolonged or even permanent supersensitization of D_2 receptors. Following the development of receptor supersensitivity, animals when challenged acutely with quinpirole display exaggerated behaviors; and this disposition to a D_2 agonist is lifelong (Kostrzewa and Brus; 1991; Kostrzewa 1995; Kostrzewa et al. 2003). Thus, in the absence of overt neuronal cell loss, and with no discernible anatomical effect, altered behavioral outcome represents an abnormal response and thereby an index of neurotoxicity.

A neurotoxin thus can be classified as such, even in the absence of neuronal apoptosis or necrosis. Therefore, a selective neurotoxin can be defined only loosely, and the definition surely will be more ambiguous over time. Nevertheless, the utility and advantages of neurotoxins will grow over the next century and beyond, and those who employ selective neurotoxins will recognize this spectrum of agents by outcomes in the experimental realm.

The topic of selective neurotoxins has been reviewed recently (Kostrzewa 1999b, 2009; Segura-Aguilar and Kostrzewa 2004; 2006; Herrera-Marschitz et al. 2007).

3 Early History of Selective Neurotoxins

The stage for selective neurotoxins was constructed in the 1930s by the noted physiologist Walter B. Canon, who promoted the thesis that the function of

a nerve can be deduced by denoting altered effect when the nerve is destroyed by surgery or other physical means (Cannon and Rosenblueth 1937). While this thesis has proven to be valuable, limitations of a surgical experimental approach relate to the fact that multiple types of neurons [phenotypically different (i.e., having different neurotransmitter systems) or with different populations of receptor types] are simultaneously damaged. Accordingly, it is not possible to ascribe a physiological alteration to a phenotypically distinct type of neuron.

The initial discovery of a selective neurotoxin was conceivably that by Lucas and Newhouse (1957) who found that the amino acid glutamate (Glu) produced overt destruction of neurons in immature mouse retina. Years later this effect was attributed to excess neuronal excitation, resulting in "excitotoxicity."

However, the age of selective neurotoxins arrived with the seminal work of the Nobel Laureate Rita Levi-Montalcini. During her fellowship with Viktor Hamburger in the 1950s, the discovery was made that there is a factor in mouse sarcoma 180 and 37, also in snake venom and mouse salivary glands, that profoundly promotes the growth of sensory and sympathetic ganglia. In collaboration with the biochemist Stanley Cohen, this neurotrophin was ultimately isolated and termed "nerve growth factor" (NGF) (Cohen et al. 1956; Levi-Montalcini et al. 1954; Levi-Montalcini and Cohen 1956; Cohen and Levi-Montalcini 1957; Levi-Montalcini and Booker 1960b; Levi-Montalcini 1964). Subsequently, an antiserum to NGF was developed and shown – when administered to neonatal mice – to prevent the development of sympathetic (noradrenergic) ganglia, thereby resulting in a relative noradrenergic denervation of peripheral organs. While intermediary ganglia (e.g., mesenteric ganglion) and terminal ganglia remained partially or largely intact, paravertebral ganglia were near-totally destroyed. The outcome was predominate removal of the sympathetic nervous system – an "immunosympathectomy" (Levi-Montalcini and Booker 1960a; Levi-Montalcini 1966; 1987 (reviews); Levi-Montalcini and Angeletti 1966). R. Levi-Montalcini and S. Cohen shared the 1987 Nobel Prize for their work. Anti-NGF ushered in the era of selective neurotoxins.

While anti-NGF was a milestone in providing discrete denervation of a phenotypically selective class of nerves, anti-NGF was not a neurotoxin per se. Rather, anti-NGF prevented the action of a specific neurotrophin, which thus prevented programmed ontogenetic development of sympathetic noradrenergic nerves. Anti-NGF had limitations: (1) Noradrenergic fibers originating in intermediary sympathetic ganglia (e.g., mesenteric ganglia) partially survived the effects of anti-NGF, and (2) sympathetic terminal ganglia, largely to genitourinary regions, largely survived anti-NGF treatment. As an experimental strategy, anti-NGF (3) had virtually no discernible effect in adult species, and (4) it had no effect on noradrenergic nerves in the brain. Anti-NGF thus was the prologue of agents that are specifically classified as selective neurotoxins.

4 The Era of Synthetic and Small Molecule Selective Neurotoxins

Beginning in the 1960s and into the present, there has been a series of discoveries of small molecule selective neurotoxins. These agents represent a valuable resource towards determining the roles of identifiable (i.e., sometimes phenotypically distinct) neurons in physiological and behavioral processes. Moreover, these neurotoxins have been useful likewise in producing animal models of human psychiatric, neurologic, and neurodegenerative disorders. The brief survey of selective neurotoxins herein provides a brief history of discovery as well as a description of their effects and resulting utility.

5 Survey of Individual Selective Neurotoxins

5.1 6-Hydroxydopamine (6-OHDA)

5.1.1 Historical Perspective

Porter et al. (1963; 1965) and Stone et al. (1963; 1964) in their mutually collaborative studies made the discovery that the compound 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine, 6-OHDA) produced long-lived depletion of NE in the heart and other tissues. However, its mechanism of action was undefined. Several years later, in search of an electrophilic marker for electron microscopic visualization of synaptic granules in sympathetic neurons, Hans Thoenen and J.P. Tranzer found a near ideal candidate in 5-hydroxydopamine (5-OHDA) (Tranzer and Thoenen 1967a). However, the startling finding was that the analog 6-OHDA produced overt neurotoxicity, destroying sympathetic (noradrenergic) neurons (Tranzer and Thoenen 1967b; 1968; Thoenen and Tranzer 1968). In short time, it was shown that 6-OHDA, when injected into brain tissue or into a brain ventricle, destroyed dopaminergic as well as noradrenergic neurons (Bloom et al. 1969; Uretsky and Iversen 1969; 1970). Many facets of 6-OHDA actions were described in review articles, shortly after discovery of 6-OHDA neurotoxicity (Thoenen and Tranzer 1973; Kostrzewa and Jacobowitz 1974). The key terms "6-hydroxydopamine" or "6-OHDA" appear in 10,846 papers in PubMed, as of January 30, 2013. 6-OHDA is still widely used today.

5.1.2 Selectivity of Action

6-OHDA is a relatively selective neurotoxin for noradrenergic and dopaminergic neurons. Owing to its chemical similarity to DA (Fig. 1), 6-OHDA (Fig. 2) has high affinity for the NET and DA transporter (DAT) and entry into NE neurons and DA neurons resulting in a high intraneuronal concentration of 6-OHDA. The K_i of 6-OHDA for the NET is <50 μM (Iversen 1970; Ma et al. 1995). When selective damage to dopaminergic nerves is the objective, an NET inhibitor such as desipramine is administered as pretreatment (Breese and Traylor 1971; Peters et al. 1977). During the process of uptake by the NET, 6-OHDA releases NE to produce a sympathomimetic effect (De Champlain and Nadeau 1971; De Champlain and Van Amerigen 1972).

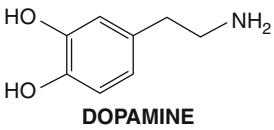


Fig. 1 (From Kostrzewa 1999b)

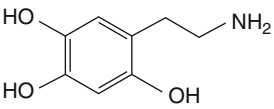


Fig. 2 (From Kostrzewa 1999b)

5.1.3 Mechanism of Action

Because 6-OHDA has micromolar affinity for the vesicular monoamine transporter-2 (VMAT-2) on NE granules and DA granules (Jonsson and Sachs 1975; Sachs et al. 1975), 6-OHDA is accumulated by granules and stored as a false transmitter.

Inside the nerve, 6-OHDA auto-oxidizes to a number of intraconvertible reactive oxygen species (ROS) including 6-OHDA-quinones, aminochrome, 5,6-dihydroxyindole, indolines, and indole-quinones (Senoh and Witkop 1959; Saner and Thoenen 1971a, b; see Kostrzewa and Jacobowitz 1974). The destructive role of 6-OHDA-derived quinoid species is likely due not so much to their binding to cellular elements, but rather to their generation of a family of other ROS which overwhelms the endogenous cellular neuroprotective processes (Graham et al. 1978). 6-OHDA-ortho-quinone and 6-OHDA-para-quinone are resonant tautomers, each of which is capable of destroying noradrenergic nerves (Heikkila et al. 1973a). However, it appears that 6-OHDA-o-semiquinone is the paramount neurotoxic auto-oxidative species producing neurotoxicity (Segura-Aguilar 2001).

Most of the above ROS generate intraneuronal hydrogen peroxide (H_2O_2), which itself is toxic (Heikkila and Cohen 1971, 1972a, b). Intraneuronal catalase inactivates H_2O_2, converting it to water. Superoxide radical also is generated by 6-OHDA auto-oxidation (Heikkila and Cohen 1973; Cohen and Heikkila 1974), as well as highly reactive hydroxyl radical (Cohen and Heikkila 1974).

5.1.4 Effects of 6-OHDA Treatment of Adult Rodents

Being ionized, 6-OHDA is unable to cross the blood-brain barrier (BBB). If administered peripherally, 6-OHDA produces marked sympathetic denervation to most peripheral organs.

To produce a CNS effect in adults, 6-OHDA must be administered into a cerebral spinal fluid compartment (i.e., intraventricular, intracisternal, or intrathecal spaces) or directly into CNS parenchyma (e.g., intrastriatal, intranigral).

5.1.5 Intraparenchymal 6-OHDA Treatments in Rat Brain

Direct injection of 6-OHDA into substantia nigra pars compacta (SNpc) – to model Parkinson's disease (PD) – results in profound (>90 %) destruction of dopaminergic perikarya, with associated near-total dopaminergic denervation of neostriatum (Ungerstedt 1968, 1971a). Because such bilateral 6-OHDA lesions are accompanied

by aphagia, adipsia, immobility, lack of grooming, and ultimate death, intranigral 6-OHDA lesions are commonly given unilaterally. Rats with a unilateral intranigral 6-OHDA lesion are able to eat, drink, groom, and otherwise remain mobile but with a circling towards the lesioned side, which abates to a large degree over time (Ungerstedt 1971d).

Intrastriatal 6-OHDA administration results in an acute moderate destruction of dopaminergic striatal terminals, followed by progressive dopaminergic denervation of this brain region over a period of 4 months, with ultimate death of 50–70 % of SNpc dopaminergic perikarya (Berger et al. 1991; Sauer and Oertel 1994; Przedborski et al. 1995; Blandini et al. 2007).

6-OHDA directly administered into the locus coeruleus results in noradrenergic denervation of neocortex, hippocampus, cerebellum, and other regions, to a lesser extent (Ungerstedt 1971c).

5.1.6 Intracisternal (i.c.) and Intraventricular (i.vtr.) 6-OHDA Treatments of Rats

In adult rats i.c. and i.vtr. 6-OHDA treatments produce modest effects on noradrenergic and dopaminergic innervation of the brain, with potency increased by pretreatment with a monoamine oxidase inhibitor (MAO-I) and with selectivity for dopaminergic neurons increased by pretreatment with the NET inhibitor desipramine (see *Handbook of Neurotoxicity* chapter by Papadeas and Breese 2013). DA D_2-receptor (D_2-R) supersensitivity is prominent in rats so-lesioned (Ungerstedt 1971b).

5.1.7 Effects of 6-OHDA Treatment of Neonatal Rodents

When administered non-centrally to perinatal rodent species, 6-OHDA and related neurotoxins have a dual action in destroying peripheral sympathetic noradrenergic nerves. 6-OHDA initially destroys sympathetic noradrenergic nerves via generation of intraneuronal ROS, and this effect results in the inability of noradrenergic nerve endings to enact the effects of NGF which would promote further ontogenetic development of these nerves. In perinatal rats, 6-OHDA crosses the blood-brain barrier and produces noradrenergic neuronal degeneration in the brain. When administered to rats on the day of birth, 6-OHDA (60 mg/kg ip) produces lifelong partial noradrenergic denervation of spinal cord (∼75 % decrease) and neocortex (∼50 %), with eventual noradrenergic sprouting and hyperinnervation of midbrain (∼50 %), pons-medulla (∼50 % increase), and cerebellum (∼100 % increase). When administered up to 5 days post-birth, 6-OHDA results in an approximate 40 % permanent decrease in noradrenergic innervation of hippocampus; when given from 3 days to 14 days post-birth, 6-OHDA decreases noradrenergic innervation of cerebellum by ∼50 %. The blood-brain barrier forms in different parts of the brain at different postnatal times, accounting for 6-OHDA access to different brain regions at different postnatal times (Kostrzewa 2007).

Both i.c. and i.vtr. 6-OHDA treatments in perinatal rodents result in near-total destruction of SNpc (Berger et al. 1985; Fernandes Xavier et al. 1994) and associated near-total DA depletion (Breese et al. 1984a, b, 1985a, b, 1987; Bruno et al. 1987; Jackson et al. 1988; Dewar et al. 1990), with near-total dopaminergic

denervation of neostriatum (Snyder et al. 1986; Descarries et al. 1992). Selectivity of 6-OHDA for dopaminergic neurons is provided by desipramine pretreatment. In the absence of desipramine perinatal 6-OHDA largely destroys the noradrenergic system as well, with locus coeruleus perikarya being susceptible to damage. Notably, neonatal 6-OHDA lesioning of dopaminergic systems results in reactive serotoninergic sprouting and eventual 5-HT neuronal hyperinnervation of forebrain (Breese et al. 1984a, b; Stachowiak et al. 1984; Snyder et al. 1986; Luthman et al. 1987; Towle et al. 1989; Descarries et al. 1992).

In adult rats that are largely DA denervated by neonatal 6-OHDA treatment, the first challenge dose of a DA D_1-R agonist induces marked perioral activity (vacuous chewing movements, VCMs), with the effect being dose related and with sensitivity to the DA D_1 agonist being 1,000-fold greater than observed in adult controls (Kostrzewa and Gong 1991). This effect is mediated or moderated by the serotoninergic system, as indicated by the fact that a 5-HT_2-R antagonist attenuates the D_1 agonist effect (Gong et al. 1992) as does 5-HT fiber denervation produced by 5, 7-dihydroxytryptamine (5,7-DHT) treatment (Brus et al. 1994). This DA D_1-R supersensitivity is accompanied by an increase in DA D_1-receptor mRNA levels in neostriatum but without a change in the number of high-affinity D_1-R in striatum (Gong et al. 1994; see Kostrzewa et al. 1998).

In the adult rats that were lesioned neonatally with 6-OHDA, the serotoninergic hyperinnervation of forebrain is accompanied by 5-HT-R supersensitivity, as evidenced by enhanced perioral activity in response to low-dose 5-HT_2 agonist treatment (Gong and Kostrzewa 1992; Gong et al. 1993).

For locomotor and classical stereotypic behaviors, there is latent D_1-R supersensitivity, as exemplified by the findings of Breese et al. (1985a, b, 1987), demonstrating that the first and second weekly adulthood challenge doses of a D_1 agonist produce no enhanced behavioral response in the neonatally 6-OHDA-lesioned rats. However, the third and all subsequent doses of a D_1-R agonist produce profound locomotor and stereotypical responses – and the effect is lifelong (Breese et al. 1985a, b, 1987). The neonatally 6-OHDA-lesioned rat represents a unique animal model for studying DA-R supersensitivity (see Kostrzewa 1995; Papadeas and Breese (2013 – this *Handbook of Neurotoxicity*)).

The effects of 6-OHDA treatments of both adult rats and neonatal rats are discussed in several reviews (Kostrzewa and Jacobowitz 1974; Breese 1975; Zigmond and Stricker 1989; Breese et al. 1990, 1994, 2005; Zigmond et al. 1993; Breese and Breese 1998; Zigmond and Keefe 1998).

5.2 6-OHDA Analogs

A number of analogs of 6-OHDA [6-OHDA = 2,4,5-trihydroxyphenethylamine] have been described (Tranzer and Thoenen 1973), having effects similar to that of 6-OHDA: 6-aminodopamine (6-ADA) (Fig. 3) (Blank et al. 1972; Heikkila et al. 1973b; Jonsson and Sachs 1973); 2-methyl-6-OHDA (Lundstrom et al. 1973; Borchardt et al. 1977); 5-methyl-6-OHDA; 2,5-dimethyl-6-OHDA

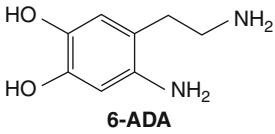


Fig. 3 (From Kostrzewa 1999b)

(Borchardt et al. 1977); 4-amino-6-OHDA (Cheng and Castagnoli 1984); 2,3,5-trihydroxyphenethylamine; 2,3,4,5-tetrahydrophenethylamine (Lundstrom et al. 1973); all eight of the trisubstituted phenyl analogs of phenethylamine; alpha-methylphenethylamine; and 1-(5-amino-2,4-dihydroxyphenyl)-2-aminopropane (Ma et al. 1995). 6-OHDA analogs that form para-quinoidal species represent the neurotoxic forms (Cheng and Castagnoli 1984); however, the ease of oxidation is not directly correlated with neurotoxic potential (Ma et al. 1995). In early studies, 2,3,4-trihydroxy-; 2,3,6-trihydroxy-; 3,4,5-trihydroxy-; 2,4,6-trihydroxy-; and N, N-dimethyl-2,4,5-trihydroxyphenethylamine had no long-term effect on noradrenergic systems (Lundstrom et al. 1973).

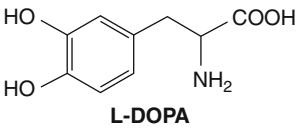
5.3 6-Hydroxydopa (6-OHDOPA)

On the basis of knowledge that L-3,4-dihydroxyphenylalanine (L-DOPA) (Fig. 4) is effective as an anti-parkinsonian agent by virtue of its being able to cross the blood-brain barrier, to then be converted in dopaminergic nerves to DA, 6-hydroxydopa (6-OHDOPA) (Fig. 5) was developed as a protoxin specifically for its ability to likewise cross the blood-brain barrier and then be converted to 6-OHDA and thereby destroy noradrenergic and/or dopaminergic nerves in the brain.

5.3.1 Historical Background

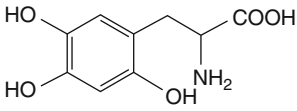
Ong et al. (1969) and Berkowitz et al. (1970) demonstrated that 6-OHDOPA effectively reduced NE content of the rat brain and that the detrimental effects of 6-OHDOPA on sympathetic noradrenergic nerves could be prevented by pretreatment with a dopa decarboxylase inhibitor. On the basis of these findings, RM Kostrzewa, under auspices of his dissertation advisor David M. Jacobowitz, undertook a series of biochemical and histochemical studies to determine the neurotoxic potential of 6-OHDOPA. Using a histofluorescence method to visualize noradrenergic neurons, the first evidence of 6-OHDOPA neurotoxicity was indicated by the loss of noradrenergic fiber number in the mouse brain, particularly forebrain – accompanied by the associated reduction in NE content in various brain regions (Jacobowitz and Kostrzewa 1971). Additionally, swollen preterminal axons were prominent in 6-OHDOPA-lesioned mice, thereby highlighting noradrenergic tracts – which ordinarily are not visible – and thereby facilitating the mapping of noradrenergic fiber tracts in the brain (Jacobowitz and Kostrzewa 1971).

Shortly thereafter, Sachs and Jonsson (1972a, b) reported analogous neurochemical findings, demonstrating NE reductions in the brains of 6-OHDOPA treated animals. While the advantage of 6-OHDOPA relates to its ability to be administered



L-DOPA

Fig. 4 (From Kostrzewa 1999b)



6-OHDOPA

Fig. 5 (From Kostrzewa 1999b)

non-centrally, 6-OHDOPA does actually produce neurotoxic effects when administered centrally (Richardson and Jacobowitz 1973). Definitive proof of 6-OHDOPA overt neurotoxicity was subsequently demonstrated by a decrease in the V_{max} for the NE transporter in brain (Kostrzewa and Garey 1976), loss of tyrosine hydroxylase activity in locus coeruleus (Kostrzewa et al. 1978), silver staining, and electron microscopic evidence of necrotic noradrenergic locus coeruleus neurons in the brain of 6-OHDOPA treated rats (Kostrzewa and Harper 1974, 1975). Cell counts of paraffin-embedded serial sections through the locus coeruleus established that neurons in the caudal half of the locus coeruleus (50 % cell loss) were most susceptible to 6-OHDOPA neurotoxicity (Clark et al. 1979).

5.3.2 Advantages of 6-OHDOPA Versus 6-OHDA

1. The protoxin 6-OHDOPA, unlike 6-OHDA, can be administered peripherally (ip, sc, iv) to adults to destroy noradrenergic nerves in the brain, thus avoiding direct intracisternal, intraventricular, or intraparenchymal injections of the brain.
2. Peripheral noradrenergic/sympathetic nerves can be protected and left virtually intact after 6-OHDOPA treatment by administering a peripherally acting dopa decarboxylase inhibitor, as pretreatment – analogous to the conventional combination of a peripheral decarboxylase inhibitor with L-DOPA therapy of Parkinson's disease.
3. 6-OHDOPA is relatively selective for noradrenergic versus dopaminergic nerves.
4. Destruction of noradrenergic terminals results in retrograde accumulation of NE in swollen axons, which become clearly visible by a histofluorescence method for NE (see below), and thus facilitates the mapping of noradrenergic tracts in the brain.

5.3.3 Disadvantages of 6-OHDOPA Versus 6-OHDA

1. Because 6-OHDOPA is relatively selective for noradrenergic neurons, dopaminergic neurons are left largely intact and unaffected by 6-OHDOPA treatment.
2. 6-OHDOPA has low neurotoxic potency for noradrenergic neurons in adults. Consequently, it is not possible to produce near-total noradrenergic denervation of regions of the brain.

3. In high dose, 6-OHDOPA produces lethal effects, thus eliminating the possibility to use a large 6-OHDOPA dose.
4. 6-OHDOPA is much more expensive than 6-OHDA.

5.3.4 Effects in the Periphery and Brain of Rodents Lesioned as Adults with 6-OHDOPA

When administered peripherally to adults, 6-OHDOPA damages sympathetic noradrenergic terminals, thereby reducing sympathetic innervation of peripheral organs such as the heart and spleen, and this is accompanied by NE depletion of the same organs. In time, because sympathetic ganglia remain intact along with preterminal axons, nerve terminals to peripheral organs regenerate, and after a period of 4–8 weeks, most or all peripheral organs are fully reinnervated by sympathetic noradrenergic nerves (Kostrzewa and Jacobowitz 1972, 1974).

5.3.5 Long-Lived Effects in the Brain of Rodents Lesioned as Neonates with 6-OHDOPA

6-OHDOPA finds greatest utility when administered to ***neonatal*** rats or mice, in order to determine developmental effects on noradrenergic neurons and to study their regenerative capacity. In prenatal mice (Kostrzewa et al. 1978) and in postnatal rats, 6-OHDOPA produces long-lived near-total noradrenergic denervation of hippocampus and >85 % long-lived noradrenergic denervation of neocortex and also spinal cord (Kostrzewa 1975; Kostrzewa and Garey 1976; Kostrzewa and Jacobowitz 1973; Sachs and Jonsson 1972a, b; Zieher and Jaim-Etcheverry 1973, 1975). These regions are provided with noradrenergic innervation by axons originating from locus coeruleus, a nucleus prominently damaged by neonatal 6-OHDOPA treatment (Kostrzewa and Harper 1974; Clark et al. 1979). Noradrenergic innervation in ventral brain is less affected by 6-OHDOPA (e.g., hypothalamus and thalamus), and recovery in these regions occurs by 8 weeks (Kostrzewa and Garey 1976).

The pons-medulla and cerebellum of neonatally 6-OHDOPA-lesioned rats display noradrenergic hyperinnervation in adulthood, subsequent to reactive noradrenergic collateral fiber sprouting (Kostrzewa 1975; Kostrzewa and Garey 1976, 1977; Kostrzewa et al. 1978). Noradrenergic fiber sprouting in brain regions proximal to the locus coeruleus appears to be related to damage to the dorsal bundle and consequent noradrenergic denervation of forebrain (Jacobowitz and Kostrzewa 1971), because the effect is duplicated by non-pharmacological surgical transection of the dorsal bundle in neonatal rats – resulting in noradrenergic fiber hyperinnervation of regions proximal to the locus coeruleus, including the pons, medulla, and cerebellum (Klisans-Fuenmayor et al. 1986).

Sympathetic noradrenergic neurons are damaged by neonatal 6-OHDOPA, but recovery from damage – evidenced by return of NE content of the heart, spleen, and other organs – is complete by 8 weeks after birth. Dopaminergic innervation is virtually unaltered by neonatal 6-OHDOPA treatment, as evidenced by retention of normal DA content of striatum throughout the developmental period, up to 8 weeks and through 1 year and longer (Kostrzewa 1975; Kostrzewa and Garey 1976).

6-OHDOPA neurotoxicity is discussed in greater detail in several reviews (Kostrzewa and Jacobowitz 1974; Kostrzewa 1989, 1998).

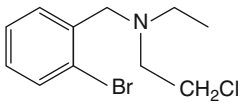
5.4 DSP-4 and Xylamine

5.4.1 Overview

2-Chloroethylamine nitrogen mustards, N-2(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) (Fig. 6) and xylamine (Fig. 7) (Ross 1976; Ross and Reny 1976; Cho et al. 1980; Kammerer et al. 1979; Kreuger and Cook 1975) are able to cross the blood-brain barrier to selectively target noradrenergic neurons. These agents form quaternary aziridinium ions which have high affinity for the NET. Both covalently bind to the NET and are accumulated by noradrenergic neurons. These agents alkylate and inactivate proteins, thus effectively reducing the number of active NET sites (Fischer and Cho 1982) and impairing the function of intracellular cytochromes, enzymes (e.g., dopamine-beta-hydroxylase) (Ross 1976), and other proteins. In the process, NE stores are depleted in both the periphery and central nervous system (CNS) (Jonsson et al. 1981; Zieher and Jaim-Etcheverry 1980; Ross 1976) and overt noradrenergic neuronal destruction ensues (Fritschy and Grzanna 1989, 1991a, b; Fritschy et al. 1990; Grzanna et al. 1989; Jaim-Etcheverry and Zieher 1980; Zieher and Jaim-Etcheverry 1980).

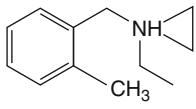
5.4.2 Mechanism of Action of DSP-4 and Xylamine

DSP-4 and xylamine are amines that intracyclize to form aziridinium ions (Ross et al. 1973) – nitrogen mustards (alkylating agents) that covalently and irreversibly bind to electrophilic sites such as thiols and amines in proteins, to effectively impair function of the protein and other molecules (Ranson et al. 1982). DSP-4 and xylamine have high affinity for the NET, and it is this property that confers selectivity of the molecules for noradrenergic neurons. Also, it is via the NET that DSP-4 and xylamine gain entry to the neurons. When DSP-4 binding to the NET is prevented by desipramine, the neurotoxic effect of DSP-4 is abated (Ross 1976). Although the precise mechanism is unknown, monoamine oxidase-B (MAO-B) inhibition by deprenyl (selegiline) also prevents DSP-4 neurotoxicity (Gibson 1987).



DSP-4

Fig. 6 (From Kostrzewa 1999b)



XYLAMINE

Fig. 7 (From Kostrzewa 1999b)

However, because the MAO-B inhibitor MDL 72974 is not neuroprotective, it is not MAO-B inhibition per se that accounts for deprenyl's neuroprotective effect (Finnegan 1993; Finnegan et al. 1990). Perhaps it is the propargylamine moiety of deprenyl that is responsible for its inhibition of DSP-4 neurotoxicity (Maruyama et al. 2000).

5.4.3 Specificity of DSP-4

Generally, because DSP-4 is preferred over xylamine, the latter is used in few studies today. Consequently, most of the following discussion relates to DSP-4.

Because of its high affinity for the NET, DSP-4 is selective for noradrenergic neurons and has little direct effect on dopaminergic, cholinergic, GABAergic, glutamatergic, and glycinergic neurons (see Dudley et al. 1990; Jaim-Etcheverry and Zieher 1983). In the brain, DSP-4 has greatest neurotoxic effects on noradrenergic nerve terminals of locus coeruleus origin (Fritschy and Grzanna 1989, 1991a). DSP-4 degenerative effects in the brain are long-lived. In contrast, in the periphery, sympathetic noradrenergic neurons recover from DSP-4 effects often in less than 1 week (Jaim-Etcheverry and Zieher 1980; Jonsson et al. 1981). Serotoninergic nerves are the only other monoaminergic nerve prominently affected by DSP-4, and the effect on serotonin (5-HT) content and serotoninergic innervation of the brain is long-lived (Jonsson et al. 1981).

5.4.4 Long-Lived Effects of DSP-4 Treatment

When adult rats are treated with a high dose of DSP-4, noradrenergic innervation of projection areas of the locus coeruleus (i.e., neocortex, hippocampus, cerebellum, spinal cord) is markedly reduced, and the effect is long-lived (Jaim-Etcheverry and Zieher 1980; Jonsson et al. 1982). Regions of the brain that are innervated by noradrenergic fibers originating from non-coerulear nuclei are little affected by DSP-4.

When neonatal rats are treated with a high dose of DSP-4, noradrenergic innervation of projection areas of the locus coeruleus is markedly reduced, as is observed in rats treated as adults. In contrast, regions of the brain proximal to the locus coeruleus (i.e., cerebellum, pons, medulla) become hyperinnervated by noradrenergic neurons, and regional NE content is thus elevated. Hyperinnervation is the consequence of collateral axonal fiber sprouting, a reactive sprouting attributable in large part to the degeneration of distal projection of locus coeruleus axons to the neocortex, hippocampus, and spinal cord. This effect is analogous to that seen after surgical transection of the dorsal bundle, the axonal tract projecting from locus coeruleus to forebrain (Kostrzewa et al. 1988). Regions of the brain innervated by non-coerulear nuclei are little affected by DSP-4, as are the sympathetic noradrenergic fibers in the periphery (Bortel et al. 2008; Jaim-Etcheverry and Zieher 1980; Jonsson et al. 1982; Nowak et al. 2006).

5.4.5 Summary on DSP-4

Although 6-OHDA is the most frequently used neurotoxin for noradrenergic neurons, there are occasional studies in which DSP-4 is advantageous. This relates to DSP-4 (1) relative selectivity, particularly for locus coeruleus neurons;

also (2) there is the outcome of long-lived noradrenergic terminal denervation and (3) lack of effect on dopaminergic neuronal fields. Additionally, (4) DSP-4 is able to cross the blood-brain barrier, and thus DSP-4 can be administered in the periphery to produced CNS effects in adults, unlike 6-OHDA. (5) When stored, DSP-4 is more stable than 6-OHDA.

DSP-4 and xylamine neurotoxicity are discussed in greater detail in several reviews (Bortel 2013 [in this *Handbook of Neurotoxicity*]; Dudley et al. 1990; Jaim-Etcheverry 1998; Jaim-Etcheverry and Zieher 1983).

5.5 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

5.5.1 Historical Perspective

In 1976, a graduate student chemist in Maryland synthesized the meperidine analog 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP), to be used as an opioid substance of abuse. However, during the process of synthesis, the chemical mixture was heated to too high a temperature, resulting in the formation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This contaminant, 1–3 % of the MPPP product, proved to be neurotoxic to dopaminergic neuronal perikarya in the pars compacta substantia nigra (SNpc). Within days, he developed the permanent symptomology spectrum of Parkinson's disease (PD) (see Shafer 1985).

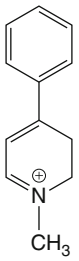
In 1979 in Southern California, a similar synthetic street drug, termed "China White" – the moniker for the purest Southeast Asia heroin – produced the same PD symptomology in substance abusers, again a permanent effect. Three years later, the same event occurred in Northern California, resulting in multiple young substance abusers developing PD-like symptoms (lifelong) and death to more than 50 people. The neurologists J. William Langston and Philip Ballard in San Jose recognized that a bad batch of a heroin-like drug was responsible, and they succeeded in alerting the public to the link between China White and PD/death. As a consequence, that street drug disappeared and the appearance of new cases of early-age PD abated (Langston and Ballard, 1984; Ballard et al. 1985).

Subsequently, MPTP was identified as the neurotoxic contaminant in China White, as testing in animals replicated the forensic finding of SNpc cell destruction found in China White substance abusers. Ultimately, MPTP became the prime neurotoxin to replicate PD in animals. MPTP is of value for probing cellular mechanisms and processes associated with PD and in studying neuroprotectants and new treatment approaches (Shafer 1985; Pasquali et al. 2013).

The key terms "MPTP" or "1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine" appear in 5,746 papers in PubMed, as of January 30, 2013. Key terms for the MPTP metabolite neurotoxic species "1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium" or "MPP^+" appear in 2,664 papers in PubMed as of January 30, 2013.

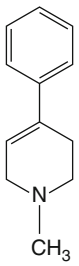
5.5.2 MPTP Mechanism and Selectivity of Action

MPTP (Fig. 8) crosses the blood-brain barrier (BBB) (Riachi et al. 1988) and is converted by monoamine oxidase-B (MAO-B) in astrocytes (Chiba et al. 1984;



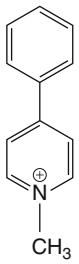
MPTP$^+$

Fig. 8 (From Kostrzewa 1999b)



MPTP

Fig. 9 (From Kostrzewa 1999b)



MPP$^+$

Fig. 10 (From Kostrzewa 1999b)

Westlund et al. 1985; Di Monte et al. 1991) [and to a lesser extent by MAO-A in other cells (Singer et al. 1986)] to the intermediate 1-methyl-4-phenyl-1,2,3-dihydropyridinium ion ($MPDP^+$) (Fig. 9) which undergoes spontaneous two-electron oxidation to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium ion (MPP^+) (Fig. 10) (Castagnoli et al. 1985). MPP^+, released from astrocytes and having high affinity for the DAT, is actively accumulated by dopaminergic neurons (Chiba et al. 1985; Javitch and Snyder 1984; Javitch et al. 1985). Inside DA nerves, the mitochondrial membrane potential actively drives accumulation of MPP^+ to a level in mitochondria as much as 40 times the concentration in cytoplasm (Wu et al. 1990). Here, MPP^+ inhibits complex I in the respiratory transport chain, between NADH dehydrogenase and coenzyme Q, thereby preventing

ATP formation (Chan et al. 1993) and promoting formation of reactive oxygen species (ROS). MPP^+ additionally inhibits alpha-ketoglutarate dehydrogenase in the tricarboxylic acid cycle, thereby preventing succinate formation, a substrate for complex II (Mizuno et al. 1987). Cellular energy depletion (Di Monte et al. 1986; Denton and Howard 1987) and ROS damage to critical elements result in neuronal damage and subsequent neuronal degeneration (Ramsay et al. 1991; see Pasquali et al. 2013). Glial-derived inflammatory cytokines add to stresses on the neuron (see Teismann et al. 2003). DA innervation of neostriatum and DA neuronal perikarya in SNpc are largely affected, although DA neurons in the ventral tegmental nucleus (VTN) and noradrenergic (melanized) neurons in locus coeruleus also are damaged (Seniuk et al. 1990).

5.5.3 Interspecies Effects of MPTP

C57 BL/6 black mice are more sensitive than other mouse strains to MPTP (Sonsalla and Heikkila 1988; Muthane et al. 1994), while rats are largely resistant to MPTP partly because of the abundance of MAO-B in the cerebrocapillary endothelium which constitutes the BBB. MPTP in rats is thereby converted to MPP^+ before crossing the BBB; MPP^+, being ionized, is unable to cross the BBB to enter the brain (Kalaria et al. 1987). Additionally, DA neurons in rats are imbued with a greater abundance of vesicular monoamine transporter-2 (VMAT-2) versus mice. This facet relates to a greater reservoir of MPP^+ inside the vesicle, which translates to a lower cytoplasmic concentration of MPP^+ in rats versus mice (Staal and Sonsalla 2000). This too accounts for a lowered neurotoxic potential for MPP^+ in rats. Nonhuman primates are highly susceptible to MPTP neurotoxicity, with MPTP resulting in effects similar to that observed in human parkinsonian patients (Jenner et al. 1984). It has become conventional to produce unilateral MPTP lesioning in nonhuman primates (e.g., intracarotid administration on one side), so that animals are able to remain mobile and eat without assistance (Bankiewicz et al. 1986; see Fox and Brotchie 2010).

Royland and Langston (1998), Teismann et al. (2003), as well as Carta et al. (2013) and Pasquali et al. (2013) in this *Handbook of Neurotoxicity* provide detailed discussion and analysis of MPTP neurotoxicity mechanisms and MPTP modeling of Parkinson's disease in rodents and nonhuman primates.

5.6 Haloperidol-Related Chemical Species

The classical antipsychotic drug haloperidol (Fig. 11), a pyridinium chemically similar in structure to MPTP, is regarded as a neurotoxin or protoxin (Roberts et al. 1995). Haloperidol and its dehydration product 4-(4-chlorophenyl)-1,4-(4-fluorophenyl)-4-oxobutyl-1,2,3,6-tetrahydropyridine (HPTP) (Fig. 12) are oxidatively metabolized to HPP^+ (Fig. 13) and 4-(4-chlorophenyl)-1,4-(4-fluorophenyl)-4-hydroxybutylpyridinium ($RHPP^+$) (Subramanyam et al. 1991; Eyles et al. 1994, 1996; Igarashi et al. 1995; Usuki et al. 1996) – analogs of MPTP. In vitro HPP^+ is

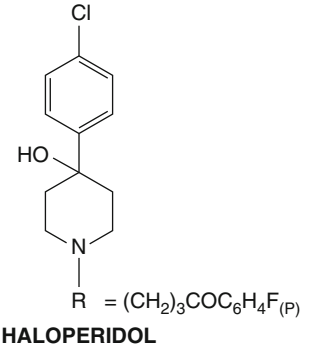


Fig. 11 (From Kostrzewa 1999b)

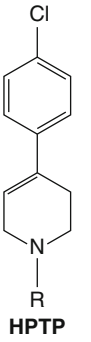


Fig. 12 (From Kostrzewa 1999b)

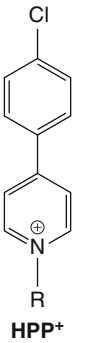
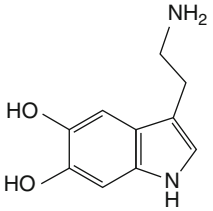


Fig. 13 (From Kostrzewa 1999b)

neurotoxic to both mesencephalic cells (Bloomquist et al. 1994) and neuroblastoma cells (Fang and Yu 1995). In vivo HPP^+ is an inhibitor of the mitochondrial respiratory chain (Rollema et al. 1994) and overtly neurotoxic to DA nerves (Subramanyam et al. 1990). It is these chemical derivatives that perhaps account for the overt neuronal destruction (Benes et al. 1984, 1985a, b) of gamma-aminobutyric acid (GABA) neurons, glutamate neurons (Meshul and Casey 1989;

Fig. 14 (From Kostrzewa 1999b)



5,6-DHT

Meshul et al. 1994), and cholinergic neurons (Mahadik et al. 1988) associated with long-term haloperidol treatment of schizophrenics, in whom tardive dyskinesia has developed. The neurological aspect of haloperidol in tardive dyskinesia is developed further in the *Handbook of Neurotoxicity* chapter on tardive dyskinesia (Kostrzewa et al. 2013).

5.7 2′-Amino-MPTP

2′-Amino-MPTP (2′-NH_2-MPTP), unlike the parent MPTP, had virtually no effect on dopaminergic neurons but did effectively reduce striatal 5-HT and 5-HIAA (Johannessen et al. 1987). Ultimately 2′-NH_2-MPTP was found to also produce a long-term reduction of NE as well as 5-HT contents in rodent forebrain (Andrews and Murphy 1993a, b; Luellen et al. 2003). A substrate for both MAO-A and MAO-B, 2′-NH_2-MPTP likely exerts its effects via the metabolite 2′-NH_2-MPP^+ which has affinity to the NET and SERT. Effects of 2′-NH_2-MPTP on NE and 5-HT in the brain are blocked by desipramine and fluoxetine, respectively (Andrews and Murphy 1993c). Intracellular 2′-NH_2-MPP^+, like MPP^+, apparently acts on mitochondria and other cellular elements to form superoxide. Mice overexpressing Cu-Zn superoxide dismutase, which converts superoxide to less reactive H_2O_2, are protected from the effects of 2′-NH_2-MPP^+ (Andrews et al. 1996). Unlike MPP^+, 2′-NH_2-MPP^+ is a poor substrate for VMAT-2 (Numis et al. 2004).

The neurotoxicity of 2′-NH_2-MPTP is discussed in detail in this *Handbook of Neurotoxicity* by Ochroch et al. (2013).

5.8 5,6- and 5,7-Dihydroxytryptamine (5,6-DHT, 5,7-DHT)

5,6-Dihydroxytryptamine (5,6-DHT) (Fig. 14), a di-/ortho-phenolic analog of 5-hydroxytryptamine (5-HT, serotonin) (Fig. 15), and 5,7-dihydroxytryptamine (5,7-DHT) (Fig. 16), a di-/meta-phenolic analog of 5-HT, were characterized as serotoninergic neurotoxins by Hans Baumgarten in early 1970s – shortly after the discovery of 6-hydroxydopamine (6-OHDA) as a neurotoxin for noradrenergic and dopaminergic neurons (Baumgarten et al. 1971, 1972a, b; Baumgarten and Schlossberger 1973; Baumgarten and Lachenmeyer 1972).

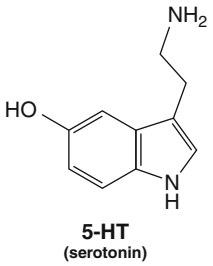


Fig. 15 (From Kostrzewa 1999b)

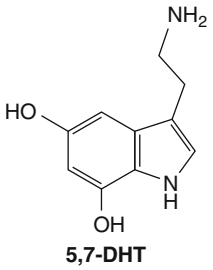


Fig. 16 (From Kostrzewa 1999b)

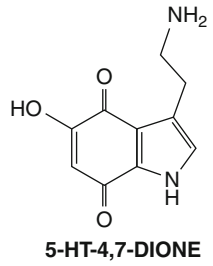


Fig. 17 (From Kostrzewa 1999b)

5.8.1 Mechanisms of Action of 5,6- and 5,7-DHT

5,6- and 5,7-DHT owe their high degree of selective serotoninergic neurotoxicity to their high affinity to the serotonin transporter (SERT) (Björklund et al. 1973a, b, 1975b), which selectively transports the DHTs into the 5-HT neuron. Once present in high intraneuronal concentration, 5,6- and 5,7-DHT intracyclize to generate quinones and additional putative neurotoxic indolamine semiquinoidal and quinoidal species, with 5-HT-4,7-dione (Fig. 17) and its dimer 6,6′-bi(5-HT-4,7-dione) being the principle derivatives (Tabatabaie and Dryhurst 1992, 1998). All are highly reactive. The resulting formation of cellular ROS (e.g., hydrogen peroxide, H_2O_2; superoxide, O_2^{-}; hydroxyl radical, $HO^{\bullet}$) further burdens the protective capacity of 5-HT neurons. Ultimate damage of mitochondrial cytochromes (Cohen and Heikkila 1978), cellular lipids, and cellular proteins thus compromises function, resulting in cellular destruction (Baumgarten et al. 1978a, 1981, 1982; Tabatabaie and Dryhurst 1992; Tabatabaie et al. 1990, 1993).

5,6- and 5,7-DHT neurotoxicity are discussed in greater detail in several reviews (Baumgarten et al. 1973, 1978a, b; Tabatabaie and Dryhurst 1998; Pranzatelli 1998; Baumgarten and Lachenmayer 2004) and in a later chapter of this *Handbook of Neurotoxicity* (Paterak and Stefański 2013).

5.8.2 Limitations of 5,6-DHT

Although the biological effects of 5,6-DHT are similar to those of 5,7-DHT, higher doses of 5,6-DHT become less selective for 5-HT neurons and produce convulsions and death (Baumgarten and Lachenmeyer 1972; Baumgarten et al. 1972b, 1973; Björklund et al. 1973b). Consequently, 5,7-DHT has supplanted 5,6-DHT as a serotoninergic neurotoxin.

5.8.3 Effects of 5,7-DHT in Adults

Long-Lived Effects in the Brain of Animals Lesioned as Adults with 5,7-DHT

Although 5,7-DHT is relatively selective for serotoninergic neurons, a low dose of 5,7-DHT will nonetheless produce both 5-HT and NE depletion in the brain (Baumgarten et al. 1973). The effects of 5,7-DHT on NE neurons can be largely prevented by pretreatment with the NET inhibitor desipramine (Björklund et al. 1975a).

A high dose of 5,7-DHT will destroy 5-HT innervation in virtually all regions of the CNS. The pons and medulla – the brain regions nearest to the most prominent 5-HT perikarya, the dorsal and midbrain raphe nuclei – are least affected by 5,7-DHT treatment, as 5-HT content is only modestly altered after 5,7-DHT. In contrast, regions of the CNS most distal to the dorsal and medial raphe are prominently affected by 5,7-DHT treatment, as 5-HT content of the neocortex, hippocampus, and spinal cord is depleted about 85–90 %. Serotoninergic denervation of striatum by 5,7-DHT is profound. These 5,7-DHT-induced alterations in 5-HT innervation in the brain are long-lived (Baumgarten et al. 1973).

Long-Lived Effects in the Brain of Animals Lesioned as Neonates with 5,7-DHT

When administered to neonatal rats, a single high dose of 5,7-DHT produces lifelong, marked (~90 %) 5-HT denervation and 5-HT depletion of the neocortex, hippocampus, and striatum (Jonsson et al. 1978). In neonatally 5,7-DHT-lesioned rats studied in adulthood, there is prominent DA D_2-receptor supersensitivity, as evidenced by enhanced behavioral responses to the D_2 agonist quinpirole (Brus et al. 1995). In these neonatally 5,7-DHT-lesioned rats, there is also a reduction in histamine levels in the brain and enhanced histamine H_3 receptor antagonist-evoked oral and locomotor activity – indicative of alterations in histaminergic neuronal systems and their effects (Jośko et al. 2011).

In rats co-lesioned as neonates with 5,7-DHT and the noradrenergic neurotoxin DSP4, prominent D_2-receptor supersensitivity is evident in adulthood (Nowak et al. 2009).

In rats lesioned neonatally with 6-OHDA, there is lifelong supersensitization of DA D_1 receptors, as evidenced by enhanced locomotor and oral activity responses and stereotypic responses to the D_1 agonist SKF 38393 (Kostrzewa and Gong 1991;

Gong et al. 1993). However, when 5,7-DHT is administered neonatally, simultaneous with 6-OHDA treatment, there is long-term (into adulthood) suppression of D_1-receptor supersensitivity (Brus et al. 1994). Spontaneous locomotor activity is normally increased in adulthood rats that were lesioned as neonates with 6-OHDA; and there is even greater spontaneous locomotor activity in those rats simultaneously lesioned as neonates with 6-OHDA and 5,7-DHT. Rats so-lesioned as neonates with 6-OHDA and 5,7-DHT have been proposed as an animal model of attention deficit hyperactivity disorder (ADHD) (Kostrzewa et al. 1994).

Summary on 5,7-DHT

5,7-DHT, a relatively selective neurotoxin for 5-HT neurons, provides a means to reasonably study acute and long-lived effects of 5-HT denervation – in both neonatal and adult animals. 5,7-DHT likewise is useful for determining the influence of prominent 5-HT denervation on the effects of other neuronal phenotypic systems and in elucidating the role of 5-HT per se in a variety of functions, including behaviors.

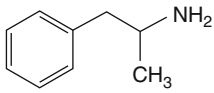
5.9 Amphetamines

5.9.1 Amphetamine (AMPH)

Amphetamine (AMPH) (Fig. 18), chemically similar to DA and NE, has high affinity for the DAT, NET and also for the serotonin transporter, SERT. By virtue of these effects, AMPH gains entry into DA-, NE-, and 5-HT neurons. Intraneuronal AMPH accumulates in both cytoplasm and vesicles, displacing these monoamines and reversing the transporters to evoke monoamine release. In additon, as a consequence of AMPH competition with DA and NE for the DAT and NET, respectively, extraneuronal and synaptic effects of both DA and NE are prolonged and enhanced. Nevertheless, acute treatment or intermittent AMPH treatments are not neurotoxic (Westfall and Westfall 2006).

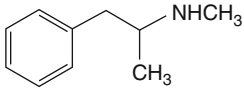
However, when continuously administered for a prolonged period ($\geq$3d), AMPH becomes overtly neurotoxic to striatal dopaminergic nerves, as evidenced by long-term striatal DA depletion (Wagner et al. 1980), loss of tyrosine hydroxylase (TOH) activity (Ellison et al. 1978), loss of DAT number and DAT protein (Scheffel et al. 1996; Krasnova et al. 2001), presence of swollen histofluorescence axons, reduced numbers of dopaminergic terminals, and overt indices of neurodegeneration – deposition of argyrophilic granules (silver degeneration staining) and ultrastructural evidence of terminal damage (Ellison et al. 1978; Nwanze and Jonsson 1981; Ryan et al. 1988, 1990; Ellison 1992). AMPH neurotoxicity extends to TOH-positive mesencephalic neurons and cortical cells (Stumm et al. 1999; Jakab and Bowyer 2002).

AMPH neurotoxicity is associated with hyperthermia (Freh 1975; Clausing and Bowyer 1999) which magnifies the effect of AMPH-induced production of superoxide and hydroxyl radical (Lin et al. 1991; Kil et al. 1996; Huang et al. 1997a; Krasnova et al. 2001) as well as DA-quinone (LaVoie and Hastings 1999).



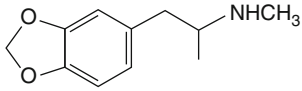
AMPHETAMINE

Fig. 18 (From Kostrzewa 1999b)



METHAMPHETAMINE

Fig. 19 (From Kostrzewa 1999b)



MDMA

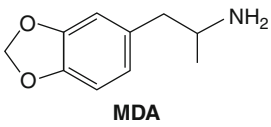
Fig. 20 (From Kostrzewa 1999b)

5.9.2 Methamphetamine (METH, Speed, Crank)

The overall acute effects of methamphetamine (METH) (Fig. 19) are analogous to those of AMPH (see Krasnova and Cadet 2009). Likewise, neurotoxic effects are similarly related to generation of ROS including DA-quinones (LaVoie and Hastings 1999), hydrogen peroxide, and superoxide (Stokes et al. 1999; Miyazaki et al. 2006). However, when METH was added to an in vivo microdialysis perfusate into striatum, the hydroxyl radical level was not elevated (Pereira et al. 2004). It appears that METH-induced glutamate exocytosis (Marshall et al. 1993; Abekawa et al. 1994; Mark et al. 2004) may be largely responsible for generation of nitric oxide (NO) and other ROS (Itzhak et al. 1998; Imam et al. 2001; Itzhak and Ali 2006). Serotoninergic neuronal degeneration is prominent with METH (Ricaurte et al. 1982). The DA D_1-receptor antagonist SCH 23390 [R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine] attenuates neurotoxic effects of METH on DA and 5-HT systems, while the DA D_2 antagonist sulpiride attenuates METH neurotoxic effects on DA systems (Sonsalla et al. 1986).

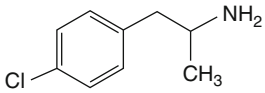
5.9.3 Methylenedioxymethamphetamine (MDMA, Ecstasy)

Methylenedioxymethamphetamine (MDMA) (Fig. 20), a substrate for the 5-HT transporter (SERT), is accumulated by 5-HT nerves; then MDMA is accumulated by vesicles via the vesicular monoamine transporter (VMAT), whereby it releases 5-HT from vesicles and into the synapse (Baumann and Rothman 2009). This action ultimately results in DA exocytosis. MDMA is selectively neurotoxic to 5-HT terminals in rats and nonhuman primates (Green et al. 1995). In mice, MDMA damages nigrostriatal dopaminergic fibers (Commins et al. 1987; Cadet et al. 1995). Methylenedioxyamphetamine (MDA) (Fig. 21) acts similarly to MDMA.



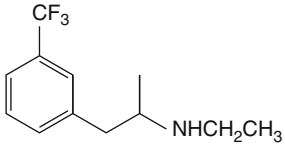
MDA

Fig. 21 (From Kostrzewa 1999b)



PCA

Fig. 22 (From Kostrzewa 1999b)



FENFLURAMINE

Fig. 23 (From Kostrzewa 1999b)

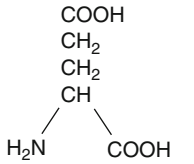
5.9.4 Parachloroamphetamine (PCA)

Parachloroamphetamine (PCA) (Fig. 22) was initially found to produce long-term reduction in tryptophan hydroxylase (Trp-OH) activity and 5-HT in the rat brain (Sanders-Bush et al. 1972a, b). Silver staining later established that PCA actions were related to overt neurotoxic effects, primarily on the serotoninergic B9 cell group (Harvey et al. 1975). PCA neurotoxicity is dependent on 5-HT release; prior 5-HT depletion with parachlorophenylalanine and reserpine prevents PCA neurotoxic actions (Berger et al. 1992). However, prior destruction of striatal dopaminergic innervation does not negate the neurotoxic effect of PCA (Perry et al. 1995).

AMPH, METH, MDA, and analogs have been the subject of many reviews (Cadet et al. 2007; Yamamoto and Raudensky 2008; Gouzoulis-Mayfrank and Daumann 2009; Yamamoto et al. 2010; Steinkellner et al. 2011) including the chapter by Bisagno and Cadet (2013) in this *Handbook of Neurotoxicity*.

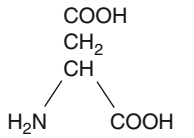
5.9.5 Fenfluramine

Another analog of amphetamine, namely, fenfluramine (Fig. 23), also is neurotoxic towards serotoninergic fibers. Fenfluramine, in high dose or in repeated doses, depletes 5-HT in brain (Harvey and McMaster 1975; Steranka and Sanders-Bush 1979; Schuster et al. 1986), also reduces tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis (Steranka and Sanders-Bush 1979), additionally reduces SERT number (Schuster et al. 1986; Appel et al. 1990; Zaczek et al. 1990) and reduces the numbers of 5-HT immunoreactive fibers in the brain (Appel et al. 1989; Molliver and Molliver 1990). The combination of phentermine with fenfluramine as an anorectic agent was withdrawn from the market because of the association with valvular heart disease (Connolly et al. 1997).



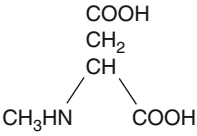
GLUTAMIC ACID

Fig. 24 (From Kostrzewa 1999b)



ASPARTIC ACID

Fig. 25 (From Kostrzewa 1999b)



NMDA

Fig. 26 (From Kostrzewa 1999b)

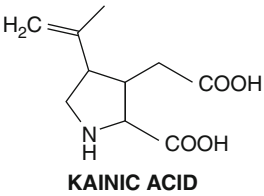
5.10 Excitatory Amino Acids

5.10.1 Historical Perspective

In 1957, Lucas and Newhouse (1957) reported that iontophoretic application of glutamic acid (GLU) (Fig. 24) produced overt destruction of the inner layers of immature mouse retina. Curtis et al. (1959) next showed that GLU and aspartic acid (ASP) (Fig. 25) are excitatory (i.e., produce depolarization) to spinal neurons. And Olney and colleagues (Olney 1969; Olney and Sharpe 1969) demonstrated that GLU is specifically neurotoxic in mice and monkeys, giving birth to the concept of "excitotoxicity" and the range of action of excitatory amino acids (EAAs) (Olney et al. 1971).

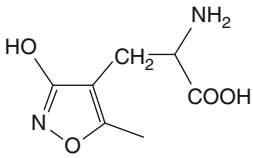
5.10.2 Mechanisms of GLU Excitotoxicity

GLU-mediated neurotoxicity is related to its agonist action at NMDA (N-methyl-D-aspartate) (Fig. 26) receptors (NMDA-R), kainate-R (Fig. 27), or AMPA-R (AMPA = alpha-amino-2,3-hydroxy-5-methyl-3-oxo-isoxazole-propionic acid) (Fig. 28), resulting in massive influx of Na^+, Cl^-, and Ca^{2+} into postsynaptic neurons (Rothman 1985; Choi 1987) and delayed activation of Ca^{2+}-sensitive proteases (Frandsen and Schousboe 1992; Tymianski et al. 1993). As a consequence of these actions and in concert with release of intracellular Ca^{2+} stores, ultimate dendrosomal swelling with vacuolization and mitochondrial edema of postsynaptic neuronal elements concludes in neuronal cell death (see Olney 1981;



KAINIC ACID

Fig. 27 (From Kostrzewa 1999b)



AMPA

Fig. 28 (From Kostrzewa 1999b)

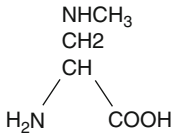
Mody and MacDonald 1995). A variety of intracellular proteins, when activated by ionic events, play a major role in cellular events relating to metabolic inhibition, apoptosis, or necrotic cellular demise (Zeevalk and Nicklas 1991; Ikonomidou and Turski 1996).

As a consequence of the above cellular events, cellular ROS are generated, and these add to the challenge of cellular viability. Neuronal $O_2^{-\cdot}$ is converted by superoxide dismutase to oxygen (O_2) and hydrogen peroxide (H_2O_2). Neuronal nitric oxide synthase (nNOS) activation leads to cellular NO formation (Dawson et al. 1996). In turn, NO reacts with $O_2^{-\cdot}$, forming peroxynitrite ($ONOO^-$) (Huie and Padmaja 1993), a highly reactive species that produces protein nitration, lipid peroxidation, impairment of GAPDH, inhibition of complexes I and II of the electron transport chain, and DNA injury (Radi et al. 1991, 1994; van der Vliet et al. 1998; Hara et al. 2005).

5.10.3 Excitotoxicity in Neurological and Neurodegenerative Disorders

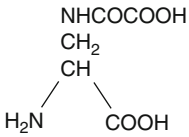
Ischemia, Anoxia, and Traumatic Brain Injury

During transient hypoxia, there is impaired uptake of GLU and ASP from extracellular spaces, leading to a 5-fold or greater increase in GLU and ASP extracellular levels (Beneviste et al. 1984; Globus et al. 1988). This is accompanied by neurotoxicity in cerebral cortex (Jorgensen and Diemer 1982), in hippocampal CA1 pyramidal neurons, and in striatal medium size neurons (Rothman and Olney 1986). Reperfusion results in augmented release of GLU and ASP (Silverstein et al. 1986), resulting in greater excitotoxicity. Prior lesioning of the afferent glutamatergic pathway prevents neurodegeneration during ischemia (Wieloch et al. 1985), as does NMDA-R antagonist treatment (Pulsinelli et al. 1993; Waner et al. 1995) including group I metabotropic GLU-R antagonists (Kingston et al. 1999). In traumatic brain injury (TBI), similarly there is an increase in extracellular glutamate (Faden et al. 1989; Nilsson et al. 1990) accompanied by neurodegeneration, with neuroprotection conferred by NMDA-R antagonists (McIntosh et al. 1989).



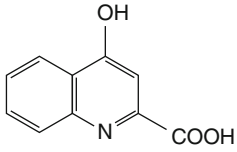
BMAA

Fig. 29 (From Kostrzewa 1999b)



BOAA

Fig. 30 (From Kostrzewa 1999b)



KYNURENIC ACID

Fig. 31 (From Kostrzewa 1999b)

Neurodegenerative Disorders

The involvement of glutamatergic systems in the neurogenesis of PD is indicated by the findings that MPP^+-induced neurodegeneration is attenuated by NMDA-R antagonists (Turski et al. 1991; Santiago et al. 1992). The naturally occurring excitatory amino acids β-N-methylamino-L-alanine (L-BMAA) (Fig. 29) and β-N-oxalylamino-L-alanine (L-BOAA) (Fig. 30) are linked, respectively, to Guam ALS-parkinsonism dementia syndrome (Spencer 1987) and neurolathyrism (Spencer et al. 1986). The kainic acid analog domoic acid, identified as the active principal in contaminated mussels, was responsible for an outbreak of toxic encephalopathy in Canada, resulting in motor neuronopathy and partial paralysis (Perl et al. 1990; see Pérez-Gómez and Tasker 2013). Kainate per se is associated with degeneration of GABA- and substance P-containing neurons (Coyle and Schwarcz 1976; McGeer and McGeer 1976).

Quinolinic acid produces a striatal lesion the models Huntington's disease (HD), associated with chorea in humans (Schwarcz et al. 1983; Beal et al. 1986), although quinolate levels are not elevated in HD (Reynolds et al. 1988; Heyes et al. 1991). Rather, animal modeling of HD is best replicated by aminooxyacetic acid (AOAA) (Urbanska et al. 1989, 1991), a synthesis inhibitor of the endogenous EAA antagonist kynurenic acid (Fig. 31) (Turski et al. 1989; see Urbanska et al. 2013). Moreover, AOAA neurotoxicity is attenuated by NMDA-R antagonists (Urbanska et al. 1991). In HD, kynurenine aminotransferases – enzymes forming kynurenine – are deficient in basal ganglia, thereby implicating dysfunctional kynurenine synthesis in the genesis of HD (Jauch et al. 1995). The overt mechanism of AOAA

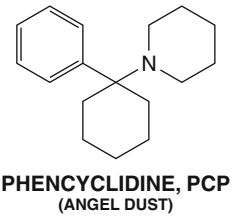


Fig. 32 (From Kostrzewa 1999b)

neurotoxicity relates to impaired cellular metabolism (Beal et al. 1991), and the mitochondrial toxin 3-nitropropionic acid (3-NP) is additionally engaged in rodents and primates to replicate the lesions attending HD (Palfi et al. 1996), with this neurotoxicity likewise being attenuated by NMDA-R antagonists (Brouillet et al. 1995).

The accumulation of extraneuronal beta-amyloid in Alzheimer disease (AD) is an incipient contributor to the neuropathology of this disorder. Beta-amyloid promotes microglial exocytosis of an unknown NMDA-R agonist (Giulian et al. 1995), stimulates NO production (Goodwin et al. 1995), enhances GLU excitotoxicity (Arias et al. 1995), impairs GLU uptake by astrocytes (Harris et al. 1996), and destabilizes intraneuronal Ca^{2+} (Koh et al. 1990).

In amyotrophic lateral sclerosis (ALS), there is an apparent loss of glial EAAT-2 glutamate transporter in the brain and spinal cord (Rothstein et al. 1992, 1995), and this perhaps accounts for elevated CSF levels of GLU in a population of ALS patients (Shaw et al. 1995). The CSF of ALS patients was found to be neurotoxic to neurons in culture, by virtue of kainate-R and AMPA-R action (Couratier et al. 1993, 1994). Motor neurons in ALS display atypical Ca^{2+}-permeable AMPA-R, conferring selective vulnerability to GLU excitotoxic action (Williams et al. 1997).

Comprehensive and insightful reviews have been written on the topic of glutamate, excitatory amino acids, and excitotoxicity (Urbanska et al. 1998; Turski and Ikonomidou 2013).

5.11 NMDA Receptor Antagonists

While NMDA-R antagonists are known to attenuate the neurotoxic effects of NMDA-R agonists (Wieloch 1985; Weiss et al. 1986; Choi et al. 1988; Park et al. 1988; Clifford et al. 1990), paradoxically NMDA-R antagonists are associated with neurotoxicity – according to the dosing schedule and stage of ontogeny at the time of treatment (Olney et al. 1989; Fix et al. 1995, 1996; Farber et al. 1995; Wozniak et al. 1996). Also, noncompetitive NMDA-R antagonists exact a greater toll than competitive NMDA-R antagonists (Ellison 1994). Dizocilpine (MK-801), ketamine, and phencyclidine (PCP) (Fig. 32) produce neurotoxicity to pyramidal neurons in cortical layers II and IV of posterior cingulate/retrosplenial cortex (Olney et al. 1989), also in striatum, subiculum and hippocampal areas CA1 and CA3 (Fix et al. 1995), cingulate cortex, fasciculus retroflexus (FR), and lateral habenula (Ellison 1995).

As proposed by Olney et al. (1991), NMDA-R antagonists disinhibit GABA neurons, which results in excess cholinergic activity. Glycine agonists and polyamine agonists attenuate the toxicity of NMDA-R antagonists without themselves causing neurotoxicity (Duval et al. 1992; Hargreaves et al. 1993). This hypothesis is based on the findings that NMDA-R neurotoxicity is prevented by muscarinic-, σ-, and α_2-R antagonists and both typical and atypical antipsychotics (Olney et al. 1991; Farber et al. 1993, 1995).

NMDA-R neurotoxicity has been the topic of several reviews (Olney et al. 1991; Rajdev and Sharp 1998; Olney 2002; Low and Roland 2004).

5.12 Cocaine

Neurotoxic effects of the abused drug cocaine have come to light, largely in the past two decades. Cocaine is known to impair mitochondrial complex I, II, and III activity and reduce state 3 respiration (Devi and Chan 1997; Yuan and Acosta 2000; Dietrich et al. 2004). This is followed by loss of the mitochondrial membrane potential and release of cytochrome c (see Cunha-Oliveira et al. 2008). At the neurotoxic stage, changes occur in Bcl-2 and Bax (Dey et al. 2007). Apoptosis is the outcome (see Cunha-Oliveira et al. 2008).

Prolonged (5-day) continuous administration of cocaine to adult rats via a cocaine-silicone pellet produced degeneration of the lateral habenula (LHb) in the dorsal thalamus and its projection – the fasciculus retroflexus (FR) – to midbrain dopaminergic nuclei (substantia nigra, ventral tegmental nucleus) and serotoninergic nuclei (dorsal and medial raphe) (Ellison 1992). AMPH, METH, MDMA, and cathinone were later found to produce similar but not identical effects as cocaine (Ellison 2002). Also, when cocaine was administered to pregnant rats for the remainder of pregnancy, starting on gestation day 17, the LHb and FR of rat pups underwent analogous neurodegeneration (Murphy et al. 1999).

The neurotoxicity of prolonged administration of cocaine and related agents (e.g., AMPH) resembles that of phencyclidine (Ellison 1995) and is thought to be related to a reduction in glucose metabolism in LHb (London et al. 1986; Wirtshafter et al. 1994), consequent to loss of GABA inhibition and associated excitotoxic neurotoxicity (Ellison 1998).

The neurotoxic effects of relatively short-term cocaine administration are discussed in papers by Cunha-Oliveira et al. (2008, 2013). The neurotoxic effects of continuous and prolonged cocaine administration are discussed by Ellison (1998).

5.13 AF64A (Ethylcholine Aziridinium Ion)

AF64A is a Trojan horse neurotoxin with relative selectivity for cholinergic nerves. Chemically similar to choline but with an aziridinium moiety on the nitrogen atom, AF64A is a nitrogen mustard (alkylating agent) that targets the choline uptake site on

acetylcholine (Ach)-containing nerves (Fisher and Hanin 1986, Hanin 1988, 1996). In high concentration (>22 μM), AF64A alkylates both low- and high-affinity choline transporter sites, impairing its own uptake by the nerve and producing nonspecific damage to any tissue but notably to the cerebral endothelium which maintains a choline transport system (Gomez-Ramos et al. 1990).

In low concentration (<5 μM), AF64A is reversibly bound to the choline transporter and then largely enters the cholinergic nerve and alkylates many sites, predominately enzymes and proteins with affinity for choline: choline acetyltransferase, choline kinase, acetylcholinesterase, and choline dehydrogenase (Curti and Marchbanks 1984; Hortnagl and Hanin 1992; Hortnagl et al. 1988; Rylett and Colhoun 1984; Uney and Marchbanks 1987). Alkylated enzymes and other macromolecules become inactivated, rendering the nerve dysfunctional (Barlow and Marchbanks 1984; Leventer et al. 1987; Potter et al. 1987; Sandberg et al. 1985). Cell death is related to alkylation at N-7 guanine sites on DNA, resulting in strand breaks and termination of RNA transcription (Futscher et al. 1992). AF641-induced alterations in cholinergic nerves are often delayed by as much as 48 h (Hanin 1996).

Unable to cross the blood-brain barrier, AF64A is commonly administered into the cerebral ventricles to model for Alzheimer's type senile dementia (Fisher and Hanin 1986). Although the levels of neurotransmitters other than Ach are acutely altered by AF64A, these changes are short-lived and thus considered to be secondary to the abrupt alteration of cholinergic neural function after AF64A treatment (see Hanin 1990).

AF64A is used infrequently, largely having been supplanted as a cholinergic neurotoxin by 192-IgG-saporin.

5.14 IgG-Saporin

The immunotoxin IgG-saporin consists of (1) a monoclonal antibody directed towards $p75^{NGF}$, the low-affinity membranous receptor for nerve growth factor (NGF), coupled with (2) saporin, a ribosome-inactivating protein derived from seeds of Saponaria officinalis (soapwort) (Wiley 1992; Wiley et al. 1991).

5.14.1 Selectivity of IgG-Saporin

IgG-saporin is a selective toxin for cholinergic neurons in basal forebrain, by virtue of their expression of the $p75^{NGF}$ receptor on the nerve membrane (Book et al. 1994). Once IgG-saporin is complexed to the $p75^{NGF}$ receptor, saporin is internalized by receptor-mediated endocytosis and then transported to the cell body where it enzymatically inactivates ribosomes to inhibit protein synthesis and produce neuronal cell death. IgG-saporin produces near-total destruction of the CBF (see Wiley 1992).

In rat basal forebrain, the cholinergic tract (CBF) is the only one expressing $p75^{NGF}$ on the plasma membrane. IgG-saporin thereby selectively destroys these

neurons and thereby produces cholinergic denervation of the CBF target regions – neocortex, hippocampus, amygdale, and olfactory bulb (Rossner et al. 1995; Schliebs 1998; Walsh et al. 1996). Other neuronal tracts of the forebrain survive IgG-saporin treatment because they lack the $p75^{NGF}$ receptor; this includes striatal cholinergic interneurons and GABAergic neurons within the striatum (Heckers et al. 1994; Wiley et al. 1995).

However, in the cerebellum, the subset of Purkinje cells, which express $p75^{NGF}$ receptors during development and into adulthood (Pioro and Cuello 1988), are damaged by IgG-saporin treatment (Berger-Sweeney et al. 1994; Heckers et al. 1994).

5.14.2 Effect of IgG-Saporin Lesioning

The CBF is the neuronal tract that degenerates in Alzheimer disease (AD), and the extent of CBF loss correlates with the degree of dementia (Perry et al. 1978). Because IgG-saporin selectively destroys the CBF, this neurotoxin is used to model AD (Schliebs et al. 1996; Wiley et al. 1995).

The neurotoxicity of IgG-saporin has been described in numerous reviews: Bassant et al. 1998; Petrosini et al. 2013 [this *Handbook of Neurotoxicity*]; Rossner 1997; Schliebs et al. 1996; Walsh and Potter 1998; Wiley (1992); Wrenn and Wiley (1998).

5.15 3-Nitropropionic Acid (3-NP)

3-Nitropropionic acid (3-NP) ($O_2NCH_2CH_2COOH$), a suicide (irreversible) inhibitor of succinate dehydrogenase (Alston et al. 1977), is a plant and fungal neurotoxin with relative selectivity for basal ganglia (caudate and putamen in humans; striatum in rodents) (Ludolph et al. 1991), although high-dose 3-NP additionally damages hippocampus. In humans who consumed foods with 3-NP and in nonhuman primates treated with 3-NP, the spectrum of neurodegeneration and motor disability, with overall spectrum of effects, resembles/models Huntington's disease (HD).

5.15.1 3-NP Mechanism of Action

Coles et al. (1979) identified 3-NP as a suicide inhibitor of succinate dehydrogenase (Huang et al. 2006). Gould and Gustine (1982) first showed that 3-NP produced overt neurotoxicity in basal ganglia.

As succinate dehydrogenase is the major element of mitochondrial respiratory chain complex II, 3-NP inactivation of this site leads to reduced ATP formation, cellular energy depletion, and ultimate preferential degeneration of medium spiny neurons in basal ganglia. NADPH-diaphorase-positive aspiny neurons and cholinergic interneurons survive 3-NP treatment (Beal et al. 1993; Brouillet et al. 1993). Neurodegenerative effects of 3-NP arise when there is prolonged partial (>50%) SDH inhibition (Alexi et al. 1998; Brouillet et al. 1998), resulting in loss of the mitochondrial membrane potential (Leventhal et al. 2000; Maciel et al. 2004), cytochrome c release from mitochondria with calpain activation (Bizat et al. 2003a, b; Nasr et al. 2003), caspase-3 activation (Bizat et al. 2003b),

translocation of Bcl-2, and ultimate neuronal apoptosis (Galas et al. 2004). Calpain activation is considered to be a signature event. Notably, 3-NP-induced calpain activation is preferential to basal ganglia, with calpain remaining active in neocortex and other brain regions where neurodegeneration is generally absent (Galas et al. 2004).

The above processes are also associated with the generation of cellular ROS including $O_2^{-\cdot}$, $HO^{\bullet}$, and NO formation (Schulz et al. 1995, 1996; Fontaine et al. 2000). Compromise of the energy status of medium spiny neurons is thought to sensitize to glutamate-mediated effects at the NMDA-R, with promotion of glutamate excitotoxicity (Hamilton and Gould 1987; Novelli et al. 1988; Zeevalk and Nicklas 1992; Kim et al. 2000). This latter event was postulated as being responsible for the neurodegeneration with 3-NP and in HD (Albin and Greenamyre 1992; Beal 1992).

3-NP is commonly administered by osmotic minipumps in rodents, producing dystonia with motor incoordination (Beal et al. 1993; Borlongan et al. 1995). In primates, daily 3-NP administration produces the characteristic striatal neurodegeneration and choreiform movements with cognitive deficits (Brouillet et al. 1995; Palfi et al. 1996). Processes and mechanisms of 3-NP have been described in detail (Brouillet et al. 2005).

5.16 Toxic Vanilloids, TRPV1 Receptor Agonists

5.16.1 Overview

Vanilloids, so-named by virtue of their vanillyl (4-hydroxy-3-methoxybenzyl) moiety, are comprised of three chemical classes: capsaicinoids, resiniferanoids, and diterpenes. Capsaicin (Fig. 33), the best known of the vanilloids, is a capsaicinoid derived from hot chili peppers (Szallasi 1998; Szallasi and Blumberg 1999) – flavoring foods by producing a spicy-hot sensation. The effect is related to activation of TRPV1 receptors (TRPV1-R) on fine-diameter unmyelinated C fibers or thinly myelinated Adelta sensory axons (Holzer 1991) of ~25 % of sensory nerves in bipolar dorsal root ganglia and trigeminal ganglia (Liu et al. 1996). Capsaicin, given repeatedly, desensitizes the TRPV1-R (Jancsó and Jancsó 1949; Buck and Burks 1986; Holzer 1991) which undergoes a conformational change in a ligand-gated cation channel (Bevan and Docherty 1993). Additionally, capsaicinoids suppress the expression of peptide neurotransmitters in sensory ganglia (Farkas-Szallasi et al. 1995) while enhancing the expression of galanin, an inhibitor of excitatory neurons (Yanagisawa et al. 1986). High-dose vanilloids are capable of producing overt destruction of sensory neurons (Jancsó et al. 1978).

Insect and animal venoms often contain peptide toxins able to activate the vanilloid receptor (Siemens et al. 2006; Bohlen and Julius 2012) or inhibit it. Capsaicinoids are used in the treatment of neuropathic pain (Rumsfield and West 1991; Winter et al 1995) and are found in “pepper” sprays and in squirrel-free bird food (birds’ TRPV1-R lack the vanilloid binding motif).

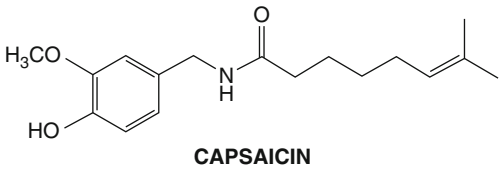


Fig. 33 (From Kostrzewa 1999b)

5.16.2 Mechanism of Vanilloid Action

Vanilloid neurotoxicity is associated with an increase in the Ca^{2+} current in sensory neurons, leading to a rise in the cytoplasmic level of Ca^{2+} (Chard et al. 1995) and activation of calcineurin and other proteases (Docherty et al. 1996) which dephosphorylate the vanilloid receptor or channel complex and thereby desensitize the receptor complex (Bevan and Docherty 1993). Capsaicin in high concentration (EC_{50}, 6.9 μM) also reduces the mitochondrial membrane potential (Dedov et al. 2001) and can produce DNA fragmentation, with apoptotic cell death.

The topic of toxic vanilloids is the subject of several reviews (Buck and Burks 1986; Holzer 1991; Bevan and Docherty 1993; Szallasi 1998; Szallasi and Blumberg 1999; Kumar et al. 2013).

5.17 Botulinum Neurotoxin

The name botulinum neurotoxin (BoNT) is derived from the Latin "botulus" (i.e., sausage), reflecting discovery of BoNT's ill effects during the Middle Ages – consequent to consumption of poorly prepared or preserved sausage. BoNT and the related tetanus neurotoxin are members of the group of anaerobic bacterial clostridial neurotoxins.

There are seven serological BoNTs, BoNT-A through BoNT-G, with A, B, E, and F subtypes having effects in humans. BoNT consists of a 100kDA heavy chain, paired by a disulfide bond with a 50 kDa light chain. One segment of the BoNT molecule, the binding domain, specifically binds to sites on the cholinergic nerve, thus imparting selectivity of BoNT neurotoxicity to cholinergic nerves (i.e., motoneurons and parasympathetic nerves). A translocation domain is considered to govern pore formation in the cholinergic plasma membrane, for entry of the catalytic domain into the cholinergic nerve. The catalytic domain inactivates V-snare or T-snare proteins, thus preventing the binding of cytoplasmic vesicles to the cholinergic plasma membrane and thereby preventing exocytosis of the neurotransmitter acetylcholine. BoNT poisoning results in skeletal muscle flaccid paralysis, with death from asphyxiation. However, in regulated dosages, BoNT has proven to be a clinically useful agent for a variety of disorders (Foster et al. 2006; Kostrzewa and Segura-Aguilar 2007; Mehanna and Jankovic 2013; Singh et al. 2013).

Mechanisms, actions, and clinical utility of BoNT are the topic of several recent reviews (Foster et al. 2006; Singh 2006; Kostrzewa and Segura-Aguilar 2007; Mehanna and Jankovic 2013; Singh et al. 2013).

5.18 Quinpirole, a Dopamine D_2-Receptor Agonist

The definition of a "selective neurotoxin" has become somewhat obscure, when taking the actions of the DA D_2-receptor agonist, quinpirole, into consideration. Quinpirole, when administered repeatedly – at specified stages of ontogeny – produces long-term sensitization (i.e., a "priming" phenomenon) of the D_2-R, so that subsequent quinpirole dosing results in exaggerated, sometimes abnormal, behavioral responses. Yet, there is no overt evidence of neurotoxicity, other than the abnormality of behavioral effects to challenge doses of this D_2-R agonist.

Eilam et al. (1989) first observed that quinpirole challenge in adult rats produced hyperactivity and perseveration in routes (restricted paths in an open environment) without perseveration in movements. Subsequently, ontogenetic postnatal daily quinpirole treatments (2.6 mg/kg/day × 32 day, base form, ip) of rats were shown to produce enhanced quinpirole-induced behavioral effects. The same abnormal stereotypic response (i.e., licking, grooming, digging, eating) to quinpirole was observed in rats that had been both 6-OHDA lesioned (134 μg, i.c.v., 3 days after birth) as neonates and challenged daily for the first 32 days after birth either with quinpirole or with the DA D_1-R agonist SKF 38393 (Kostrzewa et al. 1990).

When quinpirole (2.6 mg/kg/day) was administered ip to rats once a day for the first 28 days after birth, quinpirole challenge (25–200 μg) at 8–10 weeks produced a dose-related enhanced yawning response, without there being evidence of a change in the B_{max} or K_d for [^{3}H]spiperone binding in the striatum (Kostrzewa and Brus 1991). This enhancement of quinpirole-induced yawning was evident at 6 weeks, even in rats that had been primed by low-dose quinpirole (50 μg/kg/day) for 11 consecutive day periods: birth (P0) to P11 (11 days after birth), P12 to P22, or P23 to P33 (Kostrzewa et al. 1993a). In contrast to quinpirole which primes the D_2-R, the reputedly D_3-R agonist 7-OH-DPAT [(±)-2-(dipropylamine)-7-hydroxy-1,2,3,4-tetrahydronaphthalene] failed to prime a yawning response to either quinpirole or 7-OH-DPAT in rats (Oswiecimska et al. 2000).

Postnatal ontogenetic quinpirole priming (2.6 mg/kg/day) of rats was also associated with adulthood enhancement of a quinpirole (100 or 1000 μg/kg)-induced antinociceptive effect (hot plate response time) (Kostrzewa et al. 1991). Bizarre quinpirole-induced vertical jumping was observed during the interval of postnatal ontogenetic quinpirole priming (2.6 mg/kg/day, P0–P28) of rats, starting at P18. Quinpirole-induced vertical jumping was evident with quinpirole challenge doses in the range of 0.1–3.0 mg/kg), and the effect of high-dose quinpirole persisted for hours. Notably, vertical jumping was observed only if the cage lid was removed. Otherwise jumping was suppressed, so that rats did not harm themselves by hitting against the cage lid. Typically, paw treading against the cage wall was evident during the duration of the jumping episode (Kostrzewa et al. 1993b). In rats that had been both 6-OHDA lesioned (134 μg, i.c.v., 3 days after birth) as neonates and challenged daily from birth with quinpirole, quinpirole-induced vertical jumping was enhanced (Kostrzewa and Kostrzewa 2012).

While changes in striatal D_2-R number or affinity do not accompany D_2-R supersensitivity (Kostrzewa and Brus 1991), an acute challenge dose of amphetamine (1.0 mg/kg ip) in 3-month old rats that had been primed with quinpirole during postnatal ontogeny (50 μg/kg/day ip, P0–P11) produced a five-fold increase in striatal DA exocytosis, as evidenced in the in vivo microdialysate DA level in awake freely moving rats. In contract, a quinpirole challenge dose in adulthood did not evoke DA exocytosis. In rats that were primed from P0 to P11 by daily quinpirole treatment, there was suppression of striatal DA exocytosis by quinpirole, identical to that observed in control non-primed rats. And in rats that were primed at approximately 2 months of age by 3 increasing doses of quinpirole (25, 50, and 100 μg/kg, 1 dose per day), an acute quinpirole challenge dose (100 μg/kg) produced even greater suppression of striatal DA exocytosis (Nowak et al. 2001). These findings indicate that D_2-R supersensitivity has a postsynaptic component (i.e., D_2-R agonists produced enhanced behavioral effects) as well as a presynaptic component (i.e., AMPH-evoked exocytosis).

In neonatally 6-OHDA-lesioned rats (134 μg, i.c.v., 3 days after birth) that were additionally primed during postnatal ontogeny with quinpirole (2.6 mg/kg/day, P0–P28), a quinpirole challenge dose (2.6 mg/kg) in adulthood failed to produce an enhanced quinpirole-induced locomotor response. However, quinpirole priming did enhance D_1-R agonist SKF 38393-induced locomotor responses in adulthood (Brus et al. 2003). This effect is analogous to adulthood heterologous priming of D_1-R by weekly quinpirole challenge doses in rats that had been lesioned as neonates with 6-OHDA (Criswell et al. 1989).

While DSP-4 neonatal lesioning of noradrenergic innervation prevents ontogenetic quinpirole priming of D_2-R (Nowak et al. 2006), neonatal co-lesioning with DSP-4 and 5,7-DHT results in enhanced quinpirole-induced and 7-OHDA-induced yawning as well as enhanced apomorphine-induced stereotypies (Nowak et al. 2009). These findings indicate that both noradrenergic and serotoninergic nerves have a prominent influence on D_2-R priming.

In quinpirole-primed rats, spatial memory deficits and enhanced skilled reaching were observed in adulthood (Brown et al. 2002). Enhanced quinpirole-induced locomotor and stereotyped responses observed in adult rats that were quinpirole primed during postnatal ontogeny are attenuated by acute nicotine pretreatment in adulthood; and this effect was accompanied by increased [^{125}I]α-bungarotoxin binding in striatum and hippocampus and greater [^{3}H]cytisine binding in midbrain and cerebellum. These findings indicate that nicotinic α_7 and $\alpha_4\beta_2$ receptor parameters, respectively, are regionally altered in quinpirole-primed rats (Tizabi et al. 1999). In separate studies, nicotine adulthood treatments (0.3 mg/kg x 2/day) for 14 consecutive days reversed performance deficits in quinpirole-primed rats on the Morris water task and skilled reaching task and partially reversed a 36 % decrease of choline acetylcholine transferase in hippocampus. This finding supports the role of nicotinic receptors in quinpirole priming and projects involvement of cholinergic systems in the priming phenomenon (Brown et al. 2004).

D_2-R supersensitivity is implicated in obsessive-compulsive disorder (Eilam et al. 1989, 2006; Szechtman and Woody 2004, 2006; Alkhatib et al. 2013) and is

regarded as a major component of schizophrenia in which there is an elevation in the number of D_2^{high} receptors (Seeman et al. 2005, 2007; Seeman 2011). So-called breakthrough DA-R supersensitivity is also regarded as a factor in antipsychotic treatment failure (Samaha et al. 2007).

The process of D_2-R supersensitivity, as relating to quinpirole-induced ontogenetic priming, appears to be a lifelong permanent effect, still evident in rats approaching 2 years of age (Brus et al. 1998; Oswiecimska et al. 2000). Quinpirole induction of D_2-R supersensitivity is discussed in several reviews (Kostrzewa 1995; Kostrzewa et al. 2004, 2008a, 2011; Nowak et al. 2004; Brown et al. 2012). Permanent sensitization of D_2-R and resulting alteration of behaviors, despite lack of evidence of neuropathology, does nonetheless represent a neurotoxicity.

5.19 Other Neurotoxins

The overview of selective neurotoxin in this chapter is intended to highlight the most commonly known neurotoxins and most commonly used neurotoxins. Not all neurotoxins have been listed and/or described in detail in this overview. There are a number of other EAAs and other types of neurotoxins that are used, and some are described in greater detail in other chapters of the *Handbook of Neurotoxicity*.

6 The Future of Neurotoxins in Biomedicine

For the past 50 years, selective neurotoxins have been instrumental in:

1. *Mapping nerve tracts in the CNS*: Destruction of the nerve terminal field is often followed by axonal swelling, retrograde throughout its entire length, back to the perikaryon. A marker for this phenotypic nerve provides an excellent means to map the route of the axons. For example, by lesioning the terminal field for noradrenergic nerves and treating with a fluorescent-tagged antibody for dopamine-beta-hydroxylase, noradrenergic axons will glow brightly green and thereby show the exact course of the noradrenergic nerve tract through the brain.
2. *Demonstrating nerve fiber sprouting*: When neurotoxins destroy one axon of a bipolar (i.e., 2 axons) or multipolar nerve, the more proximal branch(es) of the nerve often sprout and ***hyperinnervate*** regions that are normally innervated by the surviving axonal branch(es). At times, the surviving branch(es) extends their geographic domain to innervate new regions which are not normally innervated by this nerve (***neo-innervation***). Neurotoxins have provided the means to uncover these phenomena. Also, by virtue of selective neurotoxicity, these findings are "pure" – not encumbered by complications ensuing, were more than one neuronal phenotype damaged.
3. *Determining the physiological role played by specific neuronal tracts*: As indicated by WB Canon, the function of a nerve can be deduced by denoting altered effect when the nerve is destroyed (Cannon and Rosenblueth 1937). Accordingly, the importance of dopaminergic innervation in nucleus accumbens

with the euphoric and other effects of drugs of abuse can be deduced. The same approach is successfully used to link other nerve phenotypes with a particular brain region and associated behavioral effect.

4. *Ascribing neuronal denervation with receptor supersensitization phenomena*: The proliferation in numbers of receptors following selective destruction of a nerve can be reasonably quantified; and possible changes in the affinity of these receptors can be studied. Also, the relative contribution of high- and low-affinity states of the receptor can be elucidated. Selective neurotoxins are especially valuable for such studies. However, since the time that selective neurotoxins have been employed for such studies, it has become clear that receptor supersensitization (i.e., ability of the receptor to produce an exaggerated response) is not necessarily related to a change in receptor number. Additional receptors do not necessarily correlate with increased receptor sensitization.
5. *Characterizing specific neuronal phenotypes with human neurological and psychiatric disorders*: Selective lesioning of particular nerve types or specified nerve tracts has been of immense value in animal modeling of human neuropathological and psychiatric states. Ideally, one seeks (a) face validity (mirroring of a behavioral deficit), (b) construct validity (conforming with a pathophysiological basis of a disorder), and (c) predictive validity (ability to project a therapeutic intervention) in an animal model. Some models relate to merely "predictive validity." For example, perinatal 6-OHDA treatment of rats leads to an adolescent and adulthood hyperactivity and attention deficit, which is responsive to amphetamine – just as human ADHD is responsive to amphetamine (see Kostrzewa et al. 2008b). Yet, human ADHD has no relationship with near-total dopaminergic denervation of forebrain – as found in this animal model. Other animal modeling of human disorders is reliant on use of selective neurotoxins.
6. *Identifying intraneuronal mechanisms and processes that lead to cell death by apoptosis, necrosis, and necroptosis*: Neurotoxins commonly evoke a number of processes that lead to cellular instability and compromised viability. Classically there is an increase in intracellular Ca^{2+} levels, also in the number of ROS and reactive nitrogen species, changes in oxidative phosphorylation accompanied by altered mitochondrial membrane potential, mitochondrial release of cytochrome c into the cytoplasm, increased phosphorylation of proteins, promotion of aggregates of abnormal proteins, activation of Bax and other proapoptotic elements along with suppression of bcl and other protective cellular elements, translocation of proteins to the nucleus, and other changes. Neurotoxins have aided immensely in the study of cell death phenomena.
7. *Recognition of the role played by microglia, astrocytes, and immune cells in neurodegeneration*: Selective neurotoxins have been instrumental in evoking neuronal damage and thereby provoking nonneural cellular responses to injury. These include astrocytes, activated microglia, and immune cells which arise in a uniquely timed and ordered array. The incentive to understand mechanisms of neurotoxicity has aroused interest in the role played by other cells in neuropathological processes.

8. *Broadening the understanding that there are multiple cellular targets whereby drugs may intervene to disrupt an otherwise programmed cell death*: There was an awakening of interest in new means by which drugs can conceivably target one or another process to facilitate recovery of an injured neuron. In addition, there is now global interest in neuroprotectants, which can prevent an otherwise deleterious effect on nerves.

   Looking ahead we can foresee the introduction of multiple kinds of selective neurotoxins, in order to broaden the horizon for these agents in elucidating the association between specified nerve and nerve tracts with biological and behavioral actions. We can envision:

   1. An increase in the number of phenotypically selective neurotoxins
   2. Introduction of neurotoxins that act on novel intracellular targets
   3. More neurotoxins tagged to antibodies
   4. Trojan horse botulinum toxins, engineered with binding proteins that target an ever-expanding field of susceptible cells, and conveying a toxic load into these cells
   5. Engineered immunotoxins, altered to target specified cells – analogous to IgG-saporin
   6. Agonists/antagonists directed towards neurotrophin receptors, so as to compromise development of specified neuronal phenotypes
   7. Antibodies for specific neurotrophins, analogous to that of anti-NGF, producing immuno-disarray of specified types of nerves
   8. Alkylating agents analogous to DSP-4
   9. Added excitatory amino acids (EAAs)
   10. Inhibitors of (endogenous) neuroprotectants
   11. Agents that desensitize nerves, akin to capsaicin
   12. A host of agents that act by unanticipated means

In the process, there will be a discovery of novel neuroprotectants, some with targeted specificity and others with generalized effects. As more becomes known of cellular processes and mechanisms in the neuronal response to injury, so too does the number of possible neuroprotectants increase.

We cannot foresee changes that will occur in the next century. So many more revolutions are certain, and someone looking back 100 years from now will likely be confused by what will probably seem to be limited scope in the assumed "direction" of this field. When tomorrow sees today's views as too narrow in scope, this will be proof that we, today, recognized how important the topic of neurotoxins is, for the development of neuroscience and an ever increasing understanding of how nerves work (adapted from Kostrzewa 1999a).

References

Abekawa, T., Ohmori, T., & Koyama, T. (1994). Effects of repeated administration of a high dose of methamphetamine on dopamine and glutamate release in rat striatum and nucleus accumbens. *Brain Research, 643*, 276–281.

Albin, R. L., & Greenamyre, J. T. (1992). Alternative excitotoxic hypotheses. *Neurology, 42*, 733–738.

Alexi, T., Hughes, P. E., Knusel, B., & Tobin, A. J. J. (1998). Metabolic compromise with systemic 3-nitropropionic acid produces striatal apoptosis in Sprague-Dawley rats but not in BALB/c ByJ mice. *Experimental Neurology, 153*, 74–93.

Alkhatib, A. H., Dvorkin-Gheva, A., & Szechtman, H. (2013). Quinpirole and 8-OH-DPAT induce compulsive checking behavior in male rats by acting on different functional parts of an OCD neurocircuit. *Behavioural Pharmacology, 24*, 65–73.

Alston, T. A., Mela, L., & Bright, H. J. (1977). 3-Nitropropionate, the toxic substance of Indigofera, is a suicide inactivator of succinate dehydrogenase. *Proceedings of the National Academy of Sciences USA, 74*, 3767–3771.

Andrews, A. M., & Murphy, D. L. (1993a). Sustained depletion of cortical and hippocampal serotonin and norepinephrine but not striatal dopamine by 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH_2-MPTP): a comparative study with 2′-CH_3-MPTP and MPTP. *Journal of Neurochemistry, 60*, 1167–1170.

Andrews, A. M., & Murphy, D. L. (1993b). 2′-NH_2-MPTP in Swiss Webster mice: evidence for long-term (6-month) depletions in cortical and hippocampal serotonin and norepinephrine, differential protection by selective uptake inhibitors or clorgyline and functional changes in central serotonin neurotransmission. *Journal of Pharmacology and Experimental Therapeutics, 267*, 1432–1439.

Andrews, A. M., & Murphy, D. L. (1993c). Fluoxetine and desipramine selectively attenuate 2′-NH_2-MPTP-induced depletions in serotonin and norepinephrine. *European Journal of Pharmacology, 250*, 215–221.

Andrews, A. M., Ladenheim, B., Epstein, C. J., Cadet, J. L., & Murphy, D. L. (1996). Transgenic mice with high levels of superoxide dismutase activity are protected from the neurotoxic effects of 2′-NH_2-MPTP on serotonergic and noradrenergic nerve terminals. *Molecular Pharmacology, 50*, 1511–1519.

Appel, N. M., Contrera, J. F., & De Souza, E. B. (1989). Fenfluramine selectively and differentially decreases the density of serotonergic nerve terminals in rat brain: evidence from immunocytochemical studies. *Journal of Pharmacology and Experimental Therapeutics, 249*, 928–943.

Appel, N. M., Mitchell, W. M., Contrera, J. F., & De Souza, E. B. (1990). Effects of high-dose fenfluramine treatment on monoamine uptake sites in rat brain: Assessment using quantitative autoradiography. *Synapse, 6*, 33–44.

Arias, C., Arrieta, I., & Tapia, R. (1995). beta-Amyloid peptide fragment 25-35 potentiates the calcium-dependent release of excitatory amino acids from depolarized hippocampal slices. *Journal of Neuroscience Research, 41*, 561–566.

Ballard, P. A., Tetrud, J. W., & Langston, J. W. (1985). Permanent human parkinsonism due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): Seven cases. *Neurology, 35*, 949–956.

Bankiewicz, K. S., Oldfield, E. H., Chiueh, C. C., Doppman, J. L., Jacobowitz, D. M., & Kopin, I. J. (1986). Hemiparkinsonism in monkeys after unilateral internal carotid artery infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Life Sciences, 39*, 7–16.

Barlow, P., & Marchbanks, R. M. (1984). Effect of ethylcholine mustard on choline dehydrogenase and other enzymes of choline metabolism. *Journal of Neurochemistry, 43*, 1568–1573.

Bassant, M. H., Jouvenceau, A., Apartis, E., Poindessous-Jazat, F., Dutar, P., & Billard, J. M. (1998). Immunolesion of the cholinergic basal forebrain: Effects on functional properties of hippocampal and septal neurons. *International Journal of Developmental Neuroscience, 16*, 613–632.

Baumann, M. H., & Rothman, R. B. (2009). Neural and cardiac toxicities associated with 3,4-methylenedioxymethamphetamine (MDMA). *International Review of Neurobiology, 88*, 257–296. doi:10.1016/S0074-7742(09)88010-0.

Baumgarten, H. G., & Lachenmeyer, L. (1972). 5,7-dihydroxytryptamine. Improvement in chemical lesioning of indoleamine neurons in the mammalian rain. *Zeitschrift für Zellforschung, 135*, 399–414.

Baumgarten, H. G., & Lachenmayer, L. (2004). Serotonin neurotoxins – past and present. *Neurotoxicity Research, 6*, 589–614.

Baumgarten, H. T., & Schlossberger, H. G. (1973). Effects of 5,6-dihydroxytryptamine on brain monoamine neurons in the rat. In J. Barchas & E. Usdin (Eds.), *Serotonin and behavior* (pp. 209–224). New York: Academic.

Baumgarten, H. G., Björklund, A., Lachenmayer, L., Nobin, A., & Stenevi, U. (1971). Long-lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine. *Acta Physiologica Scandinavica. Supplementum, 373*, 1–116.

Baumgarten, H. G., Björklund, A., Holstein, A. F., & Nobin, A. (1972a). Chemical degeneration of indoleamine axons in rat brain by 5,6-dihydroxytryptamine. Ultrastructural study. *Zeitschrift für Zellforschung, 129*, 259–271.

Baumgarten, H. G., Evetts, K. D., Holman, R. B., Iversen, L. L., Vogt, M., & Wilson, G. (1972b). Effects of 5,6-dihydroxytryptamine on monoaminergic neurons in the central nervous system of the rat. *Journal of Neurochemistry, 19*, 1587–1597.

Baumgarten, H. G., Björklund, A., Lachenmayer, L., & Nobin, A. (1973). Evaluation of the effects of 5,7-dihydroxytryptamine on serotonin and catecholamine neurons in the rat CNS. *Acta Physiologica Scandinavica. Supplementum, 391*, 1–19.

Baumgarten, H. G., Klemm, H. P., Lachenmayer, L., Björklund, A., Lovenberg, W., & Schlossberger, H. G. (1978a). Mode and mechanism of action of neurotoxic indoleamines: A review and progress report. *Annals of the New York Academy of Sciences, 305*, 3–24.

Baumgarten, H. G., Klemm, H. P., Lachenmayer, L., & Schlossberger, H. G. (1978b). Effect of drugs on the distribution of [^{14}C]-5,7-dihydroxytryptamine in rat brain. *Annals of the New York Academy of Sciences, 305*, 107–118.

Baumgarten, H. G., Jenner, S., & Klemm, H. P. (1981). Serotonin neurotoxins: recent advances in the mode of administration and molecular mechanism of action. *Journal of Physiology, Paris, 77*, 309–314.

Baumgarten, H. G., Klemm, H. P., Sievers, J., & Schlossberger, H. G. (1982). Dihydroxytryptamines as tools to study the neurobiology of serotonin. *Brain Research Bulletin, 9*, 131–150.

Beal, M. F. (1992). Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? *Annals of Neurology, 31*, 119–130.

Beal, M. F., Kowall, N. W., Ellison, D. W., Mazurek, M. F., Swartz, K. J., & Martin, J. B. (1986). Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature, 321*, 168–171.

Beal, M. F., Kowall, N. W., Swartz, K. J., Ferrante, R. J., & Martin, J. B. (1988). Systemic approaches to modifying quinolinic acid striatal lesions in rats. *Journal of Neuroscience, 8*(10), 3901–3908.

Beal, M. F., Swatz, K. J., Hyman, B. T., Storey, E., Finn, S. F., & Koroshetz, W. (1991). Aminooxyacetic acid results in excitotoxin lesions by a novel indirect mechanism. *Journal of Neurochemistry, 57*, 1068–1073.

Beal, M. F., Brouillet, E., Jenkins, B. G., Ferrante, R. J., Kowall, N. W., Miller, J. M., Storey, E., Srivastava, R., Rosen, B. R., & Hyman, B. T. (1993). Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. *Journal of Neuroscience, 13*, 4181–4192.

Benes, F. M., Paskewich, P. A., Davidson, J., & Domesick, V. B. (1984). The effects of haloperidol on synaptic patterns in the rat striatum. *Brain Research, 329*, 265–274.

Benes, F. M., Paskevich, P. A., Davidson, J., & Domesick, V. B. (1985a). Synaptic rearrangements in medial prefrontal cortex of haloperidol-treated rats. *Brain Research, 348*, 15–20.

Benes, F. M., Paskevich, P. A., Davidson, J., & Domesick, V. B. (1985b). The effects of haloperidol on synaptic patterns in the rat striatum. *Brain Research, 329*, 265–273.

Beneviste, H., Drejer, J., Schousboe, A., & Diemer, N. H. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by microdialysis. *Journal of Neurochemistry, 43*, 1369–1374.

Berger, T. W., Kaul, S., Stricker, E. M., & Zigmond, M. J. (1985). Hyperinnervation of the striatum by dorsal raphe afferents after dopamine-depleting brain lesions in neonatal rats. *Brain Research, 336*, 354–358.

Berger, K., Przedborski, S., & Cadet, J. L. (1991). Retrograde degeneration of nigrostriatal neurons induced by intrastriatal 6-hydroxydopamine injection in rats. *Brain Research Bulletin, 26*, 301–307.

Berger, U. V., Grzanna, R., & Molliver, M. E. (1992). The neurotoxic effects of p-chloroamphetamine in rat brain are blocked by prior depletion of serotonin. *Brain Research, 578*, 177–185.

Berger-Sweeney, J., Hechers, S., Mesulam, M. M., Wiley, R. G., Lappi, D. A., & Sharma, M. (1994). Differential effects on spatial navigation of immunotoxin-induced cholinergic lesions of the medial septal area and nucleus basalis. *Journal of Neuroscience, 14*, 4507–4519.

Berkowitz, B. A., Spector, S., Brossi, A., Focella, A., & Teitel, S. (1970). Preparation and biological properties of (-) and (+)-6-hydroxydopa. *Experientia, 26*, 982–983.

Bevan, S., & Docherty, R. J. (1993). Cellular mechanisms of action of capsaicin. In J. N. Wood (Ed.), *Capsaicin in the study of pain* (pp. 27–44). New York: Academic.

Bisagno, V., & Cadet, J. L. (2013). Methamphetamine and MDMA neurotoxicity: Biochemical and molecular mechanisms. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Bizat, N., Hermel, J. M., Boyer, F., Jacquard, C., Creminon, C., Ouary, S., Escartin, C., Hantraye, P., Krajewski, S., & Brouillet, E. (2003a). Calpain is a major cell death effector in selective striatal degeneration induced in vivo by 3-nitropropionate: implications for Huntington's disease. *Journal of Neuroscience, 23*, 5020–5030.

Bizat, N., Hermel, J. M., Humbert, S., Jacquard, C., Creminon, C., Escartin, C., Saudou, F., Krajewski, S., Hantraye, P., & Brouillet, E. (2003b). In vivo calpain/caspase cross-talk during 3-nitropropionic acid-induced striatal degeneration: implication of a calpain-mediated cleavage of active caspase-3. *Journal of Biological Chemistry, 278*, 43245–43253.

Björklund, A., Nobin, A., & Stenevi, U. (1973a). Effects of 5,6-dihydroxytryptamine on nerve terminal serotonin and serotonin uptake in the rat brain. *Brain Research, 53*, 117–127.

Björklund, A., Nobin, A., & Stenevi, U. (1973b). The use of neurotoxic dihydroxytryptamines as tools for morphological studies and localized lesioning of central indolamine neurons. *Zeitschrift für Zellforschung, 145*, 479–501.

Björklund, A., Baumgarten, H. G., & Rensch, A. (1975a). 5,7-Dihydroxytryptamine: improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. *Journal of Neurochemistry, 24*, 833–835.

Björklund, A., Horn, A. S., Baumgarten, H. G., Nobin, A., & Schlossberger, H. G. (1975b). Neurotoxicity of hydroxylated tryptamines: structure-activity relationships. 2. In vitro studies on monoamine uptake inhibition and uptake impairment. *Acta Physiologica Scandinavica. Supplementum, 429*, 29–60.

Blandini, F., Levandis, G., Bazzini, E., Nappi, G., & Armentero, M. T. (2007). Time-course of nigrostriatal damage, basal ganglia metabolic changes and behavioural alterations following intrastriatal injection of 6-hydroxydopamine in the rat: new clues from an old model. *European Journal of Neuroscience, 25*, 397–405. doi:10.1111/j.1460-9568.2006.05285.x.

Blank, C. L., Murrill, E., & Adams, R. N. (1972). Central nervous system effects of 6-aminodopamine and 6-hydroxydopamine. *Brain Research, 45*, 635–637.

Bloom, F. E., Algeri, S., Groppetti, A., Revuelta, A., & Costa, E. (1969). Lesions of central norepinephrine terminals with 6-OH-dopamine: biochemistry and fine structure. *Science, 166*(3910), 1284–1286.

Bloomquist, J., King, E., Wright, A., Mytilineou, C., Kimura, K., Castagnoli, K., & Castagnoli, N., Jr. (1994). 1-methyl-4-phenyl-pyridinium-like neurotoxicity of a pyridinium metabolite derived from haloperidol: Cell culture and neurotransmitter uptake studies. *Journal of Pharmacology and Experimental Therapeutics, 20*, 822–830.

Bohlen, C. J., & Julius, D. (2012). Receptor-targeting mechanisms of pain-causing toxins: how ow? *Toxicon, 60*, 254–264.

Book, A. A., Wiley, R. G., & Schweitzer, J. B. (1994). ^{192}IgG-saporin: I. Specific lethality for cholinergic neurons in the basal forebrain of the rat. *Journal of Neuropathology and Experimental Neurology, 53*, 95–102.

Borchardt, R. T., Burgess, S. K., Reid, J. R., Liang, Y. O., & Adams, R. N. (1977). Effects of 2- and/or 5-methylated analogues of 6-hydroxydopamine on norepinephrine and dopamine-containing neurons. *Molecular Pharmacology, 13*, 805–818.

Borlongan, C. V., Koutouzis, T. K., Randall, T. S., Freeman, T. B., Cahill, D. W., & Sanberg, P. R. (1995). Systemic 3-nitropropionic acid behavioral deficits and striatal damage in adult rats. *Brain Research Bulletin, 36*, 549–556.

Bortel, A. (2013). The nature of DSP-4-induced neurotoxicity. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Bortel, A., Slomian, L., Nitka, D., Swierszcz, M., Jaksz, M., Adamus-Sitkiewicz, B., Nowak, P., Josko, J., Kostrzewa, R. M., & Brus, R. (2008). Neonatal N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) treatment modifies the vulnerability to phenobarbital- and ethanol-evoked sedative-hypnotic effects in adult rats. *Pharmacological Reports, 60*, 331–338.

Breese, G. R. (1975). Chemical and immunochemical lesions by specific neurotoxic substances and antisera. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), *Handbook of Psychopharmacology* (Vol. 1, pp. 137–189). New York: Plenum.

Breese, C. R., & Breese, G. R. (1998). The use of neurotoxins to lesion catecholamine-containing neurons to model clinical disorders: approach for defining adaptive neural mechanisms and role of neurotrophic factors in brain. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 19–73). Totowa: Humana Press.

Breese, G. R., & Traylor, T. D. (1971). Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. *British Journal of Pharmacology, 42*, 88–99.

Breese, G. R., Baumeister, A. A., McCown, T. J., Emerick, S. G., Frye, G. D., Crotty, K., & Mueller, R. A. (1984a). Behavioral differences between neonatal and adult 6-hydroxydopamine-treated rats to dopamine agonists: Relevance to neurological symptoms in clinical syndromes with reduced brain dopamine. *Journal of Pharmacology and Experimental Therapeutics, 231*, 343–354.

Breese, G. R., Baumeister, A. A., McCown, T. J., Emerick, S. G., Frye, G. D., & Mueller, R. A. (1984b). Neonatal-6-hydroxydopamine treatment: Model of susceptibility for self-mutilation in the Lesch-Nyhan syndrome. *Pharmacology Biochemistry and Behavior, 21*, 459–461.

Breese, G. R., Baumeister, A., Napier, T. C., Frye, G. D., & Mueller, R. A. (1985a). Evidence that D-1 dopamine receptors contribute to the supersensitive behavioral responses induced by L-dihydroxyphenylalanine in rats treated neonatally with 6-hydroxydopamine. *Journal of Pharmacology and Experimental Therapeutics, 235*, 287–295.

Breese, G. R., Napier, T. C., & Mueller, R. A. (1985b). Dopamine agonist-induced locomotor activity in rats treated with 6-hydroxydopamine at differing ages: Functional supersensitivity of D-1 dopamine receptors in neonatally lesioned rats. *Journal of Pharmacology and Experimental Therapeutics, 234*, 447–455.

Breese, G. R., Duncan, G. E., Napier, T. C., Bondy, S. C., Iorio, L. C., & Mueller, R. A. (1987). 6-hydroxydopamine treatments enhance behavioral responses to intracerebral microinjection of D_1- and D_2-dopamine agonists into nucleus accumbens and striatum without changing dopamine antagonist binding. *Journal of Pharmacology and Experimental Therapeutics, 240*, 167–176.

Breese, G. R., Criswell, H. E., Duncan, G. E., & Mueller, R. A. (1990). A dopamine deficiency model of Lesch-Nyhan disease – the neonatal-6-OHDA-lesioned rat. *Brain Research Bulletin, 25*, 477–484. Review.

Breese, G. R., Criswell, H. E., Johnson, K. B., O'Callaghan, J. P., Duncan, G. E., Jensen, K. F., Simson, P. E., & Mueller, R. A. (1994). Neonatal destruction of dopaminergic neurons. *Neurotoxicology, 15*, 149–159. Review.

Breese, G. R., Knapp, D. J., Criswell, H. E., Moy, S. S., Papadeas, S. T., & Blake, B. L. (2005). The neonate-6-hydroxydopamine-lesioned rat: A model for clinical neuroscience and neurobiological principles. *Brain Research Reviews, 48*, 57–73. Review.

Brouillet, E., Jenkins, B. G., Hyman, B. T., Ferrante, R. J., Kowall, N. W., Srivastava, R., Roy, D. S., Rosen, B. R., & Beal, M. F. (1993). Age-dependent vulnerability of the striatum to the mitochondrial toxin 3-nitropropionic acid. *Journal of Neurochemistry, 60*, 356–359.

Brouillet, E., Hantraye, P., Ferrante, R. J., Dolan, R., Leroy-Willig, A., Kowall, N. W., & Beal, M. F. (1995). Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. *Proceedings of the National Academy of Sciences USA, 92*, 7105–7109.

Brouillet, E., Guyot, M. C., Mittoux, V., Altairac, S., Conde, F., Palfi, S., & Hantraye, P. (1998). Partial inhibition of brain succinate dehydrogenase by 3-nitropropionic acid is sufficient to initiate striatal degeneration in rat. *Journal of Neurochemistry, 70*, 794–805.

Brouillet, E., Jacquard, C., Bizat, N., & Blum, D. (2005). 3-Nitropropionic acid: a mitochondrial toxin to uncover physiopathological mechanisms underlying striatal degeneration in Huntington's disease. *Journal of Neurochemistry, 95*, 1521–1540.

Brown, R. W., Gass, J. T., & Kostrzewa, R. M. (2002). Ontogenetic quinpirole treatments produce spatial memory deficits and enhance skilled reaching in adult rats. *Pharmacology Biochemistry and Behavior, 72*, 591–600.

Brown, R. W., Thompson, K. D., Thompson, K. N., Ward, J. J., Thacker, S. K., Williams, M. T., & Kostrzewa, R. M. (2004). Adulthood nicotine treatment alleviates behavioural impairments in rats neonatally treated with quinpirole: Possible roles of acetylcholine function and neurotrophic factor expression. *European Journal of Neuroscience, 19*, 1634–1642.

Brown, R. W., Maple, A. M., Perna, M. K., Sheppard, A. B., Cope, Z. A., & Kostrzewa, R. M. (2012). Schizophrenia and substance abuse comorbidity: Nicotine addiction and the neonatal quinpirole model. *Developmental Neuroscience, 34*, 140–151. Epub 2012 Jul 6.

Bruno, J. P., Jackson, D., Zigmond, M. J., & Stricker, E. M. (1987). Effect of dopamine-depleting brain lesions in rat pups: role of striatal serotonergic neurons in behavior. *Behavioral Neuroscience, 101*, 806–811.

Brus, R., Kostrzewa, R. M., Perry, K. W., & Fuller, R. W. (1994). Supersensitization of the oral response to SKF 38393 in neonatal 6-hydroxydopamine-lesioned rats is eliminated by neonatal 5,7-dihydroxytryptamine treatment. *Journal of Pharmacology and Experimental Therapeutics, 268*, 231–237.

Brus, R., Plech, A., & Kostrzewa, R. M. (1995). Enhanced quinpirole response in rats lesioned neonatally with 5,7-dihydroxytryptamine. *Pharmacology Biochemistry and Behavior, 50*, 649–653.

Brus, R., Szkilnik, R., Nowak, P., Kasperska, A., Oswiecimska, J., Kostrzewa, R. M., & Shani, J. (1998). Locomotor sensitization of dopamine receptors by their agonists quinpirole and SKF-38393, during maturation and aging in rats. *Pharmacology Reviews and Communications, 10*, 25–30.

Brus, R., Kostrzewa, R. M., Nowak, P., Perry, K. W., & Kostrzewa, J. P. (2003). Ontogenetic quinpirole treatments fail to prime for D_2 agonist-enhancement of locomotor activity in 6-hydroxydopamine-lesioned rats. *Neurotoxicity Research, 5*, 329–338.

Buck, S. H., & Burks, T. F. (1986). The neuropharmacology of capsaicin: A review of some recent observations. *Pharmacological Reviews, 38*, 179–226.

Cadet, J. L., Ladenheim, B., Hirata, H., Rothman, R. B., Ali, S., Carlson, E., Epstein, C., & Moran, T. H. (1995). Superoxide radicals mediate the biochemical effects of methylenedioxymethamphetamine (MDMA): Evidence from using CuZn-superoxide dismutase transgenic mice. *Synapse, 21*, 169–176.

Cadet, J. L., Krasnova, I. N., Jayanthi, S., & Lyles, J. (2007). Neurotoxicity of substituted amphetamines: Molecular and cellular mechanisms. *Neurotoxicity Research, 11*, 183–202. Review.

Cannon, W. B., & Rosenblueth, A. (1937). *Autonomic Neuroeffector Systems*. New York: Macmillan.

Carta, A. R., Pisanu, A., Barcia, C., & Herrero Ezquerro, M. T. (2013) MPTP: Advances from an *evergreen* neurotoxin. In: Kostrzewa RM (Ed.), *Handbook of Neurotoxicity*. Springer, New York.

Castagnoli, N., Jr., Chiba, K., & Trevor, A. J. (1985). Potential bioactivation pathways for the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Life Sciences, 36*, 225–230.

Chan, P., Langston, J. W., Irwin, I., DeLanney, L. E., & Di Monte, D. A. (1993). 2-deoxyglucose enhances 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine-induced ATP loss in the mouse brain. *Journal of Neurochemistry, 61*, 610–616.

Chard, P. S., Bleakman, D., Savidge, J. R., & Miller, R. J. (1995). Capsaicin-induced neurotoxicity in cultured dorsal root ganglion neurons: Involvement of calcium-activated proteases. *Neuroscience, 65*, 1099–1108.

Cheng, A. C., & Castagnoli, N., Jr. (1984). Synthesis and physicochemical and neurotoxicity studies of 1-(4-substituted-2,5-dihydroxyphenyl)-2-aminoethane analogues of 6-hydroxydopamine. *Journal of Medicinal Chemistry, 27*, 513–520.

Chiba, K., Trevor, A., & Castagnoli, N., Jr. (1984). Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochemical and Biophysical Research Communications, 120*, 574–578.

Chiba, K., Trevor, A., & Castagnoli, N., Jr. (1985). Active uptake of MPP^+, a metabolite of MPTP, by brain synaptosomes. *Biochemical and Biophysical Research Communications, 128*, 1229–1232.

Cho, A. K., Ranson, R. W., Fischer, J. B., & Kammerer, R. C. (1980). The effects of xylamine, a nitrogen mustard, on [^{3}H]norepinephrine accumulation in rabbit aorta. *Journal of Pharmacology and Experimental Therapeutics, 214*, 324–327.

Choi, D. W. (1987). Ionic dependence of glutamate neurotoxicity. *Journal of Neuroscience, 7*, 369–379.

Choi, D. W., Koh, J. Y., & Peters, S. (1988). Pharmacology of glutamate neurotoxicity in cortical cell culture: Attenuation by NMDA antagonists. *Journal of Neuroscience, 8*, 185–196.

Clark, M. B., King, J. C., & Kostrzewa, R. M. (1979). Loss of nerve cell bodies in caudal locus coeruleus following treatment of neonates with 6 hydroxydopa. *Neuroscience Letters, 13*, 331–336.

Clausing, P., & Bowyer, J. F. (1999). Time course of brain temperature and caudate/putamen microdialysate levels of amphetamine and dopamine in rats after multiple doses of D-amphetamine. *Annals of the New York Academy of Sciences, 890*, 495–504.

Clifford, D. B., Olney, J. W., Benz, A. M., Fuller, T. A., & Zorumski, C. F. (1990). Ketamine, phencyclidine, and MK-801 protect against kainic acid-induced seizure-related brain damage. *Epilepsia, 31*, 382–390.

Cohen, G., & Heikkila, R. E. (1974). The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. *Journal of Biological Chemistry, 249*, 2447–2452.

Cohen, G., & Heikkila, R. E. (1978). Mechanisms of action of hydroxylated phenylethylamine and indoleamine neurotoxins. *Annals of the New York Academy of Sciences, 305*, 74–84.

Cohen, S., & Levi-Montalcini, R. (1957). Purification and properties of a nerve growth-promoting factor isolated from mouse sarcoma 180. *Cancer Research, 17*, 15–20.

Cohen, S., Levi-Montalcini, R., & Hamburger, V. (1956). A nerve growth-stimulating factor isolated from sarcomas 37 AND 180. *Proceedings of the National Academy of Sciences of the United States of America, 40*, 1014–1018.

Coles, C. J., Edmondson, D. E., & Singer, T. P. (1979). Inactivation of succinate dehydrogenase by 3-nitropropionate. *Journal of Biological Chemistry, 254*, 5161–5167.

Commins, D. L., Vosmer, G., Virus, R. M., Woolverton, W. L., Schuster, C. R., & Seiden, L. (1987). Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. *Journal of Pharmacology and Experimental Therapeutics, 241*, 338–345.

Connolly, H. M., Crary, J. L., McGoon, M. D., Hensrud, D. D., Edwards, B. S., Edwards, W. D., & Schaff, H. V. (1997). Valvular heart disease associated with fenfluramine-phentermine. *The New England Journal of Medicine, 337*, 581–588.

Couratier, P., Hugon, J., Sindou, P., Vallat, J. M., & Dumas, M. (1993). Cell culture evidence for neuronal degeneration in amyotrophic lateral sclerosis being linked to glutamate AMPA/kainate receptors. *Lancet, 341*, 265–268.

Couratier, P., Sindou, P., Esclaire, F., & Hugon, J. (1994). Neuroprotective effects of riluzole in ALS CSF toxicity. *Neuroreport, 5*, 1012–1014.

Coyle, J. T. (1996). The glutamatergic dysfunction hypothesis for schizophrenia. *Harvard Review of Psychiatry, 3*, 241–253. Review.

Coyle, J. T., & Schwarcz, R. (1976). Lesion of striatal neurons with kainic acid provides a model for Huntington's chorea. *Nature, 263*, 244–246.

Criswell, H., Mueller, R. A., & Breese, G. R. (1989). Priming of D_1-dopamine receptor responses: long-lasting behavioral supersensitivity to a D_1-dopamine agonist following repeated administration to neonatal 6-OHDA-lesioned rats. *Journal of Neuroscience, 9*, 125–133.

Cunha-Oliveira, T., Rego, A. C., & Oliveira, C. R. (2008). Cellular and molecular mechanisms involved in the neurotoxicity of opioid and psychostimulant drugs. *Brain Research Reviews, 58*, 192–208.

Cunha-Oliveira, T., Rego, A. C., & Oliveira, C. R. (2013). Cocaine as a neurotoxin. In RM Kostrzewa (Ed.), *Handbook of Neurotoxicity*. Springer: New York.

Curti, D., & Marchbanks, R. M. (1984). Kinetics of irreversible inhibition of choline transport in synaptosomes by ethylcholine mustard aziridinium. *Journal of Membrane Biology, 82*, 259–268.

Curtis, D. R., Phillis, J. W., & Watkins, J. C. (1959). Chemical excitation of spinal neurones. *Nature, 183*, 611–612.

Dawson, V. L., Kizushi, V. M., Huang, P. L., Snyder, S. H., & Dawson, T. M. (1996). Resistance to neurotoxicity in cortical cultures from neuronal nitric oxide synthase-deficient mice. *Journal of Neuroscience, 16*, 2479–2487.

De Champlain, J., & Nadeau, R. (1971). 6-hydroxydopamine, 6-hydroxydopa and degeneration of adrenergic nerves. *Federation Proceedings, 30*, 877–885.

De Champlain, J., & Van Amerigen, M. R. (1972). Regulation of blood pressure by sympathetic nerve fibers and adrenal medulla in normotensive and hypertensive rats. *Circulation Research, 31*, 617–628.

Dedov, V. N., Mandadi, S., Armati, P. J., & Verkhratsky, A. (2001). Capsaicin-induced depolarisation of mitochondria in dorsal root ganglion neurons is enhanced by vanilloid receptors. *Neuroscience, 103*, 219–226.

Denton, T., & Howard, B. D. (1987). A dopaminergic cell line variant resistant to the neurotoxin 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine. *Journal of Neurochemistry, 49*, 622–629.

Descarries, L., Soghomonian, J. J., Garcia, S., Doucet, G., & Bruno, J. P. (1992). Ultrastructural analysis of the serotonin hyperinnervation in adult rat neostriatum following neonatal dopamine denervation with 6-hydroxydopamine. *Brain Research, 569*, 1–13.

Devi, B. G., & Chan, A. W. (1997). Impairment of mitochondrial respiration and electron transport chain enzymes during cocaine-induced hepatic injury. *Life Sciences, 60*, 849–855.

Dewar, K. M., Soghomonian, J. J., Bruno, J. P., Descarries, L., & Reader, T. A. (1990). Elevation of dopamine D_2 but not D_1 receptors in adult rat neostriatum after neonatal 6-hydroxydopamine denervation. *Brain Research, 536*, 287–296.

Dey, S., Mactutus, C. F., Booze, R. M., & Snow, D. M. (2007). Cocaine exposure in vitro induces apoptosis in fetal locus coeruleus neurons by altering the Bax/Bcl-2 ratio and through caspase-3 apoptotic signaling. *Neuroscience, 144*, 509–521.

Di Monte, D., Jewell, S. A., Ekstromm, G., Sandy, M. S., & Smith, M. T. (1986). 1-Methyl-4-phenyl-l,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridine (MPP^+) cause rapid ATP depletion in isolated hepatocytes. *Biochemical and Biophysical Research Communications, 137*, 310–315.

Di Monte, D. A., Wu, E. Y., Irwin, I., De Lanney, L. E., & Langston, J. W. (1991). Biotransformation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in primary cultures of mouse astrocytes. *Journal of Pharmacology and Experimental Therapeutics, 248*, 594–600.

Dietrich, J. B., Poirier, R., Aunis, D., & Zwiller, J. (2004). Cocaine downregulates the expression of the mitochondrial genome in rat brain. *Annals of the New York Academy of Sciences, 1025*, 345–350.

Docherty, R. J., Yeats, J. C., Bevan, S., & Boddeke, H. W. (1996). Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurons from adult rats. *Pflügers Archiv, 431*, 828–837.

Dudley, M. W., Howard, B. D., & Cho, A. K. (1990). The interaction of the beta-haloethyl benzylamines, xylamine, and DSP-4 with catecholaminergic neurons. *Annual Review of Pharmacology and Toxicology, 30*, 387–403.

Duval, D., Roome, N., Gauffeny, C., Nowicki, J. P., & Scatton, B. (1992). SL82,0715, an NMDA antagonist acting at the polyamine site, does not induce neurotoxic effects on rat cortical neurons. *Neuroscience Letters, 137*, 193–197.

Eilam, D., Golani, I., & Szechtman, H. (1989). D_2-agonist quinpirole induces perseveration of routes and hyperactivity but no perseveration of movements. *Brain Research, 490*, 255–267.

Eilam, D., Zor, R., Szechtman, H., & Hermesh, H. (2006). Rituals, stereotypy and compulsive behavior in animals and humans. *Neuroscience and Biobehavioral Reviews, 30*, 456–471.

Ellison, G. (1992). Continuous amphetamine and cocaine have similar neurotoxic effects in lateral habenular nucleus and fasciculus retroflexus. *Brain Research, 598*, 353–356.

Ellison, G. (1994). Competitive and non-competitive NMDA antagonists induce similar limbic degeneration. *Neuroreport, 5*, 2688–2692.

Ellison, G. (1995). The NMDA antagonists phencyclidine, ketamine, and dizocilpine as both behavioral and anatomical models of the dementias. *Brain Research Reviews, 20*, 250–267.

Ellison, G. (1998). The neurotoxic effects of continuous cocaine and amphetamine in habenula. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 211–233). Totowa: Humana Press.

Ellison, G. (2002). Neural degeneration following chronic stimulant abuse reveals a weak link in brain, fasciculus retroflexus, implying the loss of forebrain control circuitry. *European Neuropsychopharmacology, 12*, 287–297.

Ellison, G. D., Eison, M., Huberman, H., & Daniel, F. (1978). Long term changes in dopaminergic innervation of caudate nucleus after continuous amphetamine administration. *Science, 201*, 276–278.

Eyles, D. W., McLennan, H. R., Jones, A., McGrath, J. J., Stedman, T. J., & Pond, S. M. (1994). Quantitative analysis of two pyridinium metabolites of haloperidol patients with schizophrenia. *Clinical Pharmacology and Therapeutics, 56*, 512–520.

Eyles, D. W., McGrath, J. J., & Pond, S. M. (1996). Formation of pyridinium species of haloperidol in human liver and brain. *Psychopharmacology, 125*, 214–219.

Faden, A. I., Demediuk, P., Panter, S. S., & Vink, R. (1989). The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science, 244*, 798–800.

Fang, J., & Yu, P. H. (1995). Effect of haloperidol and its metabolites on dopamine and noradrenaline uptake in rat brain slices. *Psychopharmacology, 121*, 379–384.

Farber, N. B., Price, M. T., Labruyere, J., Nemnich, J., St Peter, H., Wozniak, D. F., & Olney, J. W. (1993). Antipsychotic drugs block phencyclidine receptor-mediated neurotoxicity. *Biological Psychiatry, 34*, 119–121.

Farber, N. B., Wozniak, D. F., Price, M. T., Labruyere, J., Huss, J., St Peter, H., & Olney, J. W. (1995). Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: potential relevance to schizophrenia? *Biological Psychiatry, 38*, 788–796.

Farkas-Szallasi, T., Lundberg, J. M., Wiesenfeld-Hallin, Z., & Szallasi, A. (1995). Increased levels of GMAP, VIP and nitric oxide synthase and their mRNAs, in lumbar dorsal root ganglia of the rat following systemic resiniferatoxin treatment. *Neuroreport, 6*, 2230–2234.

Fernandes Xavier, F. G., Doucet, G., Geffard, M., & Descarries, L. (1994). Dopamine neoinnervation in the substantia nigra and hyperinnervation in the interpeduncular nucleus of adult rat following neonatal cerebroventricular administration of 6-hydroxydopamine. *Neuroscience, 59*, 77–87.

Finnegan, K. T. (1993). Neurotoxins and monoamine oxidase inhibition: New aspects. *Movement Disorders, 8*(Suppl 1), S14–S19.

Finnegan, K. T., Skratt, J. J., Irwin, I., DeLanney, L. E., & Langston, J. W. (1990). Protection against DSP-4-induced neurotoxicity by deprenyl is not related to its inhibition of MAO-B. *European Journal of Pharmacology, 184*, 119–126.

Fischer, J. B., & Cho, A. K. (1982). Inhibition of [^{3}H]norepinephrine uptake in organ cultured rat superior cervical ganglia by xylamine. *Journal of Pharmacology and Experimental Therapeutics, 220*, 115–119.

Fisher, A., & Hanin, I. (1986). Potential animal models for senile dementia of Alzheimer's type, with emphasis on AF64A-induced cholinotoxicity. *Annual Review of Pharmacology and Toxicology, 26*, 161–181.

Fix, A. S., Wozniak, D. F., Truex, L. L., McEwen, M., Miller, J. P., & Olney, J. W. (1995). Quantitative analysis of factors influencing neuronal necrosis induced by MK-801 in the rat posterior cingulate/retrosplenial cortex. *Brain Research, 696*, 194–204.

Fix, A. S., Ross, J. F., Statzel, S. R., & Switzer, R. C. (1996). Integrated evaluation of central nervous system lesions: Stains for neurons, astrocytes, and microglia reveal the spatial and temporal features of MK-801-induced neuronal necrosis in the rat cerebral cortex. *Toxicologic Pathology, 24*, 291–304.

Fontaine, M. A., Geddes, J. W., Banks, A., & Butterfield, D. A. (2000). Effect of exogenous and endogenous antioxidants on 3-nitropionic acid-induced in vivo oxidative stress and striatal lesions: insights into Huntington's disease. *Journal of Neurochemistry, 75*, 1709–1715.

Foster, K. A., Bigalke, H., & Aoki, K. R. (2006). Botulinum neurotoxin – from laboratory to bedside. *Neurotoxicity Research, 9*, 133–140.

Fox, S. H., & Brotchie, J. M. (2010). The MPTP-lesioned non-human primate models of Parkinson's disease. Past, present, and future. *Progress in Brain Research, 184*, 133–157.

Frandsen, A., & Schousboe, A. (1992). Mobilization of dantrolene-sensitive intracellular calcium pools is involved in the cytotoxicity induced by quisqualate and N-methyl-D-aspartate but not 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate and kainate in cultured cerebral cortical neurons. *Proceedings of the National Academy of Sciences USA, 89*, 2590–2594.

Freh, H. H. (1975). Hyperthermia induced by amphetamine, p-chloroamphetamine and fenfluramine in the rat-1. *Pharmacology, 13*, 163–176.

Fritschy, J. M., & Grzanna, R. (1989). Immunohistochemical analysis of the neurotoxic effects of DSP-4 identifies two populations of noradrenergic axon terminals. *Neuroscience, 30*, 181–197.

Fritschy, J. M., & Grzanna, R. (1991a). Experimentally-induced neuron loss in the locus coeruleus of adult rats. *Experimental Neurology, 111*, 123–127.

Fritschy, J. M., & Grzanna, R. (1991b). Selective effects of DSP-4 on locus coeruleus axons: are there pharmacologically different types of noradrenergic axons in the central nervous system? *Progress in Brain Research, 88*, 257–268.

Fritschy, J. M., Geffard, M., & Grzanna, R. (1990). The response of noradrenergic axons to systemically administered DSP-4 in the rat: an immunohistochemical study using antibodies to noradrenaline and dopamine-beta-hydroxylase. *Journal of Chemical Neuroanatomy, 3*, 309–321.

Futscher, B. W., Pieper, R. O., Barnes, D. M., Hanin, I., & Erickson, L. C. (1992). DNA-damaging and transcription terminating lesions induced by AF64A in vivo. *Journal of Neurochemistry, 58*, 1504–1509.

Galas, M. C., Bizat, N., Cuvelier, L., Bantubungi, K., Brouillet, E., Schiffmann, S. N., & Blum, D. (2004). Death of cortical and striatal neurons induced by mitochondrial defect involves differential molecular mechanisms. *Neurobiology of Disease, 15*, 152–159.

Gibson, C. J. (1987). Inhibition of MAO-B, but not MAO-A, blocks DSP-4 toxicity on central NE neurons. *European Journal of Pharmacology, 141*, 135–138.

Giulian, D., Haverkamp, L. J., Li, J., Karshin, W. L., Yu, J., Tom, D., Li, X., & Kirkpatrick, J. B. (1995). Senile plaques stimulate microglia to release a neurotoxin found in Alzheimer brain. *Neurochemistry International, 27*, 119–137.

Globus, M. Y., Busto, R., Dietrich, W. D., Martinez, E., Valdes, I., & Ginsberg, M. D. (1988). Intra-ischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. *Neuroscience Letters, 91*, 36–40.

Gomez-Ramos, P., Galea, E., & Estrada, C. (1990). Neuronal and microvascular alterations induced by the cholinergic toxin AF64A in the rat retina. *Brain Research, 520*, 151–158.

Gong, L., & Kostrzewa, R. M. (1992). Supersensitized oral response to a serotonin agonist in neonatal 6-OHDA treated rats. *Pharmacology Biochemistry and Behavior, 41*, 621–623.

Gong, L., Kostrzewa, R. M., Fuller, R. W., & Perry, K. W. (1992). Supersensitization of the oral response to SKF 38393 in neonatal 6-OHDA-lesioned rats is mediated through a serotonin system. *Journal of Pharmacology and Experimental Therapeutics, 261*, 1000–1007.

Gong, L., Kostrzewa, R. M., Brus, R., Fuller, R. W., & Perry, K. W. (1993). Ontogenetic SKF 38393 treatments sensitize dopamine D_1 receptors in neonatal 6-OHDA-lesioned rats. *Developmental Brain Research, 76*, 59–65.

Gong, L., Kostrzewa, R. M., & Li, C. (1994). Neonatal 6-OHDA and adult SKF 38393 treatments alter dopamine D_1 receptor mRNA levels: Absence of other neurochemical associations with the enhanced behavioral responses of lesioned rats. *Journal of Neurochemistry, 63*, 1282–1290.

Goodwin, J. L., Uemura, E., & Cunnick, J. E. (1995). Microglial release of nitric oxide by the synergistic action of beta-amyloid and IFN-gamma. *Brain Research, 692*, 207–214.

Gould, D. H., & Gustine, D. L. (1982). Basal ganglia degeneration, myelin alterations, and enzyme inhibition induced in mice by the plant toxin 3-nitropropanoic acid. *Neuropathology and Applied Neurobiology, 8*, 377–393.

Gouzoulis-Mayfrank, E., & Daumann, J. (2009). Neurotoxicity of drugs of abuse–the case of methylenedioxyamphetamines (MDMA, ecstasy), and amphetamines. *Dialogues in Clinical Neuroscience, 11*, 305–317. Review.

Graham, D. G., Tiffany, S. M., Bell, W. R., Jr., & Gutknecht, W. F. (1978). Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Molecular Pharmacology, 14*, 644–653.

Green, A. R., Cross, A. J., & Goodwin, G. M. (1995). Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy"). *Psychopharmacology, 119*, 247–260.

Grzanna, R., Berger, U., Fritschy, J. M., & Geffard, M. (1989). Acute action of DSP-4 on central norepinephrine axons: Biochemical and immunohistochemical evidence for differential effects. *Journal of Histochemistry and Cytochemistry, 37*, 1435–1442.

Hamilton, B. F., & Gould, D. H. (1987). Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: A type of hypoxic (energy deficient) brain damage. *Acta Neuropathologia (Berlin), 72*, 286–297.

Hanin, I. (1988). Role of the aziridinium moiety in the in vivo cholinotoxicity of ethylcholine aziridinium ion (AF64A). *Journal of Neuroscience Methods, 23*, 107–113.

Hanin, I. (1990). Cholinergic neurotransmission: Functional and Clinical Aspects, Aquilonius S-M, Gillberg P-G, (Eds.), (Elsevier, Amsterdam). *Progress in Brain Research, 84*, 289–299.

Hanin, I. (1996). The AF64A model of cholinergic hypofunction: An update. *Life Sciences, 58*, 1955–1964.

Hara, M. R., Agrawal, N., Kim, S. F., Cascio, M. B., Fujimuro, M., Ozeki, Y., Takahashi, M., Cheah, J. H., Tankou, S. K., Hester, L. D., Ferris, C. D., Hayward, S. D., Snyder, S. H., & Sawa, A. (2005). S-Nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nature Cell Biology, 7*, 665–674.

Hargreaves, R. J., Rigby, M., Smith, D., & Hill, R. G. (1993). Lack of effect of L-687,414 ((+)-cis-4-methyl-HA-966), an NMDA receptor antagonist acting at the glycine site, on cerebral glucose metabolism and cortical neuronal morphology. *British Journal of Pharmacology, 110*, 36–42.

Harris, M. E., Wang, Y., Pedigo, N. W., Jr., Hensley, K., Butterfield, D. A., & Carney, J. M. (1996). Amyloid beta peptide (25-35) inhibits Na^+-dependent glutamate uptake in rat hippocampal astrocyte cultures. *Journal of Neurochemistry, 67*, 277–286.

Harvey, J. A., & McMaster, S. E. (1975). Fenfluramine: evidence for a neurotoxic action on midbrain and a long-term depletion of serotonin. *Psychopharmacol Commun, 1*, 217–228.

Harvey, J. A., McMaster, S. E., & Yunger, L. M. (1975). p-Chloramphetamine: Selective neurotoxic action in brain. *Science, 187*(4179), 841–843.

Heckers, S., Ohtake, T., Wiley, R. G., Lappi, D. A., Geula, C., & Mesulam, M. M. (1994). Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. *Journal of Neuroscience, 14*, 1271–1289.

Heikkila, R. E., & Cohen, G. (1971). Inhibition of biogenic amine uptake by hydrogen peroxide: A mechanism for toxic effects of 6-hydroxydopamine. *Science, 172*(3989), 1257–1258.

Heikkila, R. E., & Cohen, G. (1972a). Further studies on the generation of hydrogen peroxide by 6-hydroxydopamine. Potentiation by ascorbic acid. *Molecular Pharmacology, 8*, 241–248.

Heikkila, R. E., & Cohen, G. (1972b). In vivo generation of hydrogen peroxide from 6-hydroxydopamine. *Experientia, 28*, 1197–1198.

Heikkila, R. E., & Cohen, G. (1973). 6-Hydroxydopamine: Evidence for superoxide radical as an oxidative intermediate. *Science, 181*(4098), 456–457.

Heikkila, R. E., Mytilineou, C., Côté, L., & Cohen, G. (1973a). Evidence for degeneration of sympathetic nerve terminals caused by the ortho- and para-quinones of 6-hydroxydopamine. *Journal of Neurochemistry, 20*, 1345–1350.

Heikkila, R. E., Mytilineou, C., Côté, L., & Cohen, G. (1973b). The biochemical and pharmacological properties of 6-aminodopamine: Similarity with 6-hydroxydopamine. *Journal of Neurochemistry, 21*, 111–116.

Herrera-Marschitz, M., Bustamante, D., Morales, P., & Goiny, M. (2007). Exploring neurocircuitries of the basal ganglia by intracerebral administration of selective neurotoxins. *Neurotoxicity Research, 11*, 169–182. Review.

Heyes, M. P., Swartz, K. J., Markey, S. P., & Beal, M. F. (1991). Regional brain and cerebrospinal fluid quinolinic acid concentrations in Huntington's disease. *Neuroscience Letters, 122*, 265–269.

Holzer, P. (1991). Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacological Reviews, 43*, 143–201.

Hortnagl, H., & Hanin, I. (1992). Toxins affecting the cholinergic system. In H. Herken & F. Hucho (Eds.), *Handbook of Experimental Pharmacology: Selective Neurotoxicity* (Vol. 102, pp. 293–331). Berlin: Springer.

Hortnagl, H., Potter, P. E., Happe, K., Goldstein, S., Leventer, S., Wulfert, E., & Hanin, I. (1988). Role of the aziridinium moiety in the in vivo cholinotoxicity of ethylcholine aziridinium ion (AF64A). *Journal of Neuroscience Methods, 23*, 107–113.

Huang, N. K., Wan, F. J., Tseng, C. J., & Tung, C. S. (1997a). Amphetamine induces hydroxyl radical formation in the striatum of rats. *Life Sciences, 61*, 2219–2229.

Huang, N. Y., Kostrzewa, R. M., Li, C., Perry, K. W., & Fuller, R. W. (1997b). Persistent spontaneous oral dyskinesias in haloperidol-withdrawn rats neonatally lesioned with 6-hydroxydopamine: Absence of an association with the B_{max} for [^{3}H]raclopride binding to neostriatal homogenates. *Journal of Pharmacology and Experimental Therapeutics, 280*, 268–276.

Huang, L. S., Sun, G., Cobessi, D., Wang, A. C., Shen, J. T., Tung, E. Y., Anderson, V. E., & Berry, E. A. (2006). 3-Nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme. *Journal of Biological Chemistry, 281*(9), 5965–5972.

Huie, R. E., & Padmaja, S. (1993). The reaction of NO with superoxide. *Free Radical Research Communications, 18*, 195–199.

Igarashi, K., Kasuya, F., Fukui, M., Usuki, E., & Castagnoli, N., Jr. (1995). Studies on the metabolism of haloperidol (HP): The role of CYP3A in the production of the neurotoxic pyridinium metabolite HPP^+ found in rat brain following IP administration of HP. *Life Sciences, 57*, 2439–2446.

Ikonomidou, C., & Turski, L. (1996). Neurodegenerative disorders: Clues from glutamate and energy metabolism. *Critical Reviews in Neurobiology, 10*, 239–263.

Imam, S. Z., Newport, G. D., Itzhak, Y., Cadet, J. L., Islam, F., Slikker, W., Jr., & Ali, S. F. (2001). Peroxynitrite plays a role in methamphetamine-induced dopaminergic neurotoxicity: Evidence from mice lacking neuronal nitric oxide synthase gene or overexpressing copper-zinc superoxide dismutase. *Journal of Neurochemistry, 76*, 745–749.

Itzhak, Y., & Ali, S. F. (2006). Role of nitrergic system in behavioral and neurotoxic effects of amphetamine analogs. *Pharmacology and Therapeutics, 109*, 246–262.

Itzhak, Y., Gandia, C., Huang, P. L., & Ali, S. F. (1998). Resistance of neuronal nitric oxide synthase-deficient mice to methamphetamine-induced dopaminergic neurotoxicity. *Journal of Pharmacology and Experimental Therapeutics, 284*, 1040–1047.

Iversen, L. L. (1970). Inhibition of catecholamine uptake by 6-hydroxydopamine in rat brain. *European Journal of Pharmacology, 10*, 408–410.

Jackson, D., Bruno, J. P., Stachowiak, M. K., & Zigmond, M. J. (1988). Inhibition of striatal acetylcholine release by serotonin and dopamine after the intracerebral administration of 6-hydroxydopamine to neonatal rats. *Brain Research, 457*, 267–273.

Jacobowitz, D., & Kostrzewa, R. (1971). Selective action of 6-hydroxydopa on noradrenergic terminals: mapping of preterminal axons of the brain. *Life Sciences, 10*, 1329–1341.

Jaim-Etcheverry, G. (1998). 2-Chloroethylamines (DSP4 and xylamine): Toxic actions on noradrenergic neurons. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 131–140). Totowa: Humana Press.

Jaim-Etcheverry, G., & Zieher, L. M. (1980). DSP-4: A novel compound with neurotoxic effects on noradrenergic neurons of adult and developing rats. *Brain Research, 188*, 513–523.

Jaim-Etcheverry, G., & Zieher, L. M. (1983). 2-Chloroethylamines: New chemical tools for the study of the noradrenergic neurons. *Trends in Pharmacological Sciences, 4*, 473–475.

Jakab, R. L., & Bowyer, J. F. (2002). Parvalbumin neuron circuits and microglia in three dopamine-poor cortical regions remain sensitive to amphetamine exposure in the absence of hyperthermia, seizure and stroke. *Brain Research, 958*, 52–69.

Jancsó, N., & Jancsó, A. (1949). Desensitization of sensory nerve endings. *Ki´se´rletes Orvostudoma´ny, 2*(Suppl), 15.

Jancsó, G., Király, E., & Jancsó-Gábor, A. (1978). Pharmacologically induced selective degeneration of chemosensitive sensory neurons. *Nature, 270*, 741–743.

Jauch, D., Urbanska, E. M., Guidetti, P., Bird, E. D., Vonsattel, J. P., Whetsell, W. O., Jr., & Schwarcz, R. (1995). Dysfunction of brain kynurenic acid metabolism in Huntington's disease: Focus on kynurenine aminotransferases. *Journal of Neurological Sciences, 130*, 39–47.

Javitch, J. A., & Snyder, S. H. (1984). Uptake of MPP^+ by dopamine neurons explains selectivity of parkinsonism-inducing neurotoxin, MPTP. *European Journal of Pharmacology, 106*, 455–456.

Javitch, J. A., D'Amato, R. J., Strittmatter, S. M., & Snyder, S. H. (1985). Parkinsonism-inducing neurotoxin, N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proceedings of the National Academy of Sciences USA, 82*, 2173–2177.

Jenner, P., Rupniak, N. M., Rose, S., Kelly, E., Kilpatrick, G., Lees, A., & Marsden, C. D. (1984). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the common marmoset. *Neuroscience Letters, 50*, 85–90.

Johannessen, J. N., Savitt, J. M., Markey, C. J., Bacon, J. P., Weisz, A., Hanselman, D. S., & Markey, S. P. (1987). The development of amine substituted analogues of MPTP as unique tools for the study of MPTP toxicity and Parkinson's disease. *Life Sciences, 40*, 697–704.

Jonsson, G., & Sachs, C. (1973). 6-Aminodopamine-induced degeneration of catecholamine neurons. *Journal of Neurochemistry, 21*, 117–124.

Jonsson, G., & Sachs, C. (1975). Actions of 6-hydroxydopamine quinones on catecholamine neurons. *Journal of Neurochemistry, 25*, 509–516.

Jonsson, G., Pollare, H., Hallman, H., & Sachs, C. (1978). Developmental plasticity of central serotonin neurons after 5,7-dihydroxytryptamine treatment. *Annals of the New York Academy of Sciences, 305*, 328–345.

Jonsson, G., Hallman, H., Ponzio, F., & Ross, S. (1981). DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) – a useful denervation tool for central and peripheral noradrenaline neurons. *European Journal of Pharmacology, 72*, 173–188.

Jonsson, G., Hallman, H., & Sundström, E. (1982). Effects of the noradrenaline neurotoxin DSP4 on the postnatal development of central noradrenaline neurons in the rat. *Neuroscience, 7*, 2895–2907.

Jorgensen, M. B., & Diemer, N. H. (1982). Selective neuron loss after cerebral ischemia in the rat: Possible role of transmitter glutamate. *Acta Neurologica Scandinavica, 66*, 536–546.

Jośko, J., Drab, J., Jochem, J., Nowak, P., Szkilnik, R., Korossy-Mruk, E., Boroń, D., Kostrzewa, R. M., Brus, H., & Brus, R. (2011). Ontogenetic serotoninergic lesioning alters histaminergic activity in rats in adulthood. *Neurotoxicity Research, 20*, 103–108.

Kalaria, R. N., Mitchell, M. J., & Harik, S. I. (1987). Correlation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity with blood-brain barrier monoamine oxidase activity. *Proceedings of the National Academy of Sciences USA, 84*, 3521–3525.

Kammerer, R. C., Amiri, B., & Cho, A. K. (1979). Inhibition of uptake of catecholamines by benzylamine derivatives. *Journal of Medicinal Chemistry, 22*, 352–355.

Kil, H. Y., Zhang, J., & Piantadosi, C. A. (1996). Brain temperature alters hydroxyl radical production during cerebral ischemia/reperfusion in rats. *Journal of Cerebral Blood Flow and Metabolism, 16*, 100–106.

Kim, G. W., Copin, J. C., Kawase, M., Chen, S. F., Sato, S., Gobbel, G. T., & Chan, P. H. (2000). Excitotoxicity is required for induction of oxidative stress and apoptosis in mouse striatum by the mitochondrial toxin, 3-nitropropionic acid. *Journal of Cerebral Blood Flow and Metabolism, 20*, 119–129.

Kingston, A. E., O'Neill, M. J., Bond, A., Bruno, V., Battaglia, G., Nicoletti, F., Harris, J. R., Clark, B. P., Monn, J. A., Lodge, D., & Schoepp, D. D. (1999). Neuroprotective actions of novel and potent ligands of group I and group II metabotropic glutamate receptors. *Annals of the New York Academy of Sciences, 890*, 438–449.

Klisans Fuenmayor, D., Harston, C. T., & Kostrzewa, R. M. (1986). Alterations in noradrenergic innervation of the brain following dorsal bundle lesions in neonatal rats. *Brain Research Bulletin, 16,* 47–54.

Koh, J. Y., Yang, L. L., & Cotman, C. W. (1990). beta-Amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage. *Brain Research, 533*, 315–320.

Kostrzewa, R. M. (1975). Effects of neonatal 6 hydroxydopa treatment on monoamine content of rat brain and peripheral tissues. *Research Communications in Chemical Pathology and Pharmacology, 11*, 567–579.

Kostrzewa, R. M. (1989). Neurotoxins that affect central and peripheral catecholamine neurons. In A. A. Boulton, G. B. Baker, & A. V. Juorio (Eds.), *Neuromethods* (Neuropharmacology Section, Vol. 12, pp. 1–48). Totowa: Humana Press.

Kostrzewa, R. M. (1995). Dopamine receptor supersensitivity. *Neuroscience and Biobehavioral Reviews, 19*, 1–17.

Kostrzewa, R. M. (1998). 6-Hydroxydopa, a catecholamine neurotoxin and endogenous excitotoxin at non-NMDA receptors. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 109–129). Totowa: Humana Press.

Kostrzewa, R. M. (1999a). Editorial. *Neurotoxicity Research, 1*, 1–2.

Kostrzewa, R. M. (1999b). Selective neurotoxins, chemical tools to probe the mind: The first thirty years and beyond. *Neurotoxicity Research, 1*, 3–25.

Kostrzewa, R. M. (2007). The blood-brain barrier for catecholamines – revisited. *Neurotoxicity Research, 11*, 261–272.

Kostrzewa, R. M. (2009). Evolution of neurotoxins: from research modalities to clinical realities. *Current Protocols in Neuroscience*, Wiley Interscience. Chapter 1:Unit1.18, pp. 1.18.1-1.18.10.

Kostrzewa, R. M., & Brus, R. (1991). Ontogenic homologous supersensitization of quinpirole-induced yawning in rats. *Pharmacology Biochemistry and Behavior, 39*, 517–519.

Kostrzewa, R. M., & Garey, R. E. (1976). Effects of 6 hydroxydopa on noradrenergic neurons in developing rat brain. *Journal of Pharmacology and Experimental Therapeutics, 197*, 105–118.
Kostrzewa, R. M., & Garey, R. E. (1977). Sprouting of noradrenergic terminals in rat cerebellum following neonatal treatment with 6 hydroxydopa. *Brain Research, 124*, 385–391.
Kostrzewa, R. M., & Gong, L. (1991). Supersensitized D_1 receptors mediate enhanced oral activity after neonatal 6-OHDA. *Pharmacology Biochemistry and Behavior, 39*(3), 677–682.
Kostrzewa, R. M., & Harper, J. W. (1974). Effects of 6 hydroxydopa on catecholamine-containing neurons in brains of newborn rats. *Brain Research, 69*, 174–181.
Kostrzewa, R. M., & Harper, J. W. (1975). Comparison of the neonatal effects of 6 hydroxydopa and 6 hydroxydopamine on growth and development of noradrenergic neurons in the central nervous system. In T. Malmfors, G. Jonsson, & C. Sachs (Eds.), *Chemical Tools in Catecholamine Research* (pp. 181–189). Amsterdam: North Holland.
Kostrzewa, R., & Jacobowitz, D. (1972). The effect of 6-hydroxydopa on peripheral adrenergic neurons. *Journal of Pharmacology and Experimental Therapeutics, 183*, 284–297.
Kostrzewa, R., & Jacobowitz, D. (1973). Acute effects of 6-hydroxydopa on central monoaminergic neurons. *European Journal of Pharmacology, 21*, 70–80.
Kostrzewa, R. M., & Jacobowitz, D. M. (1974). Pharmacological actions of 6-hydroxydopamine. *Pharmacological Reviews, 26*, 199–288.
Kostrzewa, R. M., & Kostrzewa, F. P. (2012). Neonatal 6-hydroxydopamine lesioning enhances quinpirole-induced vertical jumping in rats that were quinpirole primed during postnatal ontogeny. *Neurotoxicity Research, 21*, 231–235. Epub 2011 Aug 19.
Kostrzewa, R. M., & Segura-Aguilar, J. (2007). Botulinum neurotoxin: Evolution from poison, to research tool – onto medicinal therapeutic and future pharmaceutical panacea. *Neurotoxicity Research, 12*, 275–290.
Kostrzewa, R. M., Klara, J. W., Robertson, J., & Walker, L. C. (1978). Studies on the mechanism of sprouting of noradrenergic terminals in rat and mouse cerebellum after neonatal 6-hydroxydopa. *Brain Research Bulletin, 3*, 525–531.
Kostrzewa, R. M., Hardin, J. C., & Jacobowitz, D. M. (1988). Destruction of cells in the midportion of the locus coeruleus by a dorsal bundle lesion in neonatal rats. *Brain Research, 442*, 321–328.
Kostrzewa, R. M., Hamdi, A., & Kostrzewa, F. P. (1990). Production of prolonged supersensitization of dopamine D_2 receptors. *European Journal of Pharmacology, 183*, 1411–1412.
Kostrzewa, R. M., Brus, R., & Kalbfleisch, J. (1991). Ontogenetic homologous sensitization to the antinociceptive action of quinpirole in rats. *European Journal of Pharmacology, 209*, 157–161.
Kostrzewa, R. M., Brus, R., Rykaczewska, M., & Plech, A. (1993a). Low dose quinpirole ontogenically sensitizes to quinpirole-induced yawning in rats. *Pharmacology Biochemistry and Behavior, 44*, 487–489.
Kostrzewa, R. M., Guo, J., & Kostrzewa, F. P. (1993b). Ontogenetic quinpirole treatments induce vertical jumping activity in rats. *European Journal of Pharmacology, 239*, 183–187.
Kostrzewa, R. M., Brus, R., Kalbfleisch, J. H., Perry, K. W., & Fuller, R. W. (1994). Proposed animal model of attention deficit hyperactivity disorder. *Brain Research Bulletin, 34*, 161–167.
Kostrzewa, R. M., Reader, T. A., & Descarries, L. (1998). Serotonin neural adaptations to ontogenetic loss of dopamine neurons in rat brain. *Journal of Neurochemistry, 70*, 889–898.
Kostrzewa, R. M., Kostrzewa, J. P., & Brus, R. (2003). Dopamine receptor supersensitivity: an outcome and index of neurotoxicity. *Neurotoxicity Research, 5*, 111–118.
Kostrzewa, R. M., Kostrzewa, J. P., Nowak, P., Kostrzewa, R. A., & Brus, R. (2004). Dopamine D_2 agonist priming in intact and dopamine-lesioned rats. *Neurotoxicity Research, 6*, 457–462.
Kostrzewa, R. M., Kostrzewa, J. P., Brown, R. W., Nowak, P., & Brus, R. (2008a). Dopamine receptor supersensitivity: Development, mechanisms, presentation, and clinical applicability. *Neurotoxicity Research, 14*, 121–128. Review.
Kostrzewa, R. M., Kostrzewa, J. P., Kostrzewa, R. A., Nowak, P., & Brus, R. (2008b). Pharmacological models of ADHD. *Journal of Neural Transmission, 115*, 287–298.

Kostrzewa, R. M., Kostrzewa, J. P., Kostrzewa, R. A., Kostrzewa, F. P., Brus, R., & Nowak, P. (2011). Stereotypic progressions in psychotic behavior. *Neurotoxicity Reserarch, 19*, 243–252. Review.

Kostrzewa, R. M., Kostrzewa, J. P., & Brus, R. (2013). Tardive dyskinesia: Outcome of antipsychotic treatment and brain damage? In: Kostrzewa RM (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Krasnova, I. N., & Cadet, J. L. (2009). Methamphetamine toxicity and messengers of death. *Brain Research Reviews, 60*, 379–407.

Krasnova, I. N., Ladenheim, B., Jayanthi, S., Oyler, J., Moran, T. H., Huestis, M. A., & Cadet, J. L. (2001). Amphetamine-induced toxicity in dopamine terminals in CD-1 and C57BL/6J mice: Complex roles for oxygen-based species and temperature regulation. *Neuroscience, 107*, 265–274.

Kreuger, C. A., & Cook, D. A. (1975). Synthesis and adrenergic blocking properties of some alkylating analogs of bretylium. *Archives Internationales de Pharmacodynamie et de Therapie, 218*, 96–115.

Kumar, A., Majhi, R. K., Yadav, M., Szallasi, A., & Goswami, C. (2013). TRPV1 activators ("vanilloids") as neurotoxins. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Langston, J. W., & Ballard, P. A. (1984). Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): Implications for treatment and the pathogenesis of Parkinson's disease. *Canadian Journal of Neurological Sciences, 11*, 160–165.

LaVoie, M. J., & Hastings, T. G. (1999). Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: Evidence against a role for extracellular dopamine. *Journal of Neuroscience, 19*, 1484–1491.

Leventer, S. M., Wulfert, E., & Hanin, I. (1987). Time course of ethylcholine aziridinium ion (AF64A)-induced cholinotoxicity in vivo. *Neuropharmacology, 26*, 361–365.

Leventhal, L., Sortwell, C. E., Hanbury, R., Collier, T. J., Kordower, J. H., & Palfi, S. (2000). Cyclosporin A protects striatal neurons in vitro and in vivo from 3-nitropropionic acid toxicity. *The Journal of Comparative Neurology, 425*, 471–478.

Levi-Montalcini, R. (1964). The nerve growth factor. *Annals of the New York Academy of Sciences, 118*, 149–170.

Levi-Montalcini, R. (1966). The nerve growth factor: Its mode of action on sensory and sympathetic nerve cells. *Harvey Lectures, 60*, 217–259. Review.

Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. *Science, 237*(4819), 1154–1162. Review.

Levi-Montalcini, R., & Angeletti, P. U. (1966). Second symposium on catecholamines. Modification of sympathetic function. Immunosympathectomy. *Pharmacological Reviews, 18*, 619–628.

Levi-Montalcini, R., & Booker, B. (1960a). Destruction of the sympathetic ganglia in mammals by an antiserum to a nerve-growth protein. *Proceedings of the National Academy of Sciences USA, 46*, 384–391.

Levi-Montalcini, R., & Booker, B. (1960b). Excessive growth of the sympathetic ganglia evoked by a protein isolated from mouse salivary glands. *Proceedings of the National Academy of Sciences USA, 46*, 373–384.

Levi-Montalcini, R., & Cohen, S. (1956). In vitro and in vivo effects of a nerve growth-stimulating agent isolated from snake venom. *Proceedings of the National Academy of Sciences USA, 42*, 695–699.

Levi-Montalcini, R., Meyer, H., & Hamburger, V. (1954). In vitro experiments on the effects of mouse sarcomas 180 and 37 on the spinal and sympathetic ganglia of the chick embryo. *Cancer Research, 14*, 49–57.

Lin, P. S., Quamo, S., Ho, K. C., & Gladding, J. (1991). Hyperthermia enhances the cytotoxic effects of reactive oxygen species to Chinese hamster cells and bovine endothelial cells in vivo. *Radiation Research, 126*, 43–51.

Liu, L., Wang, Y., & Simon, S. A. (1996). Capsaicin activated currents in rat dorsal root ganglion cells. *Pain, 64*, 191–195.

London, E., Wilkerson, G., Goldberg, S., & Risner, M. (1986). Effects of L-cocaine on local cerebral glucose utilization in the rat. *Neuroscience Letters, 68*, 73–78.

Low, S. J., & Roland, C. L. (2004). Review of NMDA antagonist-induced neurotoxicity and implications for clinical development. *International Journal of Clinical Pharmacology and Therapeutics, 42*, 1–14.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Archives of Ophthalmology, 58*, 193–201.

Ludolph, A. C., He, F., Spencer, P. S., Hammerstad, J., & Sabri, M. (1991). 3-Nitropropionic acid-exogenous animal neurotoxin and possible human striatal toxin. *Canadian Journal of Neurological Sciences, 18*, 492–498.

Luellen, B. A., Miller, D. B., Chisnell, A. C., Murphy, D. L., O'Callaghan, J. P., & Andrews, A. M. (2003). Neuronal and astroglial responses to the serotonin and norepinephrine neurotoxin: 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine. *Journal of Pharmacology and Experimental Therapeutics, 307*, 923–931.

Lundstrom, J., Ong, H., Daly, J., & Creveling, C. R. (1973). Isomers of 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine): Long-term effects on the accumulation of (^{3}H)-norepinephrine in mouse heart in vivo. *Molecular Pharmacology, 9*, 505–513.

Luthman, J., Bolioli, B., Tsutsumi, T., Verhofstad, A., & Jonsson, G. (1987). Sprouting of striatal serotonin nerve terminals following selective lesions of nigro-striatal dopamine neurons in neonatal rat. *Brain Research Bulletin, 19*, 269–274.

Ma, S., Lin, L., Raghavan, R., Cohenour, P., Lin, P. Y., Bennett, J., Lewis, R. J., Enwall, E. L., Kostrzewa, R., Lehr, R. E., et al. (1995). In vivo and in vitro studies on the neurotoxic potential of 6-hydroxydopamine analogs. *Journal of Medicinal Chemistry, 38*, 4087–4097.

Maciel, E. N., Kowaltowski, A. J., Schwalm, F. D., Rodrigues, J. M., Souza, D. O., Vercesi, A. E., Wajner, M., & Castilho, R. F. (2004). Mitochondrial permeability transition in neuronal damage promoted by Ca^{2+} and respiratory chain complex II inhibition. *Journal of Neurochemistry, 90*, 1025–1035.

Mahadik, S. P., Laev, H., Korenovsk, A., & Karpiak, S. E. (1988). Haloperidol alters rat CNS cholinergic system: Enzymatic and morphological analyses. *Biological Psychiatry, 24*, 199–217.

Mark, K. A., Soghomonian, J. J., & Yamamoto, B. K. (2004). High-dose methamphetamine acutely activates the striatonigral pathway to increase striatal glutamate and mediate long-term dopamine toxicity. *Journal of Neuroscience, 24*, 11449–11456.

Marshall, J. F., O'Dell, S. J., & Weihmuller, F. B. (1993). Dopamine-glutamate interactions in methamphetamine-induced neurotoxicity. *Journal of Neural Transmission. General Section, 91*, 241–254.

Maruyama, W., Akao, Y., Youdim, M. B., & Naoi, M. (2000). Neurotoxins induce apoptosis in dopamine neurons: Protection by N-propargylamine-1(R)- and (S)-aminoindan, rasagiline and TV1022. *Journal of Neural Transmission. Supplementum, 60*, 171–186.

McGeer, E. G., & McGeer, P. L. (1976). Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamic and kainic acids. *Nature, 263*, 517–519.

McIntosh, T. K., Vink, R., Soares, H., Hayes, R., & Simon, R. (1989). Effects of the N-methyl-D-aspartate receptor blocker MK-801 on neurologic function after experimental brain injury. *Journal of Neurotrauma, 6*, 247–259.

Mehanna R, Jankovic J (2013) Botulinum neurotoxins as therapeutics. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. Springer: New York.

Meshul, C. K., & Casey, D. E. (1989). Regional, reversible ultrastructural changes in rat brain with chronic neuroleptic treatment. *Brain Research, 489*, 338–346.

Meshul, C. K., Stallbaumer, R. K., Taylor, B., & Janowsky, A. (1994). Haloperidol-induced morphological changes in striatum are associated with glutamate synapses. *Brain Research, 648*, 181–195.

Miyazaki, I., Asanuma, M., Diaz-Corrales, F. J., Fukuda, M., Kitaichi, K., Miyoshi, K., & Ogawa, N. (2006). Methamphetamine-induced dopaminergic neurotoxicity is regulated by quinone-formation-related molecules. *The FASEB Journal, 20*, 571–573.

Mizuno, Y., Saito, T., & Sone, N. (1987). Inhibition of ATP synthesis by 1-methyl-4-phenylpyridinium ion (MPP^+) in isolated mitochondria from mouse brains. *Neuroscience Letters, 81*, 204–208.

Mody, I., & MacDonald, J. F. (1995). NMDA receptor-dependent excitotoxicity: the role of intracellular release. *Trends in Pharmacological Sciences, 16*, 356–359.

Molliver, D. C., & Molliver, M. E. (1990). Anatomic evidence for a neurotoxic effect of (±)-fenfluramine upon serotonergic projections in the rat. *Brain Research, 511*, 165–168.

Murphy, C. A., Ghazi, L., Kokabi, A., & Ellison, G. (1999). Prenatal cocaine produces signs of neurodegeneration in the lateral habenula. *Brain Research, 851*, 175–182.

Muthane, U., Ramsay, K. A., Jiang, H., Jackson-Lewis, V., Donaldson, D., Fernando, S., Ferreira, M., & Przedborski, S. (1994). Differences in nigral neuron number and sensitivity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium in C57/BL and CD-1 mice. *Experimental Neurology, 126*, 195–204.

Nasr, P., Gursahani, H. I., Pang, Z., Bondada, V., Lee, J., Hadley, R. W., & Geddes, J. W. (2003). Influence of cytosolic and mitochondrial Ca^{2+}, ATP, mitochondrial membrane potential, and calpain activity on the mechanism of neuron death induced by 3-nitropropionic acid. *Neurochemistry International, 43*, 89–99.

Nilsson, P., Hillered, L., Ponten, U., Pontén, U., & Ungerstedt, U. (1990). Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. *Journal of Cerebral Blood Flow and Metabolism, 10*, 631–637.

Novelli, A., Reilly, J. A., Lysko, P. G., & Henneberry, R. C. (1988). Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Research, 451*, 205–212.

Nowak, P., Brus, R., & Kostrzewa, R. M. (2001). Amphetamine-induced enhancement of neostriatal in vivo microdialysate dopamine content in rats, quinpirole-primed as neonates. *Polish Journal of Pharmacology, 53*, 319–329.

Nowak, P., Labus, L., Kostrzewa, R. M., & Brus, R. (2004). Dopamine D_2 agonist priming in intact and dopamine-lesioned rats. *Neurotoxicity Research, 6*, 457–462.

Nowak, P., Labus, Ł., Kostrzewa, R. M., & Brus, R. (2006). DSP-4 prevents dopamine receptor priming by quinpirole. *Pharmacology Biochemistry and Behavior, 84*, 3–7.

Nowak, P., Nitka, D., Kwieciński, A., Jośko, J., Drab, J., Pojda-Wilczek, D., Kasperski, J., Kostrzewa, R. M., & Brus, R. (2009). Neonatal co-lesion by DSP-4 and 5,7-DHT produces adulthood behavioral sensitization to dopamine D(2) receptor agonists. *Pharmacological Reports, 61*, 311–318.

Numis, A. L., Unger, E. L., Sheridan, D. L., Chisnell, A. C., & Andrews, A. M. (2004). The role of membrane and vesicular monoamine transporters in the neurotoxic and hypothermic effects of 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH(2)-MPTP). *Molecular Pharmacology, 66*, 718–727.

Nwanze, E., & Jonsson, G. (1981). Amphetamine neurotoxicity on dopamine nerve terminals in the caudate nucleus of mice. *Neuroscience Letters, 36*, 163–168.

Ochroch, J. B., Bressler, A. J., Yang, H., Murphy, D. L., Altieri, S. C., Andrews, A. M. (2013). 2′-NH_2-MPTP: A serotonin and norepinephrine neurotoxin. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Olney, J. W. (1969). Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science, 164*, 719–721.

Olney, J. W. (1981). Kainic acid and other excitotoxins: A comparative analysis. In G. Di Chiara & G. J. Gessa (Eds.), *Glutamate as Neurotransmitter* (pp. 375–384). New York: Raven.

Olney, J. W. (2002). New insights and new issues in developmental neurotoxicology. *Neurotoxicology, 23*, 659–668.

Olney, J. W., & Sharpe, L. G. (1969). Brain lesions in an infant rhesus monkey treated with monosodium glutamate. *Science, 166*, 386–388.

Olney, J. W., Ho, O. L., & Rhee, V. (1971). Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. *Experimental Brain Research, 14*, 61–76.

Olney, J. W., Labruyere, J., & Price, M. T. (1989). Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science, 244*, 1360–1362.

Olney, J. W., Labruyere, J., Wang, G., Wozniak, D. F., Price, M. T., & Sesma, M. A. (1991). NMDA antagonist neurotoxicity: Mechanism and prevention. *Science, 254*(5037), 1515–1518.

Olney, J. W., Wozniak, D. F., Jevtovic-Todorovic, V., Farber, N. B., Bittigau, P., & Ikonomidou, C. (2002). Drug-induced apoptotic neurodegeneration in the developing brain. *Brain Pathology, 12*, 488–498. Review.

Ong, H. H., Creveling, C. R., & Daly, J. W. (1969). The synthesis of 2,4,5-trihydroxyphenylalanine (6-hydroxydopa). A centrally active norepinephrine-depleting agent. *Jounal of Medicinal Chemistry, 12*, 458–462.

Oswiecimska, J., Brus, R., Szkilnik, R., Nowak, P., & Kostrzewa, R. M. (2000). 7-OH-DPAT, unlike quinpirole, does not prime a yawning response in rats. *Pharmacology Biochemistry and Behavior, 67*, 11–15.

Palfi, S., Ferrante, R. J., Brouillet, E., Beal, M. F., Dolan, R., Guyot, M. C., Peschanski, M., & Hantraye, P. (1996). Chronic 3-nitropropionic acid treatment in baboons replicates the cognitive and motor deficits of Huntington's disease. *Journal of Neuroscience, 16*, 3019–3025.

Papadeas, S. T., Breese, G. R. (2013). 6-Hydroxydopamine lesioning of dopamine neurons in neonatal and adult rats induces age-dependent consequences. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Park, C. K., Nehls, D. G., Graham, D. I., Teasdale, G. M., & McCulloch, J. (1988). The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat. *Annals of Neurology, 24*, 543–551.

Pasquali, L., Caldarazzo-Ienco, E., Fornai, F. (2013). MPTP neurotoxicity: Actions, mechanisms and animal modeling of Parkinson's disease. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. Kostrzewa: Springer.

Paterak, J., Stefański, R. (2013) 5,6- and 5,7-Dihydroxytryptamine as serotoninergic neurotoxins. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Peng, R. Y., Mansbach, R. S., Braff, D. L., & Geyer, M. A. (1990). A D_2 dopamine receptor agonist disrupts sensorimotor gating in rats. Implications for dopaminergic abnormalities in schizophrenia. *Neuropsychopharmacology, 3*, 211–218.

Pereira, F. C., Macedo, T. R., Imam, S. Z., Ribeiro, C. F., & Ali, S. F. (2004). Lack of hydroxyl radical generation upon central administration of methamphetamine in rat caudate nucleus: A microdialysis study. *Neurotoxicity Research, 6*, 149–152.

Pérez-Gómez, A., Tasker, R. A. (2013). Domoic acid as a neurotoxin. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Perl, T. M., Bédard, L., Kosatsky, T., Hockin, J. C., Todd, E. C., & Remis, R. S. (1990). An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *The New England Journal of Medicine, 322*(25), 1775–1780.

Perry, K. W., Kostrzewa, R. M., & Fuller, R. W. (1995). Persistence of long-lasting serotonin depletion by p-chloroamphetamine in rat brain after 6-hydroxydopamine lesioning of dopamine neurons. *Biochemical Pharmacology, 50*, 1305–1307.

Peters, D. A., Pappas, B. A., Taub, H., & Saari, M. (1977). Effect of intraventricular injections of 6-hydroxydopamine in neonatal rats on the catecholamine levels and tyrosine hydroxylase activity in brain regions at maturity. *Biochemical Pharmacology, 26*, 2211–2215.

Perry, E. K., Tomlinson, B. E., Blessed, G., Bergmann, K., Gibson, P. H., & Perry R. H. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *British Medical Journal, 2*, 1457–1459.

Petrosini, L., De Bartolo, P., Cutuli, D. (2013). Neurotoxic effects, mechanisms and outcomes of 192 IgG-Saporin. In R. M. (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Pioro, E. P., & Cuello, A. C. (1988). Purkinje cells of adult rat cerebellum express nerve growth factor receptor immunoreactivity: light microscopy observations. *Brain Research, 455*, 182–186.

Porter, C. C., Totaro, J. A., & Stone, C. A. (1963). Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. *Journal of Pharmacology and Experimental Therapeutics, 140*, 308–316.

Porter, C. C., Totaro, J. A., & Burcin, A. (1965). The relationship between radioactivity and norepinephrine concentrations in the brains and hearts of mice following administration of labeled methyldopa or 6-hydroxydopamine. *Journal of Pharmacology and Experimental Therapeutics, 150*, 17–22.

Potter, P. E., Tedford, C. E., Kindel, G. H., & Hanin, I. (1987). Inhibition of high affinity choline transport attenuates both cholinergic and noncholinergic effects of ethylcholine aziridinium (AF64A). *Brain Research, 13*, 238–244.

Pranzatelli, M. R. (1998). Use of 5,6- and 5,7-dihydroxytryptamine to lesion serotonin neurons. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 293–311). Totowa: Humana Press.

Przedborski, S., Levivier, M., Jiang, H., Ferreira, M., Jackson-Lewis, V., Donaldson, D., & Togasaki, D. M. (1995). Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine. *Neuroscience, 67*, 631–647.

Pulsinelli, W., Sarokin, A., & Buchan, A. (1993). Antagonism of the NMDA and non-NMDA receptors in global versus focal brain ischemia. *Progress in Brain Research, 96*, 125–135.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991). Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics, 288*, 484–487.

Radi, R., Rodriguez, M., Castro, L., & Telleri, R. (1994). Inhibition of mitochondrial electron transport by peroxynitrite. *Archives of Biochemistry and Biophysics, 308*, 89–95.

Rajdev, S., & Sharp, F. R. (1998). Neurotoxicity of NMDA receptor antagonists. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 355–384). Totowa: Humana Press.

Ramsay, P. R., Krueger, M. J., Youngster, S. K., Gluck, M. R., Casida, J. E., & Singer, T. P. (1991). Interaction of 1-methyl-4-phenylpyridinium ion (MPP^+) and its analogs with the rotenone/piericidin binding site of NADH dehydrogenase. *Journal of Neurochemistry, 56*, 1184–1190.

Ranson, R. W., Kammerer, R. C., & Cho, A. K. (1982). Chemical transformations of xylamine (N-2′-chloroethyl-N-ethyl-2-methylbenzylamine) in solution. Pharmacological activity of the species derived from this irreversible norepinephrine uptake inhibitor. *Molecular Pharmacology, 21*, 380–386.

Reynolds, G. P., Pearson, S. J., Halket, J., & Sandler, M. (1988). Brain quinolinic acid in Huntington's disease. *Journal of Neurochemistry, 50*, 1959–1960.

Riachi, N. J., Arora, P. K., Sayre, L. M., & Harik, S. I. (1988). Potent neurotoxic fluorinated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine analogs as potential probes in models of Parkinson's disease. *Journal of Neurochemistry, 50*, 1319–1321.

Ricaurte, G. A., Guillery, R. W., Seiden, L. S., Schuster, C. R., & Moore, R. Y. (1982). Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Research, 235*, 93–103.

Richardson, J. S., & Jacobowitz, D. M. (1973). Depletion of brain norepinephrine by intraventricular injection of 6-hydroxydopa: a biochemical, histochemical and behavioral study in rats. *Brain Research, 58*, 117–133.

Roberts, R. C., Gaither, L. A., Gao, X. M., Kashyap, S. M., & Tamminga, C. A. (1995). Ultrastructural correlates of haloperidol-induced oral dyskinesias in rat striatum. *Synapse, 20*, 234–243.

Rollema, H., Skolnik, M., D'Engelbronner, J., Igarashi, K., Usuki, E., & Castagnoli, N., Jr. (1994). MPP-like neurotoxicity of a pyridinium metabolite derived from haloperidol: In vivo microdialysis and in vitro mitochondrial studies. *Journal of Pharmacology and Experimental Therapeutics, 268*, 380–387.

Ross, S. B. (1976). Long-term effects of N-2-chlorethyl-N-ethyl-2-bromobenzylamine hydrochloride on noradrenergic neurones in the rat brain and heart. *British Journal of Pharmacology, 58*, 521–527.

Ross, S. B., & Reny, A. L. (1976). On the long-lasting inhibitory effect of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) on the active uptake of noradrenaline. *Journal of Pharmacy and Pharmacology, 28*, 458–459.

Ross, S. B., Johansson, J. G., Linborg, B., & Dahlbom, R. (1973). Cyclizing compounds. Tertiary N-(2-bromobenzyl)-N-haloalkylamines with adrenergic blocking action. *Acta Pharmaceutica Suecica, 10*, 29–42.

Rossner, S. (1997). Cholinergic immunolesions by 192IgG-saporin–useful tool to simulate pathogenic aspects of Alzheimer's disease. *International Journal of Developmental Neuroscience, 15*, 835–850.

Rossner, S., Schliebs, R., Perez-Polo, J. R., Wiley, R. G., & Bigi, V. (1995). Differential changes in cholinergic markers from selected brain regions after specific immunolesion of the rat cholinergic basal forebrain system. *Journal of Neuroscience Research, 70*, 31–43.

Rothman, S. M. (1985). The neurotoxicity of excitatory amino acids is produced by passive chloride influx. *Journal of Neuroscience, 5*, 1483–1489.

Rothman, S. M., & Olney, J. W. (1986). Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Annals of Neurology, 19*, 105–111.

Rothstein, J. D., Martin, L. J., & Kuncl, R. W. (1992). Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *New England Journal of Medicine, 326*, 1464–1468.

Rothstein, J. D., Van Kammen, M., Levey, A. I., Martin, L. J., & Kuncl, R. W. (1995). Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Annals of Neurology, 38*, 73–84.

Royland, J. E., & Langston, J. W. (1998). MPTP: A dopaminergic neurotoxin. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 141–194). Totowa: Humana Press.

Rumsfield, J. A., & West, D. P. (1991). Topical capsaicin in dermatologic and peripheral pain disorders. *DICP Annals of Pharmacotherapy, 25*, 381–387.

Ryan, L. J., Martone, M., Linder, J., & Groves, P. (1988). Cocaine, in contrast to D-amphetamine, does not cause axonal terminal degeneration in neostriatum and agranular frontal cortex of Long-Evans rats. *Life Sciences, 43*, 1403–1409.

Ryan, L. J., Linder, J. C., Martone, M. E., & Groves, P. M. (1990). Histological and ultrastructural evidence that D-amphetamine causes degeneration in neostriatum and frontal cortex of rats. *Brain Research, 518*, 67–77.

Rylett, R. J., & Colhoun, E. H. (1984). An evaluation of irreversible inhibition of synaptosomal high-affinity choline transport by choline mustard aziridinium ion. *Journal of Neurochemistry, 43*, 787–794.

Sachs, C., & Jonsson, G. (1972a). Degeneration of central and peripheral noradrenaline neurons produced by 6-hydroxy-DOPA. *Journal of Neurochemistry, 19*, 1561–1575.

Sachs, C., & Jonsson, G. (1972b). Selective 6-hydroxy-DOPA induced degeneration of central and peripheral noradrenaline neurons. *Brain Research, 40*, 563–568.

Sachs, C., Jonsson, G., Heikkila, R., & Cohen, G. (1975). Control of the neurotoxicity of 6-hydroxydopamine by intraneuronal noradrenaline in rat iris. *Acta Physiologica Scandinavica, 93*, 345–351.

Samaha, A. N., Seeman, P., Stewart, J., Rajabi, H., & Kapur, S. (2007). "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. *Journal of Neuroscience, 27*, 2979–2986.

Sandberg, K., Schnaar, R. L., McKinney, M., Hanin, I., Fisher, A., & Coyle, J. T. (1985). AF64A: An active site directed irreversible inhibitor of choline acetyltransferase. *Journal of Neurochemistry, 44*, 439–445.

Sanders-Bush, E., Bushing, J. A., & Sulser, F. (1972a). p-Chloroamphetamine–inhibition of cerebral tryptophan hydroxylase. *Biochemical Pharmacology, 21*, 1501–1510.

Sanders-Bush, E., Bushing, J. A., & Sulser, F. (1972b). Long-term effects of p-chloroamphetamine on tryptophan hydroxylase activity and on the levels of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in brain. *European Journal of Pharmacology, 20*, 385–388.

Saner, A., & Thoenen, H. (1971a). Contributions to the molecular mechanism of action of 6-hydroxydopamine. In T. Malmfors & H. Thoenen (Eds.), *6-Hydroxydopamine and Catecholamine Neurons* (pp. 265–275). Amsterdam: North-Holland.

Saner, A., & Thoenen, H. (1971b). Model experiments on the molecular mechanism of action of 6-hydroxydopamine. *Molecular Pharmacology, 7*, 147–154.

Santiago, M., Venero, J. L., Machado, A., & Cano, J. (1992). In vivo protection of striatum from MPP^+ neurotoxicity by N-methyl-D-aspartate antagonists. *Brain Research, 586*, 203–207.

Sauer, H., & Oertel, W. H. (1994). Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: A combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience, 59*, 401–415.

Scheffel, U., Steinert, C., Kim, S. E., Ehlers, M. D., Boja, J. W., & Kuhar, M. J. (1996). Effect of dopaminergic drugs on the in vivo binding of [^{3}H]WIN 35,428 to central dopamine transporters. *Synapse, 23*, 61–69.

Schliebs, R. (1998). Basal forebrain cholinergic dysfunction – experimental approaches and the diseased brain. *International Journal of Developmental Neuroscience, 16*, 591–593.

Schliebs, R., Rossner, S., & Bigl, V. (1996). Immunolesion by 192IgG-saporin of rat basal forebrain cholinergic system: A useful tool to produce cortical cholinergic dysfunction. *Progress in Brain Research, 109*, 253–264.

Schulz, J. B., Matthews, R. T., Jenkins, B. G., Ferrante, R. J., Siwek, D., Henshaw, D. R., Cipolloni, P. B., Mecocci, P., Kowall, N. W., Rosen, B. R., et al. (1995). Blockade of neuronal nitric oxide synthase protects against excitotoxicity in vivo. *Journal of Neuroscience, 15*, 8419–8429.

Schulz, J. B., Henshaw, D. R., MacGarvey, U., & Beal, M. F. (1996). Involvement of oxidative stress in 3-nitropropionic acid neurotoxicity. *Neurochemistry International, 29*, 167–171.

Schuster, C. R., Lewis, M., & Seiden, L. S. (1986). Fenfluramine: Neurotoxicity. *Psychopharmacology Bulletin, 22*, 148–151.

Schwarcz, R., Whetsell, W. O., Jr., & Mangano, R. M. (1983). Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. *Science, 219*, 316–318.

Schwarcz, R., Foster, A. C., French, E. D., Whetsell, W. O., Jr., & Köhler, C. (1984). Excitotoxic models for neurodegenerative disorders. *Life Sciences, 35*, 19–32.

Seeman, P. (2011). All roads to schizophrenia lead to dopamine supersensitivity and elevated dopamine D_2 (high) receptors. *CNS Neuroscience and Therapeutics, 17*, 118–132.

Seeman, P., Weinshenker, D., Quirion, R., Srivastava, L. K., Bhardwaj, S. K., Grandy, D. K., Premont, R. T., Sotnikova, T. D., Boksa, P., El-Ghundi, M., O'Dowd, B. F., George, S. R., Perreault, M. L., Männistö, P. T., Robinson, S., Palmiter, R. D., & Tallerico, T. (2005). Dopamine supersensitivity correlates with D_2^{High} states, implying many paths to psychosis. *Proceedings of the National Academy of Sciences of USA, 102*(9), 3513–3518.

Seeman, P., Hall, F. S., & Uhl, G. (2007). Increased dopamine D_2^{High} receptors in knockouts of the dopamine transporter and the vesicular monoamine transporter may contribute to spontaneous hyperactivity and dopamine supersensitivity. *Synapse, 61*, 573–576.

Segura-Aguilar, J. (2001). DT-Diaphorase: A protective enzyme of the dopaminergic system. In J. Segura-Aguilar (Ed.), *Mechanisms of Degeneration and Protection of the Dopaminergic System* (pp. 289–299). Johnson City: FP Graham.

Segura-Aguilar, J., & Kostrzewa, R. M. (2004). Neurotoxins and neurotoxic species implicated in neurodegeneration. *Neurotoxicity Research, 6*, 615–630.

Segura-Aguilar, J., & Kostrzewa, R. M. (2006). Neurotoxins and neurotoxicity mechanisms. An overview. *Neurotoxicity Research, 10*, 263–287.

Seniuk, N. A., Tatton, W. G., & Greenwood, C. E. (1990). Dose-dependent destruction of the coeruleus-cortical and nigral-striatal projections by MPTP. *Brain Research, 527*, 7–20.

Senoh, S., & Witkop, B. (1959). Formation and rearrangements of aminochromes from a new metabolite of dopamine and some of its derivatives. *Journal of the American Chemical Society, 81*, 6231–6235.

Shafer, J. (1985). Designer drugs. Magazine Work. http://www.jackshafer.com/magazine_work/20091128_designer_drugs.php

Shaw, P. J., Forrest, V., Ince, P. G., Richardson, J. P., & Wastell, H. J. (1995). Studies on cellular free radical protection mechanisms in the anterior horn from patients with amyotrophic lateral sclerosis. *Neurodegeneration, 4*, 209–216.

Shaywitz, B. A., Yager, R. D., & Klopper, J. H. (1976). Selective brain dopamine depletion in developing rats: an experimental model of minimal brain dysfunction. *Science, 191*(4224), 305–308.

Siemens, J., Zhou, S., Piskorowski, R., Nikai, T., Lumpkin, E. A., Basbaum, A. I., King, D., & Julius, D. (2006). Spider toxins activate the capsaicin receptor to produce inflammatory pain. *Nature, 444*, 208–212.

Silverstein, F. S., Buchanan, K., & Johnston, M. V. (1986). Perinatal hypoxia-ischemia disrupts striatal high-affinity [^{3}H]glutamate uptake into synaptosomes. *Journal of Neurochemistry, 47*, 1614–1619.

Singer, T. P., Salach, J. L., Castagnoli, N., Jr., & Trevor, A. J. (1986). Interactions of the neurotoxic amine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine with monoamine oxidases. *Biochemistry Journal, 235*, 785–789.

Singh, B. R. (2006). Botulinum neurotoxin structure, engineering, and novel cellular trafficking and targeting. *Neurotoxicity Research, 9*, 73–92.

Singh, B. R., Kumar, R., Cai, S. (2013). Molecular mechanism and effects of clostridial neurotoxin. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer, pp. 513–551.

Snyder, A. M., Zigmond, M. J., & Lund, R. D. (1986). Sprouting of serotoninergic afferents into striatum after dopamine-depleting lesions in infant rats: a retrograde transport and immunocytochemical study. *The Journal of Comparative Neurology, 245*(2), 274–281. doi:10.1002/cne.902450209.

Sonsalla, P. K., & Heikkila, R. E. (1988). Neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine in several strains of mice. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 12*, 345–354.

Sonsalla, P. K., Gibb, J. W., & Hanson, G. R. (1986). Roles of D_1 and D_2 dopamine receptor subtypes in mediating the methamphetamine-induced changes in monoamine systems. *Journal of Pharmacology and Experimental Therapeutics, 238*, 932–937.

Spencer, P. S. (1987). Guam ALS/parkinsonism-dementia: a long-latency neurotoxic disorder caused by "slow toxin(s)" in food? *Canadian Journal of Neurological Sciences, 14*(3 Suppl), 347–357.

Spencer, P. S., Roy, D. N., Ludolph, A., Hugon, J., Dwivedi, M. P., & Schaumburg, H. H. (1986). Lathyrism: Evidence for the role of the neuroexcitatory amino acid BOAA. *Lancet, 2*, 1066–1067.

Staal, R. G., & Sonsalla, P. K. (2000). Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata. *Journal of Pharmacology and Experimental Therapeutics, 293*, 336–342.

Stachowiak, M. K., Bruno, J. P., Snyder, A. M., Stricker, E. M., & Zigmond, M. J. (1984). Apparent sprouting of striatal serotonergic terminals after dopamine-depleting brain lesions in neonatal rats. *Brain Research, 291*, 164–167.

Steinkellner, T., Freissmuth, M., Sitte, H. H., & Montgomery, T. (2011). The ugly side of amphetamines: Short- and long-term toxicity of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'), methamphetamine and D-amphetamine. *Biological Chemistry, 392*, 103–115. doi:10.1515/BC.2011.016. Review.

Steranka, L. R., & Sanders-Bush, E. (1979). Long-term effects of fenfluramine on central serotonergic mechanisms. *Neuropharmacology, 18*, 895–903.

Stokes, A. H., Hastings, T. G., & Vrana, K. E. (1999). Cytotoxic and genotoxic potential of dopamine. *Journal of Neuroscience Research, 55*, 659–665.

Stone, C. A., Stavorski, J. M., Ludden, C. T., Wenger, H. C., Ross, C. A., Totaro, J. A., & Porter, C. C. (1963). Comparison of some pharmacologic effects of certain 6-substituted dopamine derivatives with reserpine, guanethidine and metaraminol. *Journal of Pharmacology and Experimental Therapeutics, 142*, 147–156.

Stone, C. A., Porter, C. C., Stavorski, J. M., Ludden, C. T., & Totaro, J. A. (1964). Antagonism of certain effects of catecholamine-depleting agents by antidepressant and related drugs. *Journal of Pharmacology and Experimental Therapeutics, 144*, 196–204.

Stumm, G., Schlegel, J., Schafer, T., Wurz, C., Mennel, H. D., Krieg, J. C., & Vedder, H. (1999). Amphetamines induce apoptosis and regulation of bcl-x splice variants in neocortical neurons. *The FASEB Journal, 13*, 1065–1072.

Subramanyam, B., Rollema, H., Woolf, T., & Castagnoli, N., Jr. (1990). Identification of a potentially neurotoxic pyridinium metabolite of haloperidol in rats. *Biochemical and Biophysical Research Communications, 166*, 238–244.

Subramanyam, B., Pond, S. M., Eyles, D. W., Whiteford, H. A., Fouda, H. G., & Castagnoli, N., Jr. (1991). Identification of a potentially neurotoxic pyridinium metabolite in the urine of schizophrenic patients treated with haloperidol. *Biochemical and Biophysical Research Communications, 181*, 573–578.

Szallasi, A. (1998). Toxic vanilloids. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 385–398). Totowa: Humana Press.

Szallasi, A., & Blumberg, P. M. (1999). Vanilloid (capsaicin) receptors and mechanisms. *Pharmacological Reviews, 51*, 159–212.

Szechtman, H., & Woody, E. (2004). Obsessive-compulsive disorder as a disturbance of security motivation. *Psychological Review, 111*, 111–127. Review.

Szechtman, H., & Woody, E. Z. (2006). Obsessive-compulsive disorder as a disturbance of security motivation: Constraints on comorbidity. *Neurotoxicity Research, 10*, 103–112. Review.

Szechtman, H., Sulis, W., & Eilam, D. (1998). Quinpirole induces compulsive checking behavior in rats: a potential animal model of obsessive-compulsive disorder (OCD). *Behavioral Neuroscience, 112*, 1475–1485.

Tabatabaie, T., Wrona, M. Z., & Dryhurst, G. (1990). Autoxidation of the serotonergic neurotoxin 5,7-dihydroxytryptamine. *Journal of Medicinal Chemistry, 33*, 667–672.

Tabatabaie, T., & Dryhurst, G. (1992). Chemical and enzyme-mediated oxidation of the serotonergic neurotoxin 5,7-dihydroxytryptamine: Mechanistic insights. *Journal of Medicinal Chemistry, 35*, 2261–2273.

Tabatabaie, T., & Dryhurst, G. (1998). Molecular mechanisms of action of 5,6- and 5,7-dihydroxytryptamine. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 269–291). Totowa: Humana Press.

Tabatabaie, T., Goyal, R. N., Blank, C. L., & Dryhurst, G. (1993). Further insights into the molecular mechanisms of action of the serotonergic neurotoxin 5,7-dihydroxytryptamine. *Journal of Medicinal Chemistry, 36*, 229–236.

Teismann, P., Tieu, K., Cohen, O., Choi, D. K., Wu, D., Marks, D., Vila, M., Jackson-Lewis, V., & Przedborski, S. (2003). Pathogenic role of glial cells in Parkinson's disease. *Movement Disorders, 18*, 121–129. Review.

Thoenen, H., & Tranzer, J. P. (1968). Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. *Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie, 261*, 271–288.

Thoenen, H., & Tranzer, J. P. (1973). The pharmacology of 6-hydroxydopamine. *Annual Review Pharmacology, 13*, 169–180. Review.

Tizabi, Y., Copeland, R. L., Jr., Brus, R., & Kostrzewa, R. M. (1999). Nicotine blocks quinpirole-induced behavior in rats: Psychiatric implications. *Psychopharmacology, 145*, 433–441.

Towle, A. C., Criswell, H. E., Maynard, E. H., Lauder, J. M., Joh, T. H., Mueller, R. A., & Breese, G. R. (1989). Serotonergic innervation of the rat caudate following a neonatal

6-hydroxydopamine lesion: an anatomical, biochemical and pharmacological study. *Pharmacology Biochemistry and Behavior, 34*, 367–374.

Tranzer, J. P., & Thoenen, H. (1967a). Electron microscopy studies on the peripheral sympathetic nervous system of the cat; physiological and pharmacological aspects. *Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie, 257*, 73–75.

Tranzer, J. P., & Thoenen, H. (1967b). Electronmicroscopic localization of 5-hydroxydopamine (3,4,5-trihydroxy-phenyl-ethylamine), a new 'false' sympathetic transmitter. *Experientia, 23*, 743–745.

Tranzer, J. P., & Thoenen, H. (1968). An electron microscopic study of selective, acute degeneration of sympathetic nerve terminals after administration of 6-hydroxydopamine. *Experientia, 24*, 155–156.

Tranzer, J. P., & Thoenen, H. (1973). Selective destruction of adrenergic nerve terminals by chemical analogues of 6-hydroxydopamine. *Experientia, 29*, 314–315.

Turski G. N., & Ikonomidou, G. (2013) Glutamate as a neurotoxin. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer

Turski, W. A., Gramsbergen, J. B., Traitler, H., & Schwarcz, R. (1989). Rat brain slices produce and liberate kynurenic acid upon exposure to L-kynurenine. *Journal of Neurochemistry, 52*, 1629–1636.

Turski, L., Bressler, K., Rettig, K. J., Loschmann, P. A., & Wachtel, H. (1991). Protection of substantia nigra from MPP^+ neurotoxicity by N-methyl-D-aspartate antagonists. *Nature, 349*, 414–418.

Tymianski, M., Charlton, M. P., Carlen, P. L., & Tator, C. H. (1993). Source specificity of early calcium neurotoxicity in cultured embryonic spinal neurons. *Journal of Neuroscience, 13*, 2085–2104.

Uney, J. B., & Marchbanks, R. M. (1987). Specificity of ethylcholine mustard aziridinium ion as an irreversible inhibitor of choline transport in cholinergic and non-cholinergic tissue. *Journal of Neurochemistry, 8*, 1673–1676.

Ungerstedt, U. (1968). 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *The European Journal of Pharmaceutical, 5*, 107–110.

Ungerstedt, U. (1971a). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiologica Scandinavica. Supplementum, 367*, 95–122.

Ungerstedt, U. (1971b). Postsynaptic supersensitivity after 6-hydroxy-dopamine induced degeneration of the nigrostriatal dopamine system. *Acta Physiologica Scandinavica. Supplementum, 367*, 69–93.

Ungerstedt, U. (1971c). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiologica Scandinavica. Supplementum, 367*, 1–48.

Ungerstedt, U. (1971d). Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. *Acta Physiologica Scandinavica. Supplementum, 367*, 49–68.

Urbanska, E., Ikonomidou, C., Sieklucka, M., & Turski, W. A. (1991). Aminooxyacetic acid produces excitotoxic lesions in the rat striatum. *Synapse, 9*, 129–135.

Urbanska, E. M., Dekundy, A., Kleinrok, Z., Turski, W. A., & Czuczwar, S. J. (1998). Glutamatergic receptor agonists and brain pathology. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins* (pp. 329–354). Totowa: Humana Press.

Urbanska, E. M., Chmiel-Perzynska, I., Perzynski, A., Derkacz, M., Owe-Larsson, B. (2013). Endogenous kynurenic acid and neurotoxicity. In R. M. Kostrzewa (Ed.), *Handbook of Neurotoxicity*. New York: Springer.

Uretsky, N. J., & Iversen, L. (1969). Effects of 6-hydroxydopamine on noradrenaline-containing neurons in the rat brain. *Nature, 221*(5180), 557–559.

Uretsky, N. J., & Iversen, L. (1970). Effects of 6-hydroxydopamine on catecholamine-containing neurons in the rat brain. *Journal of Neurochemistry, 17*, 269–278.

Usuki, E., Pearch, R., Parkinson, A., & Castagnoli, N., Jr. (1996). Studies on the conversion of haloperidol and its tetrahydropyridinium metabolites by human liver microsomes. *Chemical Research in Toxicology, 9*, 800–806.

van der Vliet, A., Hoen, P. A., Wong, P. S., Bast, A., & Cross, C. E. (1998). Formation of S-nitrosothiols via direct nucleophilic nitrosation of thiols by peroxynitrite with elimination of hydrogen peroxide. *Journal of Biological Chemistry, 273*, 30255–30262.

Waddington, J. L., Cross, A. J., Gamble, S. J., & Bourne, R. C. (1983). Spontaneous orofacial dyskinesia and dopaminergic function in rats after 6 months of neuroleptic treatment. *Science, 220*(4596), 530–532.

Wagner, G. C., Ricaurte, G. A., Johanson, C. E., Schuster, C. R., & Seiden, L. S. (1980). Amphetamine induces depletion of dopamine and loss of dopamine uptake sites in caudate. *Neurology, 30*, 547–550.

Walsh, T. J., & Potter, P. E. (1998). Selective cholinergic neurotoxins. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins* (pp. 313–327). Totowa: Humana Press.

Walsh, T. J., Herzog, C. D., Gandhi, C., Stackman, R. W., & Wiley, R. G. (1996). Injection of IgG 192-saporin into the medial septum produces cholinergic hypofunction and dose-dependent working memory deficits. *Brain Research, 726*, 69–79.

Waner, D. S., Martin, H., Ludwig, P., McAllister, A., Keana, J. F., & Weber, E. (1995). In vivo models of cerebral ischemia: Effects of parenterally administered NMDA receptor glycine site antagonists. *Journal of Cerebral Blood Flow and Metabolism, 15*, 188–196.

Weiss, J., Goldberg, M. P., & Choi, D. W. (1986). Ketamine protects cultured neocortical neurons from hypoxic injury. *Brain Research, 380*, 186–190.

Westfall, T. C., & Westfall, D. P. (2006). Neurotransmission. The autonomic and somatic motor nervous system. In L. L. Brunton, J. S. Lazo, & K. L. Parker (Eds.), *Goodman and Gilman's the Pharmacological Basis of Therapeutics* (11th ed., p. 173). New York: McGraw Hill.

Westlund, K. N., Denney, R. M., Kochersperger, L. M., Ross, R. M., & Abell, C. W. (1985). Distinct monoamine oxidase A and B populations in primate brain. *Science, 230*, 181–183.

Wieloch, T. (1985). Hypoglycemia-induced neuronal damage prevented by an N-methyl-D-aspartate antagonist. *Science, 230*, 681–683.

Wieloch, T., Lindvall, O., Bloomquist, P., & Gage, F. H. (1985). Evidence for amelioration of ischaemic neuronal damage in the hippocampal formation by lesions of the perforant path. *Neurological Research, 7*, 24–26.

Wiley, R. G. (1992). Neural lesioning with ribosome-inactivating proteins: Suicide transport and immunolesioning. *Trends in Neurosciences, 15*, 285–290.

Wiley, R. G., Oeltmann, T. N., & Lappi, D. A. (1991). Immunolesioning: selective destruction of neurons using immunotoxin to rat NGF receptor. *Brain Research, 562*, 149–153.

Wiley, R. G., Berbos, T. G., Deckwerth, T. L., Johnson, E. M., Jr., & Lappi, D. A. (1995). Destruction of the cholinergic basal forebrain using immunotoxin to rat NGF receptor: modeling the cholinergic degeneration of Alzheimer's disease. *Journal of Neurological Sciences, 128*, 157–166.

Williams, T. L., Day, N. C., Kamboj, R. K., Ince, P. G., & Shaw, P. J. (1997). Calcium-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors: a molecular determinant of selective vulnerability in amyotrophic lateral sclerosis. *Annals of Neurology, 35*, 200–207.

Winter, J., Bevan, S., & Campbell, E. A. (1995). Capsaicin and pain mechanisms. *British Journal of Anaesthesia, 75*, 157–168.

Wirtshafter, D., Asin, K., & Pitzer, M. (1994). Dopamine agonists and stress produce different patterns of fos-like immunoreactivity in the lateral habenula. *Brain Research, 633*, 21–26.

Wozniak, D. F., Brosnan-Watters, G., Nardi, A., McEwen, M., Corso, T. D., Olney, J. W., & Fix, A. S. (1996). MK-801 neurotoxicity in male mice – histologic effects and chronic impairment in spatial learning. *Brain Research, 707*, 165–179.

Wrenn, C. C., & Wiley, R. G. (1998). The behavioral functions of the cholinergic basal forebrain: lessons from 192 IgG-saporin. *International Journal of Developmental Neuroscience, 16*, 595–602.

Wu, E. Y., Smith, M. T., Bellomo, G., & Di Monte, D. A. (1990). Relationships between the mitochondrial transmembrane potential, ATP concentration, and cytotoxicity in isolated rat hepatocytes. *Archives of Biochemistry and Biophysics, 282*, 358–362.

Yamamoto, B. K., & Raudensky, J. (2008). The role of oxidative stress, metabolic compromise, and inflammation in neuronal injury produced by amphetamine-related drugs of abuse. Journal of Neuroimmune Pharmacology *3*(4), 203–217. doi: 10.1007/s11481-008-9121-7. Epub 2008 Aug 15. Review.

Yamamoto, B. K., Moszczynska, A., & Gudelsky, G. A. (2010). Amphetamine toxicities: Classical and emerging mechanisms. Annals of the New York Academy of Sciences *1187*, 101–121. doi: 10.1111/j.1749-6632.2009.05141.x. Review.

Yanagisawa, M., Yagi, N., Otsuka, M., Yanihara, A., & Yanaihara, N. (1986). Inhibitory effects of galanin on the isolated spinal cord of the newborn rat. *Neuroscience Letters, 70*, 278–282.

Yuan, C., & Acosta, D., Jr. (2000). Effect of cocaine on mitochondrial electron transport chain evaluated in primary cultures of neonatal rat myocardial cells and in isolated mitochondrial preparations. *Drug and Chemical Toxicology, 23*, 339–348.

Zaczek, R., Battaglia, G., & Culp, S. (1990). Effects of repeated fenfluramine administration on indices of monoamine function in rat brain: Pharmacokinetic, dose response, regional specificity and time course data. *Journal of Pharmacology and Experimental Therapeutics, 253*, 104–112.

Zeevalk, G. D., & Nicklas, W. J. (1991). Mechanisms underlying initiation of excitotoxicity associated with metabolic inhibition. *Journal of Pharmacology and Experimental Therapeutics, 257*, 870–878.

Zeevalk, G. D., & Nicklas, W. J. (1992). Evidence that the loss of the voltage-dependent Mg^{2+} block at the N-methyl-D-aspartate receptor underlies receptor activation during inhibition of neuronal metabolism. *Journal of Neurochemistry, 59*, 1211–1220.

Zieher, L. M., & Jaim-Etcheverry, G. (1973). Regional differences in the long-term effect of neonatal 6-hydroxydopa treatment on rat brain noradrenaline. *Brain Research, 60*, 199–207.

Zieher, L. M., & Jaim-Etcheverry, G. (1975). Different alterations in the development of the noradrenergic innervation of the cerebellum and the brain stem produced by neonatal 6-hydroxydopa. *Life Sciences, 17*, 987–991.

Zieher, L. M., & Jaim-Etcheverry, G. (1980). Neurotoxicity of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP 4) on noradrenergic neurons is mimicked by its cyclic aziridinium derivative. *European Journal of Pharmacology, 65*, 249–256.

Zigmond, M. J., & Keefe, K. A. (1998). 6-Hydroxydopamine as a tool for studying catecholamines in adult animals: Lessons from the neostriatum. In R. M. Kostrzewa (Ed.), *Highly Selective Neurotoxins: Basic and Clinical Applications* (pp. 75–107). Totowa: Humana Press.

Zigmond, M. J., & Stricker, E. M. (1989). Animal models of parkinsonism using selective neurotoxins: Clinical and basic implications. *International Review of Neurobiology, 31*, 1–79.

Zigmond, M., Abercrombie, E., Berger, T., Grace, A., & Stricker, E. (1993). Compensatory responses to partial loss of dopaminergic neurons: studies with 6-hydroxydopamine. In J. Schneider & M. Gupta (Eds.), *Current Concepts in Parkinson's Disease Research* (pp. 99–140). Toronto: Hogrefe & Huber Publication.

Necroptosis, a Potential Therapeutic Target for Neurological Disorders

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Abstract

Necrosis is considered to be an unregulated and chaotic cell death. However, recent advances in cell death strategies support necroptosis as a form of regulated programmed necrotic cell death. In response to TNF-α or Fas ligands, necroptosis can be induced by cell death receptors in multiple cell lines in the presence of a caspase inhibitor z-VAD; necroptotic cell death has been found to play an important role in normal development, immunity, inflammation, cancer, and human diseases. In this chapter, the molecular mechanisms governing necroptosis, recent findings about the upstream and downstream schema of necroptosis, and potential therapeutic targets in neurological disorders are discussed. After being activated by TNF-α (or Fas ligands) and death receptors, receptor-interacting proteins 1 and 3 (RIP1 and RIP3) form a complex, which play a central role in the induction of necroptosis. RIP3 phosphorylates and activates mitochondrial proteins mixed lineage kinase domain-like protein (MLKL) and PGAM5, resulting in the execution of necroptosis by dynamin-related protein 1, the GTPase that controls mitochondrial fission. Some small molecules such as necrostain-1 and necrosulfonamide target different steps of necroptosis and impede the progress of necroptosis. FADD, caspase-8, CLIP, and CYLD positively or negatively regulate RIP1-/RIP3-dependent necroptosis by different mechanisms. Recent studies demonstrate the involvement of necroptosis in many neurological disorders including stroke, trauma, neonatal hypoxic–ischemic encephalopathy, and Huntington's disease. As a potential therapeutic target, the understanding of necroptotic mechanisms will provide new insights to develop more potent neuroprotectants and specific therapeutic strategies for clinical treatments of neurological disorders.

Keywords

Necroptosis • Neurological diseases • RIP1 • RIP3

List of Abbreviations

AIF	Apoptosis-inducing factor
ANT	Adenine nucleotide translocase
Aur-A	Aurora-A kinase
BHA	Butylated hydroxyanisole
CCI	Controlled cortical impact
cIAP1/2	Apoptosis protein 1/2
CypA	Cyclophilin A
CypD	Cyclophilin D
DRP1	Dynamin-related protein 1
FADD	Fas-associated protein with death domain
FLIPL	FLICE-like inhibitory protein long
GLUD1	Glutamate dehydrogenase 1
GLUL	Glutamate–ammonia ligase
HI	Hypoxia–ischemia

HIE	Hypoxic–ischemic encephalopathy
HMGB1	High-mobility group box 1 protein
HSP90	Heat shock protein
IAPs	Inhibitors of apoptosis
MEFs	Mouse embryonic fibroblasts
MKRN1	Makorin Ring Finger Protein 1
MLKL	Mixed lineage kinase domain-like protein
OGD	Oxygen–glucose deprivation
Plk1	Polo-like kinase 1
RHIM	RIP homotypic interaction motif
RIP	Receptor-interacting protein
ROS	Reactive oxygen species
TNFR1	TNF receptor 1
TNF-α	Tumor necrosis factor-α
z-VAD	z-VAD-fmk

1 Introduction

Neurological disorders, from the gamut of acute medical emergencies such as stroke to debilitating chronic neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, are related to neuronal cell damage, cell repair, and final cell death or survival. For the prevention or therapeutic intervention for these neurological disorders, the process of cell death still is one of major targets.

Neuronal loss is observed in many neurological disorders including stroke, Parkinson's disease, and Alzheimer's disease (Ekshyyan and Aw 2004). However, the kinds of cell death existing in the pathological process of the multiplicity of neurological diseases are still not clear. On the basis of intracellular morphological changes, it was considered that there were three types of cell death in embryonic developmental processes (Schweichel and Merker 1973). Type I cell death is characterized by cytoplasmic shrinkage, pyknosis, nuclear condensation, and late cell fragmentation, which is identical to "apoptosis," as described by Kerr (Kerr et al. 1972). Type II cell death is characterized by substantial autophagic vacuolization in the cytoplasm, termed lately as "autophagy." Type III cell death is characterized by clumping of chromatin, swelling of organelles, and cell disintegration including the rupture of nuclei, mitochondria, lysosomes, and plasma membrane; and this is considered to be "necrosis" (Kitanaka and Kuchino 1999). In the past decades, accumulating evidences demonstrate that many alternative cell death mechanisms exist during the development and pathology of diseases including necroptosis, elimination by shedding, keratinocyte death by cornification, and cell–cell cannibalism by entosis (Yuan and Kroemer 2010). Among the reported forms of cell death, "apoptosis," "autophagy," and "necroptosis" are regarded as the major types most closely associated with neuropathology in neurological and neurodegenerative disorders

(Edinger and Thompson 2004). Apoptotic and autophagic cell death regulation pathways have been extensively studied and well established in the past two decades. Unlike apoptosis and autophagy – the forms of programmed cell death – necrosis has long been considered as an uncontrolled nonprogrammed cell death characterized by rapid chaotic breakdown of the cell under intolerable conditions. More recently, necrosis has been recognized as a programmed and regulated cell death process (Degterev et al. 2005; Edinger and Thompson 2004). Since necroptosis – a form of necrotic cell death – was proposed in 2005 (Degterev et al. 2005), the concept of regulated necrosis stirred cell death research, while regulation of signal pathway transduction became one of the hot topics in biomedical research.

Necroptosis represents a specific form of caspase-3-independent programmed necrotic cell death that is induced by cell death ligands such as TNF-α and Fas ligand via cell death receptors in multiple cell types, in the presence of an apoptosis inhibitor (Degterev et al. 2005). The increasing evidences have demonstrated the existence of necroptosis in a variety of neurological disorders. In this chapter, the regulative pathway of necroptosis and its potential therapeutic application in neurological disease will be discussed.

2 The Cellular Signal Transduction of Necroptosis

Necroptosis, as a form of necrotic cell death, was identified by Degterev and his colleagues based on the observation of cell death induced by TNF-α or Fas ligand in the presence of an apoptosis inhibitor (Degterev et al. 2005). Initially, necroptosis was not considered to share signal pathways with apoptosis and was thereby accorded as an independent cell death process distinct from apoptosis. However, recent advances have shed greater insight into the mechanisms by which necroptosis is regulated and executed. Actually, as an alternative form of cell death, necroptosis can not only cross talk with apoptosis and autophagy but under some circumstances can also recruit other cell death processes such as apoptosis or autophagy (Duprez et al. 2012; Lu and Walsh 2012; Simenc and Lipnik-Stangelj 2012; Vandenabeele et al. 2010; Zhang et al. 2009). In most pathological situations, different kinds of cell death may exist and lead to both cell loss and tissue damage (Long and Ryan 2012; Yahiro et al. 2012). However, one form of cell death will dominate the pathological process. When the signal pathways of this dominant cell death process are blocked (e.g., caspase inhibitor block of apoptosis), an alternative cell death process such as necroptosis can be increased and become the dominant form of cell death (Han et al. 2011). By understanding the conversion or switch between different forms of cell death, it becomes possible to identify new clinical targets – thus enabling the advance of new therapeutic strategies. As for necroptotic cell death, the deciding factors for the conversion from/to apoptosis are the central protein complexes RIP1 and RIP3; but positive regulators (such as deubiquitinase CYLD) and negative regulators (such as caspase-8, FADD) also closely control the fate of cells (survival, necroptosis, or other forms of cell death converted from necroptosis). The advances on regulation of necroptosis are discussed below.

2.1 The Central Signals for Necroptosis: RIP1 and RIP3

Receptor-interacting protein (RIP) kinase family has been identified as having seven members so far (Zhang et al. 2010). RIP kinase family members share a homologous kinase domain but have different functional domains. Since their discovery as a novel form apoptosis-inducing protein, RIP kinases have emerged as essential sensors of cellular stress (Stanger et al. 1995). RIP kinases not only trigger pro-survival through the activation of transcription factors such as NF-κB and AP-1 but also initiate the death-inducing programs (Meylan and Tschopp 2005). Hence, RIP kinases are regarded as crucial regulators of cell survival and death (Festjens et al. 2007). Among these family members, RIP1 and RIP3 are specifically related to necroptosis (Cho et al. 2009) (Fig. 1).

RIP1, the first member of the RIP kinase family, contains three domains: an N-terminal kinase domain, an intermediate domain, and a C-terminal death domain. Although the death domain of RIP1 was shown to bind to several death receptors, such as TNFR1 to activate NF-κB and induce apoptosis (Holler et al. 2000; Stanger et al. 1995), the serine/threonine activity of RIP1 is essential for necroptosis (Degterev et al. 2005). Moreover, RIP1 is regarded as a crucial adaptor kinase and a switch between different forms of cell death in response to extracellular stress or damaging stimuli such as TNF-α and Fas ligands (Festjens et al. 2007). TNF-α is the most extensively studied extracellular signal that leads to apoptosis or necroptosis. Under the apoptosis situation, TNF-α stimulation sequentially induces the formation of two protein complexes: complex I and complex II. In the cytoplasmic membrane, TNF-α binds to TNF receptor 1 (TNFR1) and induces the formation of protein complex I including TRADD, TRAF2, RIP1, and cIAP1. RIP1 is recruited to the protein complex via its death domain to TNFR1. After the formation of protein complex I, these proteins dissociate from TNFR1, and RIP1 enters the cytosol. In the cytosol, RIP1 forms protein complex II with caspase-8, Fas-Associated protein with Death Domain (FADD), and other proteins to activate the caspase cascade to execute apoptosis. In the presence of pancaspase inhibitor z-VAD-fmk (z-VAD), caspase-8 activity is inhibited and the apoptotic pathway is blocked. In this situation, RIP1 interacts with RIP3 to form Ripsome and initiate necroptosis as an alternative cell death. In the apoptosis-deficient condition, the expression of an inactive RIP1 or the inhibition of RIP1 activity by Nec-1 inhibits TNF-induced necroptosis (Degterev et al. 2005; Holler et al. 2000). Mice lacking RIP1 appear normal at birth but fail to thrive, displaying extensive apoptosis in both the lymphoid and adipose tissues and dying at 1–3 days of age (Kelliher et al. 1998). Therefore, RIP1 kinase activity is crucial for this alternative cell death pathway of necroptosis.

The increasing body of evidences demonstrate that RIP3 is a determinant for necroptosis (Challa and Chan 2010). Recent studies show that cells which lack RIP3 expression are resistant to necroptosis, despite the normal RIP1 expression (Cho et al. 2009; He et al. 2009). When RIP3 expression was restored with an shRNA-resistant RIP3 transgene, necroptotic cell death was reestablished (He et al. 2009). However, if RIP3 Kinase-Dead Mutant K50A abolishes RIP3 kinase

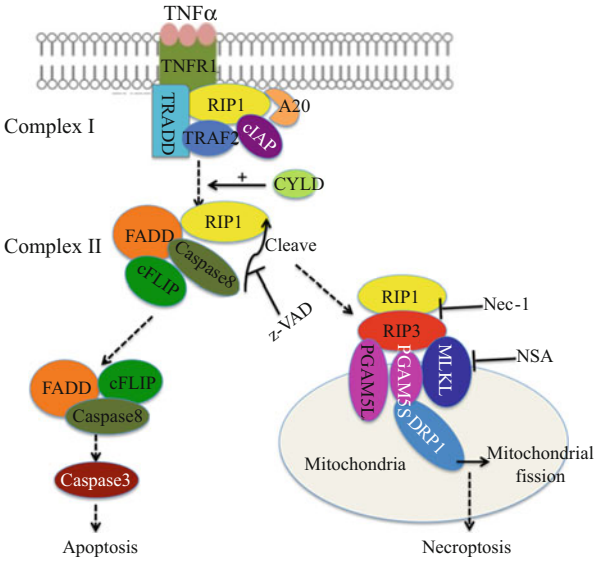

Fig. 1 TNF-α-elicited signaling pathways on apoptosis and necroptosis. Upon TNF-α stimulation, TNFR1 induces the formation of protein complex I including TRADD, TRAF2, and RIP1 at the plasma membrane. RIP1 is modified by polyubiquitination via cIAP1 and A20. After the dissociation from TNFR1, RIP1 enters the cytosol and deubiquitinates by CYLD. In the cytosol, RIP1 forms protein complex II with FADD, caspase-8, and cellular FLIP (cFLIP). The composition of protein complex II and its activation determine the directions of the cell death: apoptosis or necroptosis. FADD contains a death-effector domain, which recruits and causes the autocatalytic activation of the initiator caspase-8; caspase-8 directly cleaves intracellular substrates or activates other caspases such as caspase-3, finally resulting in apoptosis. The apoptotic machinery FADD, caspase-8, and cFLIP suppress necroptosis via the cleavage of RIP1 by activated caspase-8. In apoptosis-deficient conditions such as in the presence of pancaspase inhibitor z-VAD, caspase-8 activation is inhibited and blocks the RIP1 cleavage. RIP1 then interacts with RIP3 to form a RIP1–RIP3 complex. RIP3 phosphorylates two proteins in the outer mitochondrial membrane: a protein kinase MLKL and a phosphatase PGAM5. After the activation of MLKL, short form of PGAM5 (PGAM5S) activates DRP1, the GTPase that controls mitochondrial fission, in a hydrophobic environment, leading to mitochondrial fission and necroptosis. The necroptosis inhibitors necrostain-1 (Nec-1) and necrosulfonamide (NSA) block necroptotic cell death by targeting the interaction of RIP1 and RIP3 and MLKL, respectively

activity, necroptosis remains blocked, indicating that the kinase domain is still essential for the role of RIP3 (He et al. 2009).

The interaction of RIP1 and RIP3 is required for mutual and autophosphorylation of RIP1 and RIP3 and the activation of the downstream necrotic signaling pathway (Cho et al. 2009; He et al. 2009). Both the intermediate domain of RIP1 and the unique C-terminus of RIP3 have a RIP homotypic interaction motif (RHIM), which is required for mediating the interaction between RIP1 and RIP3 (Meylan and Tschopp 2005). The small-molecule necrostain-1, an inhibitor of RIP1 kinase activity, blocks necroptosis by impairing the interaction between RIP1 and RIP3 (Cho et al. 2009; He et al. 2009). The mutation in the

RHIM domain of RIP proteins inhibits necroptotic cell death (Cho et al. 2009), suggesting that the interaction of RIP1 and RIP3 or RIP1–RIP3 complex is crucial for death receptor-mediated necroptosis. The apoptotic pathway seems to be controlled by a caspase network, whereas necroptotic signaling is controlled by a kinase cascade and highly regulated by an intracellular protein platform (Gunther et al. 2013).

2.2 The Upstream Positive and Negative Regulators of Necroptosis

2.2.1 FADD/Caspases-8/FLIP

FADD is an adaptor molecule that bridges the Fas receptor or other death receptors such as TNFR1 to caspase-8 through its death domain to form the death-inducing signaling complex during apoptosis (Bodmer et al. 2000; Kreuz et al. 2004). FADD containing a death-effector domain can recruit and cause the autocatalytic activation of the initiators caspase-8 and caspase-10, which can directly cleave intracellular substrates or activate other caspases including caspase-3 (Tourneur and Chiocchia 2010). When caspase-8 is activated, it mediates apoptosis; at the same time, caspase-8 prevents necroptotic cell death by cleaving RIP1 and/or RIP3 or by binding FLICE-like inhibitory protein long (FLIPL) to form an "inhibitory" caspase-8–FLIPL heterodimer (Lu et al. 2011; Oberst et al. 2011; Rebe et al. 2007). While caspase-8 is inactivated by a pancaspase inhibitor z-VAD or caspase-8 knockout, the proteolytic inactivation of RIP1 and RIP3 is prevented and the inhibitory effect of caspase-8 on necroptosis is abolished. These are confirmed by in vitro and in vivo experiments. In antigen-activated T cells, the ablation of caspase-8 or FADD can lead to RIP1-dependent necroptosis (Bell et al. 2008; Ch'en et al. 2008). Similarly, caspase-8- and FADD-deficient mice demonstrated severe embryonic phenotype including cardiac malformations and hemorrhage mediated by RIP1- and RIP3-dependent necroptosis (Gunther et al. 2013; Kaiser et al. 2011). Interestedly, caspase-8/RIP1 or caspase-8/RIP3 double knockout mice are viable and survive into fertile adults; RIP1 or RIP3 knockout rescues embryonic lethality phenotype observed in caspase-8- or FADD-deficient mice (Kaiser et al. 2011; Oberst et al. 2011; Zhang et al. 2011a). These experiments demonstrate the regulation of caspase-8 and FADD on RIP1- and/or RIP3-mediated necroptosis.

FLIP has a similar structure to caspase-8, but lacks the catalytic activity. FLIP may be involved in the regulation of necroptotic cell death via RIP kinase, as FLIP knockout mice have a similar phenotype to that of caspase-8- and FADD-deficient mice (Yeh et al. 2000). However, in a recent study, RIP3 ablation rescued embryonic phenotype of FADD-deficient mice, but did not rescue the phenotype of FLIP-deficient mice (Dillon et al. 2012), suggesting distinct functions of FLIP from FADD and caspase-8 on the regulation of necroptosis. Surprisingly, embryos at E9.5–E10 in the FLIP and RIP3 double knockout mice display obvious apoptotic cell death (Dillon et al. 2012), demonstrating the antiapoptotic activity of FLIP on FADD-/caspase-8-mediated apoptosis. Therefore, in the absence of FLIP, caspase-8

may form homodimers to activate apoptotic pathways and result in cellular apoptosis; in the presence of FLIP, the heterodimer of caspase-8 and FLIP forms a complex with FADD to prevent RIP kinase-dependent necroptosis (Dillon et al. 2012; Feoktistova et al. 2011). There is constant interplay between death and survival complexes, which compete with each other until one eventually dominates and determines the cell's fate (Declercq et al. 2009). The regulation of FADD/caspase-8/FLIP on necroptosis and apoptosis demonstrates that various death pathways are closely intertwined, and cellular processes are in tight control of each other.

2.2.2 Deubiquitinase (CYLD/A20/Cezanne) and Ubiquitinase (IAPs/MKRN1)

Protein ubiquitylation and deubiquitylation are important processes to regulate numerous signal pathways and proteins. Ubiquitylation status of RIP1 determines the type of RIP1-mediated cell death. When RIP1 is recruited to TNFR1, RIP1 is heavily modified by ubiquitination; however, ubiquitinated RIP1 was not found in the RIP1–RIP3 complex (Cho et al. 2009; Teng et al. 2005). The different ubiquitination patterns imply that RIP1 is deubiquitinated in the process of necroptosis. Therefore, the enzymes for ubiquitination and deubiquitination of RIP1 protein are the regulators of necroptosis.

CYLD is a lysine-63 (K63)-specific deubiquitinase, which was identified as an important regulator of necroptosis in a genome-wide RNAi screen (Hitomi et al. 2008). Knockdown of CYLD inhibits TNF-α-induced necroptosis in cultured human Jurkat cells (Hitomi et al. 2008), which suggests that CYLD may be responsible for the deubiquitination of RIP1 during necroptosis. A recent study confirmed that RIP1 is a substrate of CYLD (Wright et al. 2007). Consistent with this, CYLD promotes assembly of the RIP1–FADD–caspase-8 complex in response to TNF-α treatment, indicating that deubiquitination of RIP1 by CYLD is an essential step in this process (Wang et al. 2008). CYLD was recently found to be cleaved by caspase-8 and thereby prevents necroptosis in mouse embryonic fibroblasts (MEFs) upon TNF-α stimulation; however, overexpression of a non-cleavable CYLD mutant rapidly promotes necroptotic cell death in response to TNF-α treatment (O'Donnell et al. 2011), which connects CYLD, caspase-8, and necroptosis together. Therefore, RIP1 polyubiquitylation may hinder the recruitment of downstream proteins of necroptosis such as RIP3; CYLD facilitates necroptosis by removal of the polyubiquitin chains on RIP1. The positive regulation of CYLD on necroptosis is also controlled by caspase-8 activity, a negative regulator of necroptosis. Similar to CYLD, ubiquitin-editing enzymes A20 and Cezanne are also reported to deubiquitinate RIP1 protein (Enesa et al. 2008; Shembade et al. 2010).

2.2.3 cIAPs

Inhibitors of apoptosis (IAPs) characterized by a variable number of highly conserved baculoviral IAP repeat motifs including cellular inhibitor of apoptosis protein 1/2 (cIAP1/2) and X-linked inhibitor of apoptosis protein (XIAP) have been thought to function primarily to inhibit apoptosis. A "Really Interesting

New Gene" (RING) domain with ubiquitin protein ligase (E3) activity in the C-terminal of IAPs implies that IAPs are multifunctional proteins to regulate the modification of its target protein via ubiquitylation. Recent studies demonstrate that IAPs also regulate RIP1 ubiquitination and RIP1-dependent necroptotic cell death (Bertrand et al. 2008; McComb et al. 2012; Tenev et al. 2011). IAPs can directly conjugate polyubiquitin chains to RIP1 and block RIP1-dependent ripoptosome formation containing the core components RIP1, FADD, and caspase-8 by targeting the components of ripoptosome for ubiquitylation and degradation. Deletion of IAPs enhances ripoptosome formation and the association of RIP1 and caspase-8, resulting in ripoptosome-mediated apoptosis or necroptosis (Tenev et al. 2011). In tumor cells, cIAP1 inhibition by its antagonists or RNA interference facilitates TNF-induced necroptosis (He et al. 2009; Vanlangenakker et al. 2011). Consistent with this, McComb et al. found that cIAPs also regulate RIP3 via ubiquitination to inhibit the RIP1–RIP3 death complex, and reduced cIAP activity in vivo by the exposure to SMAC mimetics or cIAP1/2 knockout leads to elevated macrophage necroptotic cell death (McComb et al. 2012). Therefore, cIAPs are important targets for preventing apoptosis and necroptosis in acute and chronic diseases or inducing cell death in cancer therapy.

2.2.4 Ubiquitinase on FADD–Makorin Ring Finger Protein 1 (MKRN1) E3 Ligase

A recent study shows that FADD regulates cell death and survival via a ubiquitination-induced degradation pathway (Lee et al. 2012). Makorin Ring Finger Protein 1 (MKRN1) E3 ligase, a ubiquitinase, ubiquitinates FADD; ubiquitinated FADD is subjected to degradation through a proteasomal pathway. The knockdown of MKRN1 increases the stability of FADD and facilitates caspase-8- and caspase-3-mediated apoptosis in response to death signals; however, the overexpression of MKRN1 suppresses extrinsic apoptosis by increasing FADD degradation (Lee et al. 2012). In the presence of caspase inhibitors, MKRN1 knockdown significantly increases RIP1–RIP3–FADD complex formation and enhances necroptotic cell death upon TNF-α and z-VAD treatment (Lee et al. 2012). This suggests that ubiquitinase MKRN1 also regulates apoptosis and necroptosis via the ubiquitination and degradation of FADD.

2.2.5 Polo-Like Kinase 1 (Plk1) and Aurora-A Kinase (Aur-A)

Plk1 and Aur-A are two important mitotic kinases in the regulation of multiple mitotic processes (Macurek et al. 2009). A recent study has shown that Plk1 and Aur-A are involved in the phosphorylative modification of FADD and cooperatively phosphorylate FADD at S194 and S203, respectively (Jang et al. 2011). The double-phosphorylated FADD results in its dissociation from RIP1, which is activated to necroptotic cell death; however, the single-phosphorylated FADD cannot induce cell death, suggesting that double phosphorylation of FADD is critical for RIP1-dependent necroptosis (Jang et al. 2011). Therefore, inhibition of double phosphorylation of FADD by mitotic kinases Plk1 and Aur-A may block necroptosis. In contrast, another study indicates that the small-molecule BI2536, an

inhibitor of Plk1, increases PARP-1-mediated necroptosis (Deeraksa et al. 2013). This inconsistency of two studies may be explained by the specificity of small molecules on Plk1 and the difference of cell lines. However, both studies demonstrate the involvement of mitotic kinase Plk1 on the regulation of necroptosis. More information on how Plk1 regulates RIP1-dependent necroptosis should be investigated in future studies.

2.3 Downstream Signals of Necroptosis

While the upstream signaling of RIP1–RIP3 and RIP1 ubiquitination are important to determine how necroptosis is initiated, the identification of downstream components is necessary to determine how necroptosis is executed. Caspases are the executioners for apoptotic cell death; however, these proteases have no positive role in necroptosis (Pop et al. 2011). Thus far, an analogous class of executioner proteins has not been identified for necroptosis. Some downstream signals relevant to necroptosis are reported, and this information may provide clues for future studies to unveil necroptosis.

2.3.1 Mixed Lineage Kinase Domain-Like Protein (MLKL)

MLKL belongs to the protein kinase superfamily, since a kinase domain is identified within the C-terminus of the protein; however, its function and substrates are still unknown. Recently, two research groups independently demonstrated that MLKL is a substrate of RIP3 and the direct downstream signal of RIP1–RIP3 complex-mediated necroptosis (Sun et al. 2012; Zhao et al. 2012). Sun and colleagues identified MLKL as an interacting protein of RIP3, by immunoprecipitation and mass spectrometry analysis (Sun et al. 2012). Knockdown of MLKL prevents the enlarged RIP3 punctae during necrosis progress and arrests necrosis at a specific step, which is an effect identical to that caused by necrosulfonamide, a newly identified necroptosis inhibitor and MLKL inhibitor (Sun et al. 2012). Their further findings show that the interaction of MLKL and RIP3 is initiated by autophosphorylation of RIP3, and then two critical residues of MLKL for necrosis are phosphorylated at threonine 357 and serine 358 by RIP3 (Sun et al. 2012). Zhao et al. also demonstrated that MLKL is a key downstream mediator of necrosis directly regulated by RIP3 kinase (Zhao et al. 2012). By a kinase/phosphatase shRNA library screening in a human colon adenocarcinoma cell line, they found that a lack of MLKL blocks TNF-induced necrosis but does not affect the interaction of RIP1 and RIP3, suggesting that MLKL is the downstream of RIP1–RIP3 complex (Zhao et al. 2012). Further findings indicate that MLKL is recruited to RIP1–RIP3 complex by interacting with RIP3, but not mutant RIP3 without kinase activity (Zhao et al. 2012), which is consistent with Sun et al.'s results (Sun et al. 2012). Although MLKL is proven to be directly downstream of RIP3, the downstream of signals of MLKL remains elusive.

2.3.2 PGAM5

PGAM5 is a mitochondrial protein phosphatase in the outer membrane of the mitochondria. A recent study found that PGAM5 coprecipitates with RIP3 under necroptosis-inducing conditions (Wang et al. 2012). More importantly, RIP3 phosphorylates PGAM5 and consequently increases PGAM5 phosphatase activity (Wang et al. 2012), suggesting that PGAM5 is also the substrate of RIP3. Knockdown of either splice variant of PGAM5 (PGAM5L or PGAM5S) by siRNA reduces necroptosis induced by different stimuli, indicating that both isoforms of PGAM5 are essential for necroptosis; however, only PGAM5S phosphorylation is dependent on the presence of MLKL and is blocked by the MLKL inhibitor necrosulfonamide (Wang et al. 2012). These findings suggest that PGAM5S is also recruited to the RIP1–RIP3–MLKL complex. Further study indicates that PGAM5S is phosphorylated after the necrosome (RIP1, RIP3, MLKL, and PGAM5L) is shifted to a more hydrophobic environment that contains dynamin-related protein 1 (DRP1), the GTPase that controls mitochondrial fission. Interestingly, knockdown of DRP1 prevents necroptosis, and knockdown of PGAM5S attenuates DRP1 activation (Wang et al. 2012), demonstrating that DRP1 is required for necroptosis and is regulated by PGAM5S. Therefore, PGAM5S is also a substrate and downstream signal of RIP1–RIP3 complex, and DRP1 may be one of the executioners in the necroptotic pathways.

2.3.3 Poly(ADP-Ribose) Polymerase-1/Apoptosis-Inducing Factor (PARP-1/AIF) Pathway

PARP is a family of nuclear enzymes that catalyze poly(ADP-ribosyl)ation reaction to modulate other proteins to maintain chromatin structure, transcription, and genomic integrity (Krishnakumar and Kraus 2010). It is widely known that PARP-1 activation mediates AIF translocation from the mitochondria to nucleus, resulting in caspase-independent cell death (Andrabi et al. 2008; Hong et al. 2004; van Wijk and Hageman 2005). Since the concept of programmed necrosis is accepted, PARP-1-/AIF-mediated caspase-independent cell death has begun to be regarded as necroptosis (Delavallee et al. 2011). However, how the RIP1–RIP3 complex in cytosol signals to PARP-1 in the nucleus is still unclear. In our previous studies, the relationship between PARP-1/AIF and necroptosis by using a hippocampal cell line HT-22 was explored. Glutamate-induced oxidative stress causes a caspase-3-independent necrosis-like cell death in HT-22 cells; the necroptosis-specific inhibitor necrostatin-1 almost completely blocks this kind of cell death (Xu et al. 2007). Necrostatin-1 not only prevents glutamate-induced glutathione depletion in HT-22 cells but also blocks the translocation of AIF from the mitochondria to nucleus (Xu et al. 2007). The effect of necrostain-1 on the AIF upstream signal PARP-1 activity is further examined, and it is found that necrostain-1 inhibits glutamate-induced PARP-1 activation but not total expression. However, necrostain-1 cannot directly inhibit PARP-1 activation induced by the DNA-alkylating agent MNNG (Xu et al. 2010a), indicating that PARP-1-/AIF-mediated signal pathway in HT-22 cell is

downstream of necroptosis and that necrostain-1 targets upstream of PARP-1/AIF, which is consistent with the fact that necrostain-1 inhibits RIP1 activity and blocks the interaction of RIP1 and RIP3 (He et al. 2009). Increasing evidences demonstrate that PARP-1/AIF signal pathway is involved in the downstream signals of necroptosis and may be one of the executioner proteins for necroptosis (Cabon et al. 2012; Deeraksa et al. 2013; Dunai et al. 2012; Jouan-Lanhouet et al. 2012). The mechanisms attending PARP-1/AIF regulation of necroptosis need further investigation.

2.3.4 Mitochondrial Targets

The mitochondrial protein families are involved in the regulation and execution of apoptosis; however, many mitochondrial proteins have been recently found to function in necroptosis. Adenine nucleotide translocase (ANT) and cyclophilin D (CypD), two important components of the mitochondrial permeability transition, regulate the exchange of ADP and ATP between the cytosol and the mitochondria (Halestrap and Brenner 2003). TNF-α and z-VAD-fmk treatment results in a mitochondrial defect in ADP/ATP exchange through the inhibition of ANT activity, which is dependent on RIP1 activation (Temkin et al. 2006). Mice lacking CypD are more resistant to cardiac ischemia and reperfusion injury than control animals and have reduced incidence of necrotic cell death (Nakagawa et al. 2005), suggesting that mitochondrial proteins such as ANT and CypD are important players in necroptosis and that mitochondria functions are also influenced in necroptosis.

Because mitochondria are the major producers of reactive oxygen species (ROS), which are important in executing necroptotic cell death (Festjens et al. 2006b; Moquin and Chan 2010), it is conceivable that RIP1 may directly or indirectly target the mitochondria. Treatment with butylated hydroxyanisole (BHA), a ROS scavenger, inhibits necroptosis in many cell types (Festjens et al. 2006a; Lin et al. 2004). RIP1 and RIP3 act on upstream signals to regulate ROS production during necroptosis. A recent study shows that RIP3 interacts with several mitochondrial enzymes including glycogen phosphorylase (PYGL), glutamate–ammonia ligase (GLUL), and glutamate dehydrogenase 1 (GLUD1) and leads to TNF-/z-VAD-induced ROS production. RIP3 overexpression activates these enzymes and enhances mitochondrial energy metabolism, a process that correlates with enhanced ROS production and necroptosis (Zhang et al. 2009). In contrast, RIP3 deficiency results in reduced ROS production and necroptotic cell death (Cho et al. 2009). Thus, the mitochondria and mitochondrial proteins are important targets for RIP3 or RIP1–RIP3 complex in necroptotic cell death.

2.3.5 JNK/ERK

In a siRNA screen for searching the regulators of necroptosis, transcription factors c-Jun and Sp1 are identified. However, both transcription factors can be activated by JNK signaling (Chuang et al. 2008; Coffey et al. 2002; Hitomi et al. 2008), suggesting that JNK may be downstream of necroptosis (Vanlangenakker et al. 2012). Biton and Ashkenazi recently reported that RIP1 is activated and further

activates NF-κB in the early-phase response upon extensive DNA damage; however, in the absence of caspase activation, RIP1 activates JNK3 pathway in the late-phase response, resulting in the activation of the transcription factor AP-1 and FADD-mediated caspase-8 activation (Biton and Ashkenazi 2011). As discussed above, MLKL contains a kinase-like domain which is similar to the mixed lineage kinase that functions as MAPKKs in the JNK pathway (Gallo and Johnson 2002). Therefore, after activation of MLKL by RIP3, MLKL may activate the downstream JNK pathway to execute necroptotic cell death. This is confirmed by Zhao et al.'s findings that MLKL is required for the late-phase activation of JNK in TNF-induced necroptosis in human colon adenocarcinoma HT-29 cells (Zhao et al. 2012). Although current evidences demonstrate the involvement of JNK in the downstream of necroptosis, more studies on its role in necroptosis are needed.

3 Cellular Markers of Necroptosis

For apoptosis and autophagy, many markers were used for the in situ detection in vitro and in vivo, including active caspase-3, cleaved PARP, DNA ladders, Beclin-1 and, microtubule associated protein 1 light chain 3 (Klionsky et al. 2007; Sarkar and Li 2006). Thus far, no specific morphological or biochemical markers are available for the detection of necroptosis. Regardless, many necrotic biomarker candidates and methods for the determination of necroptosis have been proposed recently.

Because the cellular nuclei, mitochondria, lysosomes, and plasma membrane are ruptured upon lethal stimuli on cells, intracellular cytoplasmic contents are released to extracellular spaces. Released proteins, such as high-mobility group box 1 protein (HMGB1), cyclophilin A (CypA), and cytokeratin-18, are regarded as candidate biomarkers of necroptosis (Christofferson and Yuan 2010; Kramer et al. 2004; Scaffidi et al. 2002). HMGB1 protein is a nuclear factor that acts as an architectural chromatin-binding factor, promoting protein assembly on specific DNA targets. When released from the cell, HMGB1 interacts with the receptors for advanced glycation end products and becomes a potent mediator of inflammation, signaling the demise of individual cells to its neighbors (Scaffidi et al. 2002). Since apoptotic cells do not release HMGB1 and fail to promote inflammation (Scaffidi et al. 2002), therefore, released HMGB1 is used to distinguish necrotic cell death from apoptotic cell death. However, its specificity is compromised because HMGB1 can also be actively secreted from immune cells or passively released from cells dying by secondary necrosis following apoptosis (Kazama et al. 2008; Scaffidi et al. 2002). Similar to HMGB1, CypA, a cytosolic peptidyl-prolyl *cis–trans* isomerase, is released in the early stage of necroptosis due to early permeabilization of the plasma membrane (Christofferson and Yuan 2010). Like HMGB1, CypA is also actively secreted; therefore, CypA release may combine with other methods together to determine the existence of necroptosis (Christofferson and Yuan 2010). Intact soluble cytokeratin-18 is released from necrotic cells; but cleaved cytokeratin-18 by caspases is released from apoptotic cells. The ratio of cleaved cytokeratin-18 to

intact cytokeratin-18 in the blood has been proposed as a marker to determine the extent of both types of cell death in patients with endometrial tumors (Kramer et al. 2004). Because not only necrotic cells contribute to intact cytokeratin-18 in the blood, this ratio may provide quantitative and qualitative information in those patients who have a significantly high level of cytokeratin-18 (Linder et al. 2010).

The combination of Tdt-mediated dUTP nick end labeling (TUNEL) staining and cleaved caspase-3 staining has been used recently to determine cell death types in dying cells (Gunther et al. 2011; Welz et al. 2011). Those cells with positive TUNEL staining and positive cleaved caspase-3 staining are considered as apoptotic cells; on the contrary, those cells with positive TUNEL staining and negative cleaved caspase-3 staining are considered as necroptotic cells. Since necroptotic cell death cannot be easily determined using a single method, it should be identified by a combination of methods.

4 Inhibitors and Interventions for Necroptotic Cell Death

Necroptosis is an important cellular death mechanism likely to be involved in human pathology, from viral infections to neurodegenerative disorders. Necroptotic features are commonly observed in a wide range of human pathologies, including stroke, myocardial infarction, brain trauma, and possibly some forms of neurodegeneration. Therefore, inhibition of necroptotic cell death by specific small-molecule inhibitors, necrostatins, may represent an exciting new direction for therapy.

4.1 Nec-1 and Its Analogs

Nec-1, a specific inhibitor of necroptosis, acts by inhibiting RIP1 kinase activity (Degterev et al. 2005). Nec-1 has become an important tool for evaluating the contribution of necrotic cell death in vitro and in many experimental animal models in vivo. By using Nec-1, necroptosis has been found in a wide range of cell death paradigms, including ischemic brain injury (Degterev et al. 2005; Xu et al. 2007). Nec-1 shows promising neuroprotection on cerebral ischemia injury for the reduction of infarct volume and the improvement of neurological deficits in middle cerebral artery occlusion (MCAO) mouse models (Degterev et al. 2005; Gu et al. 2010), suggesting that necroptosis is an alternative mechanism to activate delayed ischemic injury, due to its slow-onset kinetics. Nec-1 reduced acute cellular injury, brain tissue damage, motor dysfunction, and spatial learning deficits after controlled cortical impact (CCI) in mice; therefore, Nec-1 and other necrostatins may be promising therapeutic agents to reduce the pathological and functional impairment of traumatic brain injury (You et al. 2008). Moreover, Nec-1 rescues retinal neurons in the inner retinal layers, attenuates retinal degeneration, and reduces functional impairments after retinal ischemic injury (Rosenbaum et al. 2010).

Since the discovery of Nec-1, several structurally distinct Nec-1 analogs for necroptosis blockage have been developed: Nec-3 (Teng et al. 2008), Nec-4

(Teng et al. 2007), Nec-5 (Wang et al. 2007), and Nec-7 (Zheng et al. 2008). Nec-1, Nec-3, Nec-4, and Nec-5 all inhibit RIP1 kinase; however, Nec-7 does not, suggesting that Nec-7 may target a different regulatory pathway from Nec-1.

4.2 Necrosulfonamide

Like Nec-1, necrosulfonamide (molecular weight, 461) – another newly identified specific inhibitor of necroptosis – was selected by screening a chemical library of 200,000 compounds (Sun et al. 2012). Unlike Nec-1 which blocks the interaction of RIP1 and RIP3, necrosulfonamide specifically inhibits necroptosis by targeting MLKL, a key signaling molecule from the mitochondria in the necroptotic cell death pathway, downstream of the active RIP3 (Sun et al. 2012). Necrosulfonamide can block necroptosis at a specific step in different cells such as Jurkat cells, HeLa cells, and mouse 3T3 cells in response to TNF-α stimuli, by binding to the N-terminal of MLKL and covalently modifying MLKL at the reactive amino acid residue cysteine 86 (Sun et al. 2012). Moreover, necrosulfonamide also blocks staurosporine-induced primary necrosis in the presence of a caspase inhibitor in U937 cells (Dunai et al. 2012). Whether necrosulfonamide has inhibitory effects on necroptosis in different disease models in vivo or in vitro is unknown and needs further studies.

4.3 Geldanamycin

Because RIP1 has a critical role in RIP1–RIP3 complex formation and RIP1–RIP3-dependent necroptosis, RIP1 or RIP3 may be promising new targets for neuroprotection in neurological or other pathological disorders. Our recent study demonstrates that degradation of RIP1 offers an intriguing possibility to rescue necroptotic cells from death induced by oxygen–glucose deprivation (OGD) in the presence of the caspase inhibitor z-VAD (Chen et al. 2012b). RIP1 has low expression in normal cortical neurons, but OGD and z-VAD treatment significantly increases RIP1 expression and induces neuronal necroptosis. However, geldanamycin decreases RIP1 level and rescues OGD- and z-VAD-induced necroptosis (Chen et al. 2012b). Further study indicates that geldanamycin promotes the reduction of heat shock protein (HSP90), which is known to decrease the stability of RIP1 and facilitate the degradation of RIPK1 (Lewis et al. 2000). Therefore, geldanamycin and its analogs may be used for the treatment of stroke or other neurological disorders, by targeting the degradation of RIP1.

5 Necroptotic Cell Death in Neurological Diseases

5.1 Stroke

Stroke is the third leading cause of death and a leading cause of serious long-term disability. Due to the heavy economic and social burden caused by stroke

patients, an understanding of the pathophysiology of stroke is regarded as an urgent and critical research topic in many countries. Recent in vitro and in vivo studies demonstrated that necroptosis is an important process that contributes to neuronal cell death and functional impairment after cerebral ischemic injury (Chen et al. 2012b; Degterev et al. 2005; Meloni et al. 2011; Xu et al. 2010b).

Degterev et al. showed that the specific and potent inhibitor of necroptosis, Nec-1, when administered by intracerebroventricular injection along with the caspase inhibitor z-VAD, significantly reduces infarct volume and improves neurological functions in an MCAO animal model (Degterev et al. 2005). These findings imply that Nec-1 may protect against brain injury by blocking necroptosis when neuronal apoptosis is inhibited by caspase inhibitor z-VAD. Consistent with this, our findings indicate that the combination of apoptosis inhibitor humanin and necroptosis inhibitor Nec-1 provides synergistic protective effects on cerebral infarction and neurological function in mice (Xu et al. 2006; Xu et al. 2010b). By using an in vitro OGD ischemia model, a recent study demonstrated RIP1-dependent necroptosis induced by z-VAD treatment; geldanamycin, which promotes the degradation of RIP1, rescues OGD- and z-VAD-induced necroptotic cell death in culture cortical neurons (Chen et al. 2012b). A recent study with cell death biomarkers also identified that different forms of cell death including necroptosis exist in both acute and delayed OGD models (Meloni et al. 2011). These studies imply that necroptosis is a potential therapeutic target for the treatment of stroke.

5.2 Neonatal Hypoxic–Ischemic Encephalopathy (HIE)

Neonatal HIE is the most common cause of perinatal brain injury, resulting from pulmonary and/or cardiac dysfunction. Because of the similarity, rodent neonatal hypoxia–ischemia (HI) injury models are used to study the mechanisms of HIE (Ashwal and Pearce 2001). West et al. (West et al. 2006) demonstrates that caspase-3-deficient mice have greater tissue loss than controls after cerebral HI injury; caspase-3 knockout results in increased PARP-1-mediated caspase-independent cell death following HI. By using the same animal model, Northington et al. (Northington et al. 2011) found that Nec-1 significantly decreases brain injury in the forebrain and thalamus and decreases necrotic cell death, suggesting the existence of necroptosis in HI brain injury. They also showed that HI brain injury promotes necroptosis by increasing RIP1–RIP3 complex formation and inhibiting RIP3–FADD interaction and that mitochondrial dysfunction in neurons and astrocytes following neonatal hypoxia–ischemia is prevented by RIP1 activity inhibition (Chavez-Valdez et al. 2012; Northington et al. 2011). Therefore, the protection of necrostatin-1 on HI brain injury may represent a therapeutic strategy and new direction for therapy of neonatal HI.

5.3 Trauma

A recent study also demonstrates that RIP1-/RIP3-dependent necrosis appears to be involved in the mechanism of cell death and neurological dysfunction after traumatic brain injury (You et al. 2008). Necrostatin-1 administration significantly reduces brain lesion volume and motor deficits after controlled cortical impact in a trauma animal model but also improves spatial memory acquisition by Morris water maze performance. However, Nec-1 did not reduce caspase-3-positive cells in dentate gyrus or cortex, indicating that Nec-1 is not an apoptosis inhibitor, but a necroptosis inhibitor (You et al. 2008).

5.4 Huntington's Disease

Huntington's disease is a neurodegenerative genetic disorder characterized by movement abnormalities, psychiatric symptoms, and cognitive deficits. Zhu et al. (Zhu et al. 2011) recently examined the role of necroptosis in Huntington's disease by using Nec-1 as tool in immortalized striatal ST14A cells and R6/2 transgenic mice. In ST14A cells stably expressing mutant Htt fragment (N548-128Q), a cell model of Huntington's disease, the pancaspase inhibitor z-VAD induces caspase-3-independent cell death. Caspase-8-specific inhibitor (IETD-fmk), but not caspase-3- and caspase-9-specific inhibitor, induces a similar cell death as z-VAD in ST14A cells. Nec-1 almost completely rescues this kind of cell death and inhibits RIP1 cleavage induced by z-VAD or caspase-8 inhibitor IETD-fmk, supporting the notion that Nec-1 blocks RIP1-dependent necroptosis in ST14A cells (Zhu et al. 2011). In the R6/2 transgenic mouse model of Huntington's disease, Nec-1 treatment significantly delays the onset of Huntington's disease as well as behavioral deterioration and extends the life span, suggesting the involvement of necroptosis in the pathogenesis of Huntington's disease (Zhu et al. 2011). This study implies that necroptosis may be involved in other neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease. Moreover, Nec-1 may be a promising protector in these diseases.

5.5 Glutamate Toxicity

Glutamate excitotoxicity is linked to neurological disorders such as stroke, Parkinson's disease, Huntington's disease, and Alzheimer's dementia (Beal 1992; Lau and Tymianski 2010). Glutamate excitotoxicity also causes neuronal death in cultured primary neurons in vitro (Chen et al. 2012a; Zhang et al. 2011b). Nec-1, as a necroptosis inhibitor and in the absence of caspase inhibitors, protects against glutamate analog *N*-methyl-D-aspartate (NMDA)-induced cell death in primary cortical neurons (Li et al. 2008). In this study, Nec-1 partially blocked NMDA effects, suggesting necroptotic involvement in NMDA-induced cell death. However, necroptosis only occupies a small portion of cell death without the induction of

caspase inhibitors. Non-receptor-mediated glutamate toxicity causes necroptosis in neuronal cells, as well (Xu et al. 2007; Xu et al. 2010a). In a hippocampal HT-22 cell line that has no ionotropic glutamate receptor, again, glutamate produces caspase-3-independent cell death. Nec-1 almost completely blocks glutamate-induced cell death, suggesting the involvement of necroptosis in glutamate-induced cell death in HT-22 cells (Xu et al. 2007). Further study indicates that the PARP-1/AIF pathway mediates necroptosis induced by glutamate in HT-22 cells (Xu et al. 2007; Xu et al. 2010a). These in vitro studies imply that glutamate toxicity may cause necroptosis in many neurological disorders, thus providing a new direction for treatments.

6 Conclusion

Necroptosis is a recently identified regulated cell death that has the morphological features of necrosis. Investigation into the regulation and signaling pathways of necroptosis represents one of the hot topics in cell death research. Recent findings throw light on the importance of necroptotic cell death in normal development, immunity, inflammation, cancer, and neurological disorders (Han et al. 2011; Vanlangenakker et al. 2012; Yuan and Kroemer 2010). An explosion of recent articles begins to unravel the mysteries centering on the necroptotic process. In response to external or internal stimuli, RIP1 and/or RIP3 are the determining factors at the central of the regulation platform of necroptosis to function as the switches between apoptosis to necroptosis. Pro-apoptotic proteins such as caspase-8, FLIPL, and FADD negatively regulate RIP1–RIP3 complex formation and inhibit the process of necroptosis. However, some factors such as CYLD, which deubiquitinates or modifies RIP1 and induces RIP1–RIP3 complex formation, positively promote necroptotic cell death. Although the complete signaling pathway remains unclear, recent advances have shown that mitochondrial enzymes MLKL and PGAM5 are directly downstream from RIP3 activation and that DRP1 may be one of the executioners of necroptosis. Due to complicated signaling pathways in the cells, more investigations are needed to remove the veil over this cell death process. The unraveling of the mechanisms governing necropototic cell death will lead to a better understanding of the interplay of necroptosis and other forms of cell death and the identification of new clinical targets.

Because of the lack of clear and distinctive biomarkers for necroptosis, it is difficult to determine the existence of necroptosis in animal disease models. In addition, it is hard to tell whether necroptosis is the only form of necrotic cell death. More specifically biomarkers need to be developed to effectively discern necroptosis in vivo. Thus far, only Nec-1 and NSA are considered as specific inhibitors for necroptosis – used as tools to examine necroptosis. Because these inhibitors may also block other cell death processes, it is possible that there is an overestimate of the portion of cell death deemed to be necroptosis.

Recent studies have examined the existence of necroptotic cell death in many neurological disorders such as stroke, trauma, and Huntington's disease. Because no specific biomarker is available, most studies used the neuroprotection of Nec-1

on cell death as the evidence for necroptosis. In addition, it remains difficult to understand the contribution to neurological pathogenesis; how great a percentage of necroptosis occurring in the pathology of neurological disorders is still difficult to estimate. The extensive studies on necroptosis hopefully will lead to the identification of specific necrotic biomarkers to resolve the many questions. Because caspase inhibitors promote necroptosis in vitro, does necroptosis play a critical role in the pathology of neurological disorders under the circumstances wherein there is no caspase inhibition? This remains an unanswered question. Recent studies demonstrate that mitochondrial dysfunction is involved in the mechanisms of necroptosis (Sun et al. 2012; Wang et al. 2012; Zhang et al. 2009; Zhao et al. 2012) and that mitochondrial dysfunction contributes to the pathophysiology of acute neurological disorders such as stroke and neurodegenerative disorders such as Parkinson's disease (Perez-Pinzon et al. 2012; Schon and Przedborski 2011; Vosler et al. 2009). Therefore, the connection between necroptosis and neuronal cell death in these disorders is in need of further investigation. Because of its potential involvement in neurological disorders, necroptosis has become a potential therapeutic target for these disorders. A clearer understanding of necroptotic mechanisms will provide new targets and exciting opportunities to develop more potent neuroprotective agents such as Nec-1 or necrosulfonamide for clinical treatments of neurological disorders.

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References

Andrabi, S. A., Dawson, T. M., & Dawson, V. L. (2008). Mitochondrial and nuclear cross talk in cell death: Parthanatos. *Annals of the New York Academy of Sciences, 1147*, 233–241.

Ashwal, S., & Pearce, W. J. (2001). Animal models of neonatal stroke. *Current Opinion in Pediatrics, 13*, 506–516.

Beal, M. F. (1992). Role of excitotoxicity in human neurological disease. *Current Opinion in Neurobiology, 2*, 657–662.

Bell, B. D., Leverrier, S., Weist, B. M., Newton, R. H., Arechiga, A. F., Luhrs, K. A., Morrissette, N. S., & Walsh, C. M. (2008). FADD and caspase-8 control the outcome of autophagic signaling in proliferating T cells. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 16677–16682.

Bertrand, M. J., Milutinovic, S., Dickson, K. M., Ho, W. C., Boudreault, A., Durkin, J., Gillard, J. W., Jaquith, J. B., Morris, S. J., & Barker, P. A. (2008). cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. *Molecular Cell, 30*, 689–700.

Biton, S., & Ashkenazi, A. (2011). NEMO and RIP1 control cell fate in response to extensive DNA damage via TNF-alpha feedforward signaling. *Cell, 145*, 92–103.

Bodmer, J. L., Holler, N., Reynard, S., Vinciguerra, P., Schneider, P., Juo, P., Blenis, J., & Tschopp, J. (2000). TRAIL receptor-2 signals apoptosis through FADD and caspase-8. *Nature Cell Biology, 2*, 241–243.

Cabon, L., Galan-Malo, P., Bouharrour, A., Delavallee, L., Brunelle-Navas, M. N., Lorenzo, H. K., Gross, A., & Susin, S. A. (2012). BID regulates AIF-mediated caspase-independent necroptosis by promoting BAX activation. *Cell Death and Differentiation, 19*, 245–256.

Ch'en, I. L., Beisner, D. R., Degterev, A., Lynch, C., Yuan, J., Hoffmann, A., & Hedrick, S. M. (2008). Antigen-mediated T cell expansion regulated by parallel pathways of death. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 17463–17468.

Challa, S., & Chan, F. K. (2010). Going up in flames: Necrotic cell injury and inflammatory diseases. *Cellular and Molecular Life Sciences, 67*, 3241–3253.

Chavez-Valdez, R., Martin, L. J., Flock, D. L., & Northington, F. J. (2012). Necrostatin-1 attenuates mitochondrial dysfunction in neurons and astrocytes following neonatal hypoxia-ischemia. *Neuroscience, 219*, 192–203.

Chen, T., Fei, F., Jiang, X. F., Zhang, L., Qu, Y., Huo, K., & Fei, Z. (2012a). Down-regulation of Homer1b/c attenuates glutamate-mediated excitotoxicity through endoplasmic reticulum and mitochondria pathways in rat cortical neurons. *Free Radical Biology & Medicine, 52*, 208–217.

Chen, W. W., Yu, H., Fan, H. B., Zhang, C. C., Zhang, M., Zhang, C., Cheng, Y., Kong, J., Liu, C. F., Geng, D., & Xu, X. (2012b). RIP1 mediates the protection of geldanamycin on neuronal injury induced by oxygen-glucose deprivation combined with zVAD in primary cortical neurons. *Journal of Neurochemistry, 120*, 70–77.

Cho, Y. S., Challa, S., Moquin, D., Genga, R., Ray, T. D., Guildford, M., & Chan, F. K. (2009). Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell, 137*, 1112–1123.

Christofferson, D. E., & Yuan, J. (2010). Cyclophilin A release as a biomarker of necrotic cell death. *Cell Death and Differentiation, 17*, 1942–1943.

Chuang, J. Y., Wang, Y. T., Yeh, S. H., Liu, Y. W., Chang, W. C., & Hung, J. J. (2008). Phosphorylation by c-Jun NH2-terminal kinase 1 regulates the stability of transcription factor Sp1 during mitosis. *Molecular Biology of the Cell, 19*, 1139–1151.

Coffey, E. T., Smiciene, G., Hongisto, V., Cao, J., Brecht, S., Herdegen, T., & Courtney, M. J. (2002). c-Jun N-terminal protein kinase (JNK) 2/3 is specifically activated by stress, mediating c-Jun activation, in the presence of constitutive JNK1 activity in cerebellar neurons. *The Journal of Neuroscience, 22*, 4335–4345.

Declercq, W., Vanden Berghe, T., & Vandenabeele, P. (2009). RIP kinases at the crossroads of cell death and survival. *Cell, 138*, 229–232.

Deeraksa, A., Pan, J., Sha, Y., Liu, X. D., Eissa, N. T., Lin, S. H., & Yu-Lee, L. Y. (2013). Plk1 is upregulated in androgen-insensitive prostate cancer cells and its inhibition leads to necroptosis. *Oncogene*. *32*(24), 2973–2983. doi: 10.1038/onc.2012.309. [Epub ahead of print].

Degterev, A., Huang, Z., Boyce, M., Li, Y., Jagtap, P., Mizushima, N., Cuny, G. D., Mitchison, T. J., Moskowitz, M. A., & Yuan, J. (2005). Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nature Chemical Biology, 1*, 112–119.

Delavallee, L., Cabon, L., Galan-Malo, P., Lorenzo, H. K., & Susin, S. A. (2011). AIF-mediated caspase-independent necroptosis: A new chance for targeted therapeutics. *IUBMB Life, 63*, 221–232.

Dillon, C. P., Oberst, A., Weinlich, R., Janke, L. J., Kang, T. B., Ben-Moshe, T., Mak, T. W., Wallach, D., & Green, D. R. (2012). Survival function of the FADD-CASPASE-8-cFLIP(L) complex. *Cell Reports, 1*, 401–407.

Dunai, Z. A., Imre, G., Barna, G., Korcsmaros, T., Petak, I., Bauer, P. I., & Mihalik, R. (2012). Staurosporine induces necroptotic cell death under caspase-compromised conditions in U937 cells. *PLoS One, 7*, e41945. doi:10.1371/journal.pune.0041945. Epub 2012 Jul 31.

Duprez, L., Bertrand, M. J., Vanden Berghe, T., Dondelinger, Y., Festjens, N., & Vandenabeele, P. (2012). Intermediate domain of receptor-interacting protein kinase 1 (RIPK1) determines switch between necroptosis and RIPK1 kinase-dependent apoptosis. *Journal of Biological Chemistry, 287*, 14863–14872.

Edinger, A. L., & Thompson, C. B. (2004). Death by design: Apoptosis, necrosis and autophagy. *Current Opinion in Cell Biology, 16*, 663–669.

Ekshyyan, O., & Aw, T. Y. (2004). Apoptosis: A key in neurodegenerative disorders. *Current Neurovascular Research, 1*, 355–371.

Enesa, K., Zakkar, M., Chaudhury, H., le Luong, A., Rawlinson, L., Mason, J. C., Haskard, D. O., Dean, J. L., & Evans, P. C. (2008). NF-kappaB suppression by the deubiquitinating enzyme Cezanne: A novel negative feedback loop in pro-inflammatory signaling. *Journal of Biological Chemistry, 283*, 7036–7045.

Feoktistova, M., Geserick, P., Kellert, B., Dimitrova, D. P., Langlais, C., Hupe, M., Cain, K., MacFarlane, M., Hacker, G., & Leverkus, M. (2011). cIAPs block Ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms. *Molecular Cell, 43*, 449–463.

Festjens, N., Kalai, M., Smet, J., Meeus, A., Van Coster, R., Saelens, X., & Vandenabeele, P. (2006a). Butylated hydroxyanisole is more than a reactive oxygen species scavenger. *Cell Death and Differentiation, 13*, 166–169.

Festjens, N., Vanden Berghe, T., & Vandenabeele, P. (2006b). Necrosis, a well-orchestrated form of cell demise: Signalling cascades, important mediators and concomitant immune response. *Biochimica et Biophysica Acta, 1757*, 1371–1387.

Festjens, N., Vanden Berghe, T., Cornelis, S., & Vandenabeele, P. (2007). RIP1, a kinase on the crossroads of a cell's decision to live or die. *Cell Death and Differentiation, 14*, 400–410.

Gallo, K. A., & Johnson, G. L. (2002). Mixed-lineage kinase control of JNK and p38 MAPK pathways. *Nature Reviews Molecular Cell Biology, 3*, 663–672.

Gu, X. H., Xu, R., Yuan, G. L., Lu, H., Gu, B. R., & Xie, H. P. (2010). Preparation of chlorogenic acid surface-imprinted magnetic nanoparticles and their usage in separation of traditional Chinese medicine. *Analytica Chimica Acta, 675*, 64–70.

Gunther, C., Martini, E., Wittkopf, N., Amann, K., Weigmann, B., Neumann, H., Waldner, M. J., Hedrick, S. M., Tenzer, S., Neurath, M. F., & Becker, C. (2011). Caspase-8 regulates TNF-alpha-induced epithelial necroptosis and terminal ileitis. *Nature, 477*, 335–339.

Gunther, C., Neumann, H., Neurath, M. F., & Becker, C. (2013). Apoptosis, necrosis and necroptosis: Cell death regulation in the intestinal epithelium. *Gut, 62*(7), 1062–1071. Epub 2012 Jun 11. Review.

Halestrap, A. P., & Brenner, C. (2003). The adenine nucleotide translocase: A central component of the mitochondrial permeability transition pore and key player in cell death. *Current Medicinal Chemistry, 10*, 1507–1525.

Han, J., Zhong, C. Q., & Zhang, D. W. (2011). Programmed necrosis: Backup to and competitor with apoptosis in the immune system. *Nature Immunology, 12*, 1143–1149.

He, S., Wang, L., Miao, L., Wang, T., Du, F., Zhao, L., & Wang, X. (2009). Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. *Cell, 137*, 1100–1111.

Hitomi, J., Christofferson, D. E., Ng, A., Yao, J., Degterev, A., Xavier, R. J., & Yuan, J. (2008). Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell, 135*, 1311–1323.

Holler, N., Zaru, R., Micheau, O., Thome, M., Attinger, A., Valitutti, S., Bodmer, J. L., Schneider, P., Seed, B., & Tschopp, J. (2000). Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nature Immunology, 1*, 489–495.

Hong, S. J., Dawson, T. M., & Dawson, V. L. (2004). Nuclear and mitochondrial conversations in cell death: PARP-1 and AIF signaling. *Trends in Pharmacological Sciences, 25*, 259–264.

Jang, M. S., Lee, S. J., Kang, N. S., & Kim, E. (2011). Cooperative phosphorylation of FADD by Aur-A and Plk1 in response to taxol triggers both apoptotic and necrotic cell death. *Cancer Research, 71*, 7207–7215.

Jouan-Lanhouet, S., Arshad, M. I., Piquet-Pellorce, C., Martin-Chouly, C., Le Moigne-Muller, G., Van Herreweghe, F., Takahashi, N., Sergent, O., Lagadic-Gossmann, D., Vandenabeele, P., Samson, M., & Dimanche-Boitrel, M. T. (2012). TRAIL induces necroptosis involving RIPK1/RIPK3-dependent PARP-1 activation. *Cell Death and Differentiation, 19*, 2003–2014.

Kaiser, W. J., Upton, J. W., Long, A. B., Livingston-Rosanoff, D., Daley-Bauer, L. P., Hakem, R., Caspary, T., & Mocarski, E. S. (2011). RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature, 471*, 368–372.

Kazama, H., Ricci, J. E., Herndon, J. M., Hoppe, G., Green, D. R., & Ferguson, T. A. (2008). Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. *Immunity, 29*, 21–32.

Kelliher, M. A., Grimm, S., Ishida, Y., Kuo, F., Stanger, B. Z., & Leder, P. (1998). The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. *Immunity, 8*, 297–303.

Kerr, J. F., Wyllie, A. H., & Currie, A. R. (1972). Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer, 26*, 239–257.

Kitanaka, C., & Kuchino, Y. (1999). Caspase-independent programmed cell death with necrotic morphology. *Cell Death and Differentiation, 6*, 508–515.

Klionsky, D. J., Cuervo, A. M., & Seglen, P. O. (2007). Methods for monitoring autophagy from yeast to human. *Autophagy, 3*, 181–206.

Kramer, G., Erdal, H., Mertens, H. J., Nap, M., Mauermann, J., Steiner, G., Marberger, M., Biven, K., Shoshan, M. C., & Linder, S. (2004). Differentiation between cell death modes using measurements of different soluble forms of extracellular cytokeratin 18. *Cancer Research, 64*, 1751–1756.

Kreuz, S., Siegmund, D., Rumpf, J. J., Samel, D., Leverkus, M., Janssen, O., Hacker, G., Dittrich-Breiholz, O., Kracht, M., Scheurich, P., & Wajant, H. (2004). NFkappaB activation by Fas is mediated through FADD, caspase-8, and RIP and is inhibited by FLIP. *The Journal of Cell Biology, 166*, 369–380.

Krishnakumar, R., & Kraus, W. L. (2010). The PARP side of the nucleus: Molecular actions, physiological outcomes, and clinical targets. *Molecular Cell, 39*, 8–24.

Lau, A., & Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflügers Archiv, 460*, 525–542.

Lee, E. W., Kim, J. H., Ahn, Y. H., Seo, J., Ko, A., Jeong, M., Kim, S. J., Ro, J. Y., Park, K. M., Lee, H. W., Park, E. J., Chun, K. H., & Song, J. (2012). Ubiquitination and degradation of the FADD adaptor protein regulate death receptor-mediated apoptosis and necroptosis. *Nature Communications, 3*, 978.

Lewis, J., Devin, A., Miller, A., Lin, Y., Rodriguez, Y., Neckers, L., & Liu, Z. G. (2000). Disruption of hsp90 function results in degradation of the death domain kinase, receptor-interacting protein (RIP), and blockage of tumor necrosis factor-induced nuclear factor-kappaB activation. *Journal of Biological Chemistry, 275*, 10519–10526.

Li, Y., Yang, X., Ma, C., Qiao, J., & Zhang, C. (2008). Necroptosis contributes to the NMDA-induced excitotoxicity in rat's cultured cortical neurons. *Neuroscience Letters, 447*, 120–123.

Lin, Y., Choksi, S., Shen, H. M., Yang, Q. F., Hur, G. M., Kim, Y. S., Tran, J. H., Nedospasov, S. A., & Liu, Z. G. (2004). Tumor necrosis factor-induced nonapoptotic cell death requires receptor-interacting protein-mediated cellular reactive oxygen species accumulation. *Journal of Biological Chemistry, 279*, 10822–10828.

Linder, S., Olofsson, M. H., Herrmann, R., & Ulukaya, E. (2010). Utilization of cytokeratin-based biomarkers for pharmacodynamic studies. *Expert Review of Molecular Diagnostics, 10*, 353–359.

Long, J. S., & Ryan, K. M. (2012). New frontiers in promoting tumour cell death: Targeting apoptosis, necroptosis and autophagy. *Oncogene, 31*, 5045–5060.

Lu, J. V., & Walsh, C. M. (2012). Programmed necrosis and autophagy in immune function. *Immunological Reviews, 249*, 205–217.

Lu, J. V., Weist, B. M., van Raam, B. J., Marro, B. S., Nguyen, L. V., Srinivas, P., Bell, B. D., Luhrs, K. A., Lane, T. E., Salvesen, G. S., & Walsh, C. M. (2011). Complementary roles of Fas-associated death domain (FADD) and receptor interacting protein kinase-3 (RIPK3) in T-cell homeostasis and antiviral immunity. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 15312–15317.

Macurek, L., Lindqvist, A., & Medema, R. H. (2009). Aurora-A and hBora join the game of Polo. *Cancer Research, 69*, 4555–4558.

McComb, S., Cheung, H. H., Korneluk, R. G., Wang, S., Krishnan, L., & Sad, S. (2012). cIAP1 and cIAP2 limit macrophage necroptosis by inhibiting Rip1 and Rip3 activation. *Cell Death and Differentiation, 19*, 1791–1801.

Meloni, B. P., Meade, A. J., Kitikomolsuk, D., & Knuckey, N. W. (2011). Characterisation of neuronal cell death in acute and delayed in vitro ischemia (oxygen-glucose deprivation) models. *Journal of Neuroscience Methods, 195*, 67–74.

Meylan, E., & Tschopp, J. (2005). The RIP kinases: Crucial integrators of cellular stress. *Trends in Biochemical Sciences, 30*, 151–159.

Moquin, D., & Chan, F. K. (2010). The molecular regulation of programmed necrotic cell injury. *Trends in Biochemical Sciences, 35*, 434–441.

Nakagawa, T., Shimizu, S., Watanabe, T., Yamaguchi, O., Otsu, K., Yamagata, H., Inohara, H., Kubo, T., & Tsujimoto, Y. (2005). Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature, 434*, 652–658.

Northington, F. J., Chavez-Valdez, R., Graham, E. M., Razdan, S., Gauda, E. B., & Martin, L. J. (2011). Necrostatin decreases oxidative damage, inflammation, and injury after neonatal HI. *Journal of Cerebral Blood Flow and Metabolism, 31*, 178–189.

O'Donnell, M. A., Perez-Jimenez, E., Oberst, A., Ng, A., Massoumi, R., Xavier, R., Green, D. R., & Ting, A. T. (2011). Caspase 8 inhibits programmed necrosis by processing CYLD. *Nature Cell Biology, 13*, 1437–1442.

Oberst, A., Dillon, C. P., Weinlich, R., McCormick, L. L., Fitzgerald, P., Pop, C., Hakem, R., Salvesen, G. S., & Green, D. R. (2011). Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. *Nature, 471*, 363–367.

Perez-Pinzon, M. A., Stetler, R. A., & Fiskum, G. (2012). Novel mitochondrial targets for neuroprotection. *Journal of Cerebral Blood Flow and Metabolism, 32*, 1362–1376.

Pop, C., Oberst, A., Drag, M., Van Raam, B. J., Riedl, S. J., Green, D. R., & Salvesen, G. S. (2011). FLIP(L) induces caspase 8 activity in the absence of interdomain caspase 8 cleavage and alters substrate specificity. *Biochemical Journal, 433*, 447–457.

Rebe, C., Cathelin, S., Launay, S., Filomenko, R., Prevotat, L., L'Ollivier, C., Gyan, E., Micheau, O., Grant, S., Dubart-Kupperschmitt, A., Fontenay, M., & Solary, E. (2007). Caspase-8 prevents sustained activation of NF-kappaB in monocytes undergoing macrophagic differentiation. *Blood, 109*, 1442–1450.

Rosenbaum, D. M., Degterev, A., David, J., Rosenbaum, P. S., Roth, S., Grotta, J. C., Cuny, G. D., Yuan, J., & Savitz, S. I. (2010). Necroptosis, a novel form of caspase-independent cell death, contributes to neuronal damage in a retinal ischemia-reperfusion injury model. *Journal of Neuroscience Research, 88*, 1569–1576.

Sarkar, F. H., & Li, Y. (2006). Markers of apoptosis. *Methods in Molecular Medicine, 120*, 147–160.

Scaffidi, P., Misteli, T., & Bianchi, M. E. (2002). Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature, 418*, 191–195.

Schon, E. A., & Przedborski, S. (2011). Mitochondria: The next (neurode)generation. *Neuron, 70*, 1033–1053.

Schweichel, J. U., & Merker, H. J. (1973). The morphology of various types of cell death in prenatal tissues. *Teratology, 7*, 253–266.

Shembade, N., Ma, A., & Harhaj, E. W. (2010). Inhibition of NF-kappaB signaling by A20 through disruption of ubiquitin enzyme complexes. *Science, 327*, 1135–1139.

Simenc, J., & Lipnik-Stangelj, M. (2012). Staurosporine induces apoptosis and necroptosis in cultured rat astrocytes. *Drug and Chemical Toxicology, 35*, 399–405

Stanger, B. Z., Leder, P., Lee, T. H., Kim, E., & Seed, B. (1995). RIP: A novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. *Cell, 81*, 513–523.

Sun, L., Wang, H., Wang, Z., He, S., Chen, S., Liao, D., Wang, L., Yan, J., Liu, W., Lei, X., & Wang, X. (2012). Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell, 148*, 213–227.

Temkin, V., Huang, Q., Liu, H., Osada, H., & Pope, R. M. (2006). Inhibition of ADP/ATP exchange in receptor-interacting protein-mediated necrosis. *Molecular and Cellular Biology, 26*, 2215–2225.

Tenev, T., Bianchi, K., Darding, M., Broemer, M., Langlais, C., Wallberg, F., Zachariou, A., Lopez, J., MacFarlane, M., Cain, K., & Meier, P. (2011). The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. *Molecular Cell, 43*, 432–448.

Teng, X., Degterev, A., Jagtap, P., Xing, X., Choi, S., Denu, R., Yuan, J., & Cuny, G. D. (2005). Structure-activity relationship study of novel necroptosis inhibitors. *Bioorganic & Medicinal Chemistry Letters, 15*, 5039–5044.

Teng, X., Keys, H., Jeevanandam, A., Porco, J. A., Jr., Degterev, A., Yuan, J., & Cuny, G. D. (2007). Structure-activity relationship study of [1,2,3]thiadiazole necroptosis inhibitors. *Bioorganic & Medicinal Chemistry Letters, 17*, 6836–6840.

Teng, X., Keys, H., Yuan, J., Degterev, A., & Cuny, G. D. (2008). Structure-activity relationship and liver microsome stability studies of pyrrole necroptosis inhibitors. *Bioorganic & Medicinal Chemistry Letters, 18*, 3219–3223.

Tourneur, L., & Chiocchia, G. (2010). FADD: A regulator of life and death. *Trends in Immunology, 31*, 260–269.

van Wijk, S. J., & Hageman, G. J. (2005). Poly(ADP-ribose) polymerase-1 mediated caspase-independent cell death after ischemia/reperfusion. *Free Radical Biology & Medicine, 39*, 81–90.

Vandenabeele, P., Galluzzi, L., Vanden Berghe, T., & Kroemer, G. (2010). Molecular mechanisms of necroptosis: An ordered cellular explosion. *Nature Reviews Molecular Cell Biology, 11*, 700–714.

Vanlangenakker, N., Vanden Berghe, T., Bogaert, P., Laukens, B., Zobel, K., Deshayes, K., Vucic, D., Fulda, S., Vandenabeele, P., & Bertrand, M. J. (2011). cIAP1 and TAK1 protect cells from TNF-induced necrosis by preventing RIP1/RIP3-dependent reactive oxygen species production. *Cell Death and Differentiation, 18*, 656–665.

Vanlangenakker, N., Vanden Berghe, T., & Vandenabeele, P. (2012). Many stimuli pull the necrotic trigger, an overview. *Cell Death and Differentiation, 19*, 75–86.

Vosler, P. S., Graham, S. H., Wechsler, L. R., & Chen, J. (2009). Mitochondrial targets for stroke: Focusing basic science research toward development of clinically translatable therapeutics. *Stroke, 40*, 3149–3155.

Wang, K., Li, J., Degterev, A., Hsu, E., Yuan, J., & Yuan, C. (2007). Structure-activity relationship analysis of a novel necroptosis inhibitor, Necrostatin-5. *Bioorganic & Medicinal Chemistry Letters, 17*, 1455–1465.

Wang, L., Du, F., & Wang, X. (2008). TNF-alpha induces two distinct caspase-8 activation pathways. *Cell, 133*, 693–703.

Wang, Z., Jiang, H., Chen, S., Du, F., & Wang, X. (2012). The mitochondrial phosphatase PGAM5 functions at the convergence point of multiple necrotic death pathways. *Cell, 148*, 228–243.

Welz, P. S., Wullaert, A., Vlantis, K., Kondylis, V., Fernandez-Majada, V., Ermolaeva, M., Kirsch, P., Sterner-Kock, A., van Loo, G., & Pasparakis, M. (2011). FADD prevents RIP3-mediated epithelial cell necrosis and chronic intestinal inflammation. *Nature, 477*, 330–334.

West, T., Atzeva, M., & Holtzman, D. M. (2006). Caspase-3 deficiency during development increases vulnerability to hypoxic-ischemic injury through caspase-3-independent pathways. *Neurobiology of Disease, 22*, 523–537.

Wright, A., Reiley, W. W., Chang, M., Jin, W., Lee, A. J., Zhang, M., & Sun, S. C. (2007). Regulation of early wave of germ cell apoptosis and spermatogenesis by deubiquitinating enzyme CYLD. *Developmental Cell, 13*, 705–716.

Xu, X., Chua, C. C., Gao, J., Hamdy, R. C., & Chua, B. H. (2006). Humanin is a novel neuroprotective agent against stroke. *Stroke, 37*, 2613–2619.

Xu, X., Chua, C. C., Kong, J., Kostrzewa, R. M., Kumaraguru, U., Hamdy, R. C., & Chua, B. H. (2007). Necrostatin-1 protects against glutamate-induced glutathione depletion and caspase-independent cell death in HT-22 cells. *Journal of Neurochemistry, 103*, 2004–2014.

Xu, X., Chua, C. C., Zhang, M., Geng, D., Liu, C. F., Hamdy, R. C., & Chua, B. H. (2010a). The role of PARP activation in glutamate-induced necroptosis in HT-22 cells. *Brain Research, 1343*, 206–212.

Xu, X., Chua, K. W., Chua, C. C., Liu, C. F., Hamdy, R. C., & Chua, B. H. (2010b). Synergistic protective effects of humanin and necrostatin-1 on hypoxia and ischemia/reperfusion injury. *Brain Research, 1355*, 189–194.

Yahiro, K., Satoh, M., Nakano, M., Hisatsune, J., Isomoto, H., Sap, J., Suzuki, H., Nomura, F., Noda, M., Moss, J., & Hirayama, T. (2012). Low-density Lipoprotein Receptor-related Protein-1 (LRP1) mediates autophagy and apoptosis caused by Helicobacter pylori VacA. *Journal of Biological Chemistry, 287*, 31104–31115.

Yeh, W. C., Itie, A., Elia, A. J., Ng, M., Shu, H. B., Wakeham, A., Mirtsos, C., Suzuki, N., Bonnard, M., Goeddel, D. V., & Mak, T. W. (2000). Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. *Immunity, 12*, 633–642.

You, Z., Savitz, S. I., Yang, J., Degterev, A., Yuan, J., Cuny, G. D., Moskowitz, M. A., & Whalen, M. J. (2008). Necrostatin-1 reduces histopathology and improves functional outcome after controlled cortical impact in mice. *Journal of Cerebral Blood Flow and Metabolism, 28*, 1564–1573.

Yuan, J., & Kroemer, G. (2010). Alternative cell death mechanisms in development and beyond. *Genes & Development, 24*, 2592–2602.

Zhang, D. W., Shao, J., Lin, J., Zhang, N., Lu, B. J., Lin, S. C., Dong, M. Q., & Han, J. (2009). RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science, 325*, 332–336.

Zhang, D., Lin, J., & Han, J. (2010). Receptor-interacting protein (RIP) kinase family. *Cellular & Molecular Immunology, 7*, 243–249.

Zhang, H., Zhou, X., McQuade, T., Li, J., Chan, F. K., & Zhang, J. (2011a). Functional complementation between FADD and RIP1 in embryos and lymphocytes. *Nature, 471*, 373–376.

Zhang, Z., Shi, R., Weng, J., Xu, X., Li, X. M., Gao, T. M., & Kong, J. (2011b). The proapoptotic member of the Bcl-2 family Bcl-2/E1B-19 K-interacting protein 3 is a mediator of caspase-independent neuronal death in excitotoxicity. *FEBS Journal, 278*, 134–142.

Zhao, J., Jitkaew, S., Cai, Z., Choksi, S., Li, Q., Luo, J., & Liu, Z. G. (2012). Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 5322–5327.

Zheng, W., Degterev, A., Hsu, E., Yuan, J., & Yuan, C. (2008). Structure-activity relationship study of a novel necroptosis inhibitor, necrostatin-7. *Bioorganic & Medicinal Chemistry Letters, 18*, 4932–4935.

Zhu, S., Zhang, Y., Bai, G., & Li, H. (2011). Necrostatin-1 ameliorates symptoms in R6/2 transgenic mouse model of Huntington's disease. *Cell Death and Disease, 2*, e115.

RCSN Cell System for Identifying Dopaminergic Neurotoxicity

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Contents

Abstract

Dopaminergic toxicity represents a potential mechanism underlying Parkinson's disease (PD) neuropathology. Nevertheless, the study of such a mechanism is hampered by the lack of permanent and stable in vitro models that bear relevant cellular traits, namely, neuronal dopaminergic function. Although various permanent cell lines exhibiting variable dopaminergic properties do exist, such properties are not necessarily stable and may require the application of complex and costly differentiation protocols for induction. The latter is particularly true when inducing in vitro differentiation from more undifferentiated tissue, such as stem cells. Also, cell lines may lose viability or eventually undergo permanent differentiation. This chapter discusses

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a permanently growing cell line, named RCSN-3, which was established from the substantia nigra of an adult Fisher 344 rat. The cell line retains dopaminergic traits, including dopamine production and secretion, and the presence of catecholamine reuptake transporters. Notably, these properties have remained expressed in RCSN-3 cells for decades. This chapter also addresses the contribution of RCSN-3 to dopaminergic-mediated toxic phenomena, in particular, and to the identification of potential therapeutical targets in dopaminergic neurons. Finally, RCSN-3 cells are also presented as a model for cell transplant therapy, in animal models of PD, and their contribution in this field is discussed in relation to more recently available cell sources, such as stem cells and induced pluripotent stem cells (iPSCs).

1 Neurons in Culture: What Can They Do for Us?

The culture of neuronal cells has been widely used, as it presents an unparalleled opportunity to provide model systems for ready and unrestricted access to living tissue. Neurons, however, exhibit quite distinct features when taken from an in vivo to in vitro environments, which must be addressed to provide adequate answers for the questions to be asked. Indeed, neuronal tissue inherently has greater demands (i.e., oxygen, nutrients, trophic factors), which must be provided to insure adequate survival and maintenance of differentiated function in culture conditions (Freshney 2000). Among them, one can count the use of special matrixes for attachment and differentiation (collagen, laminin, fibronectin, extracellular matrix preparations, etc.), along with the supplementation of soluble factors in the media, which have a very wide range of complexity, ranging from monovalent ions to molecules of high molecular weight (Levi-Montalcini 1979; Marchionni 1993). These demands, associated with limitations in the quantities of procurable tissue, particularly from human origin, have prompted the interest in developing immortalized cell lines from neurons, which would potentially provide a limitless amount of cells and, hopefully, stable models for years to come. However, many such cell lines tend to exhibit problems in their stability and/or viability, with end results ranging from death to irreversible loss of differentiated functions (Cárdenas et al. 1999). Yet, if such drawbacks can be overcome, they appear as a feasible alternative, as they can generate genetically homogeneous cell populations that can be suitable models to explore physiological and pathophysiological phenomena at the cell level and also identify pharmacological targets which can be used for drug screening.

Following this line of thought, the use of tissue culture in dopaminergic systems appears as an adequate tool to study problems of clinical relevance, especially those related to relevant degenerative diseases such as Parkinson's disease (PD). Indeed, the discrete localization of the neuropathological hallmark

in this condition – namely, the degeneration of the *substantia nigra pars compacta* (SN) – presents dopaminergic culture systems as ideal models for the study of PD cell pathophysiology. This chapter will refer to such models, and it will particularly address a specific neuronal cell line, named RCSN-3, which was established from the substantia nigra of an adult Fisher 344 rat. Evidence of its use in the study of potential toxic mechanisms relevant to PD will be discussed, along with future directions.

2 Parkinson's Disease: Where We Stand...

Parkinson's disease is a neurodegenerative disorder affecting an estimated 0.3 % of the population in the industrialized world (de Lau and Breteler 2006), the elderly being the highest population at risk. The disease is progressive in nature, and there are no effective treatments to arrest or revert the evolution of the disease. The clinical manifestations of PD are several, and presentations vary greatly among patients. The most evident include signs and symptoms that affect voluntary movement, which include resting tremor in the extremities and muscular rigidity, which leads to difficulties in walking with a characteristic shuffling gait. Also, impaired writing, speaking, and masking of facial expression are present. Bradykinesia, or slowness in initiating and executing movements, and stooped posture and instability are also commonly observed signs (Sian et al. 1999). Neuropsychiatric manifestations (mood disorders, altered thought process, cognitive decline) may also be present (Jankovic 2008), particularly in later stages of the disease, thus presenting PD as a far more complex condition than one that merely compromises motor function. At the neuropathological level, the most remarkable finding is the degeneration of the dopaminergic neurons present in the SN. These neurons project to the striatum, providing the latter with a basal dopaminergic input necessary for the adequate function of the regulatory circuit of the basal nuclei. The degeneration of the SN determines an imbalance in the regulation of the circuitry of the basal ganglia, whose GABAergic output to the thalamus is increased, resulting in an overall inhibition of this projection nucleus (Lozano et al. 1998). Hence, messages processed in the basal ganglia which project to the cerebral cortex via the thalamus have an increased inhibitory component in PD, especially those coding for voluntary movement, resulting in reduced motility.

The mechanisms for the progressive degeneration of the SN in PD remain largely unknown, in particular for sporadic forms of PD. A small minority of patients with mutations in certain genes such as alpha-synuclein, parkin, leucine-rich repeat kinase 2 (LRRK2), or PTEN-induced putative kinase 1 (PINK1) will develop the disease with a high degree of certainty (Davie 2008; Lesage and Brice 2009). Yet, the mechanisms of toxicity remain obscure, in particular at the cellular and molecular level. Therefore, the development of neuronal in vitro cell models

that resemble SN function is of the essence in order to address such questions and in turn identify relevant intracellular therapeutical targets.

3 Ex Vivo Dopaminergic Cells: What can They do to Help?

Many strategies are being pursued to develop new therapies for PD patients, mainly targeting to the prevention of the damage of the nigrostriatal system (neuroprotection or rescue) and/or to replacing neurons that were lost due to the underlying condition. Efforts in this regard have included the use of trophic factors (Takayama et al. 1995) and viral vectors (Choi-Lundberg et al. 1997) for protection and rescue, to the transplantation of primary xenogeneic or allogeneic tissue for repair (Deacon et al. 1997). Nevertheless, progress in the knowledge of mechanisms is hampered, firstly, by the lack of animal models that adequately reflect the human condition. Indeed, PD does not occur naturally in other mammals, and most animal models involve acute destruction of the SN by use of toxins, most of which share structural resemblance to dopamine (DA) (i.e., 6-OH-dopamine intoxicated rats; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-intoxicated monkeys) (see Fig. 1). Hence, such models do not reproduce the inherent chronic damage of the SN in PD and tend to target a reduced number of many potential mechanisms involved in dopaminergic degeneration. Indeed, such toxins result in rapid and sizable oxidative stress and/or energy depletion, which are most probably not solely responsible for cell damage. Other models have incorporated transgenics for known PD-related genes, including knockouts of alpha-synuclein (SNCA) or overexpression of mutated forms of this protein (Blandini and Armentero 2012). In spite of such progress, no such models reproduce all the characteristics of PD, and the aforementioned models tend to address specific questions of a given mechanism.

The first approach to a given condition must address alterations at the cellular level, a goal that is difficult to attain in a full animal, where many variables escape from the control of the researcher. Initially, in vitro models comprised primary cultured cells, which tend to reflect more closely the traits of the tissue of origin. Yet, the inherent variability of such preparations and limitations in the amounts of tissue procurable conspire against presenting these models as a universal preparation. The use of neuronal cell lines overcame the noted limitations in quantities of tissue, yet long-term instability and eventual permanent dedifferentiation are but two factors that can hinder their usefulness. Nevertheless, dopaminergic cell lines have been used for quite some time. The PC12 cell line, which is derived from the pheochromocytoma of a rat (Greene and Tischler 1976), has been and is still largely used to study dopaminergic and neuronal function, yet it is tumorigenic, it is difficult to grow, and it requires nerve growth factor (NGF) for neuronal differentiation in vitro. Another interesting cell line, SH-SY5Y, derived from the neuroblastoma of a child, has also been used to study dopaminergic function, especially since it has dopamine-β-hydroxylase activity (Oyarce and Fleming 1991), thus suggesting the presence of catecholaminergic function, yet it does not derive from a dopaminergic precursor.

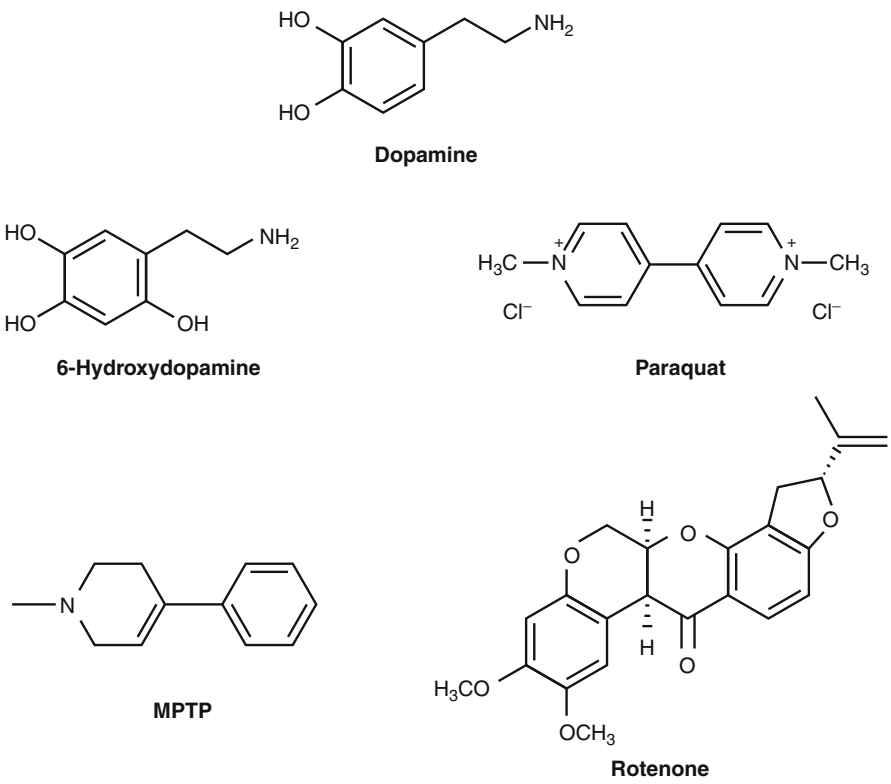

Fig. 1 Dopamine and toxins of dopaminergic system. Many known toxins of dopaminergic neurons share structural similarities with dopamine, thus entering dopaminergic neurons via dopaminergic reuptake transporters. 6-Hydroxydopamine generates reactive oxygen species such as superoxide radical inside the cells. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is the precursor of the neurotoxin MPP^+ (1-methyl-4-phenylpyridinium), which, once inside dopaminergic neurons, interferes with complex I of the electron transport chain. Rotenone interferes with the electron transport chain in mitochondria, particularly by inhibiting the transfer of electrons from iron-sulfur centers in complex I to ubiquinone, in turn interfering with NADH in the creation of ATP. Paraquat, a known herbicide, can catalyze the formation of reactive oxygen species (ROS), particularly superoxide

Lately, stem cells, derived from the nervous system (neural stem cells), have been used to generate sufficient cell mass to later differentiate to dopaminergic cells. Yet, the reduced amount of cells, slow in vitro proliferation, and the ethical and practical difficulties involved in procuring viable human nervous tissue do introduce difficulties in this line of work. At present, one of the most interesting lines of research involves reprogramming human fibroblasts from patients to generate induced pluripotent stem cells or iPSCs. Such cells can generate patient-specific cells that can later be differentiated to dopaminergic neurons (Swistowski et al. 2010).

4 RCSN-3: A Permanent In Vitro Neuronal Dopaminergic Model

All of the aforementioned cell models are and have been useful in the study of the neuronal physiology and pathophysiology of PD. Yet, limitations in growth rate, in differentiation, and in the source of tissue define most of their limitations, whereas lack of stable, relevant dopaminergic functional traits is another drawback. Hence, a stable dopaminergic neuronal cell line, derived from SN tissue that retains differentiated traits, would provide a valuable model to test PD-related phenomena at the cell level. In this regard, RCSN-3 appears as an interesting candidate for such tasks.

RCSN-3 was established in the early 1980s, from primary cultures of the SN dissected from a 4-month-old Fisher 344 rat (Arriagada et al. 2002). The cell line was established from the said primary cultures by means of the UCHT1 immortalization protocol (Caviedes et al. 1993, 1994; Cárdenas et al. 1999, 2002). This original procedure has managed to successfully generate neuronal cell lines in permanent culture from diverse origins, obtained from both normal and pathological subjects (Caviedes et al. 1993; Liberona et al. 1997; Allen et al. 2000). Briefly, the immortalization procedure entails maintaining neuronal primary cultures in the presence of media conditioned by the rat thyroid cell line UCHT1. This treatment induces tissue immortalization in variable periods of time (Caviedes et al. 1993). This procedure has allowed the establishment of continuously growing cell lines that express stable neuronal phenotypes and lack glial characteristics. These and other lines so established retain differentiated traits, some for decades (Caviedes et al. 1993; Liberona et al. 1997; Cárdenas et al. 1999; Allen et al. 2000 and 2005).

After a period of 7–8 months of UCHT1 treatment, transformation foci of rapid proliferation became evident, after which the cultures were weaned from UCHT1 media. The resulting motherline, named RCSN, was subsequently cloned. Of these, RCSN clone 3, or RCSN-3, has been the line that stably retains dopaminergic traits. The RCSN-3 cell line grows in monolayers and can also be induced to grow in suspended, attachment-free clusters (Fig. 2) (Caviedes et al. 2012). RCSN-3 has a doubling time of approximately 50 h, a plating efficiency of 21 %, and a saturation density of 50,000 cells/cm^2 when grown in basal growth media composed of DMEM/HAM-F12 nutrient mixture (1:1), 10 % adult bovine serum, 2.5–5 % fetal bovine serum, and 40 mg/l gentamicin (Dagnino-Subiabre et al. 2000; Paris et al. 2001; Martinez-Alvarado et al. 2001).

Regarding their neuronal trails, RCSN-3 cells are immunohistochemically positive for neuron-specific enolase (NSE), MAP-2, 200 kD neurofilament, and parvalbumin. Further, RCSN-3 cells exhibit intracellular melanin deposits, as evidenced by Fe^{2+} ion capture cytology studies (Arriagada et al. 2002). On the other hand, glial markers such as GFAP and S-100 are negative (Arriagada et al. 2002). Regarding dopaminergic function, the cells express tyrosine hydroxylase (TH) in basal culture conditions, with increased labeling after differentiation with serum deprivation (Arriagada et al. 2002; Paris et al. 2008). They also

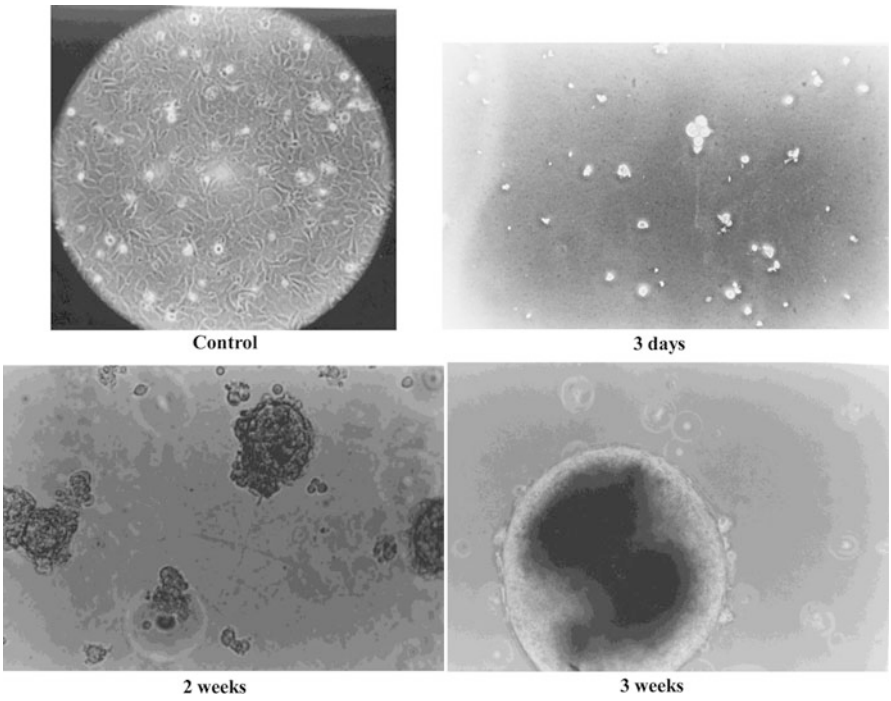

Fig. 2 RCSN-3 cells grow in monolayers when kept in standard growth media. When subjected to culture protocols that disfavor attachment (Caviedes et al. 2012), RCSN-3 cells grow in suspension and cluster progressively

show fluorescent labeling in the cytoplasm after treatment with paraformaldehyde-glyoxylic acid, suggesting the presence of catecholamine content. Experimental evidence of active DA release by RCSN-3 cells has been obtained with HPLC studies of culture supernatant and single cell amperometry with carbon electrodes, both of which are highly suggestive that RCSN-3 is capable of secreting this neurotransmitter. Further, RCSN-3 cells express a number of transporters, such as norepinephrine, serotonin, and, interestingly, DA (Paris et al. 2008). Hence, this is the only known dopaminergic cell line that originated from the SN of an adult animal and thus an attractive model to study PD-related mechanisms, in particular those related to dopaminergic toxicity.

5 Can Dopamine be Harmful? How?

Firstly, the causes for the discrete compromise of dopaminergic neurons of the SN in PD patients remain a mystery. Yet, DA itself may have a role. As a catecholamine, DA has a catechol ring with two hydroxyl groups in positions 4 and 5 (Fig. 1). In physiological conditions, such hydroxyl groups may remain

reduced due to several mechanisms, such as inclusion in low-pH secretory vesicles, reduction by several scavenging enzymes, and formation of neuromelanin. An imbalance in such mechanisms could shift the balance of the redox state of such hydroxyl groups to a more oxidative stage – namely, superoxide – this introducing a potential pro-oxidative state. Considering the specificity of the lesions in PD, it is possible then that the high oxidative state associated with DA metabolism may cause deterioration of dopaminergic neurons. The mechanism underlying increased oxidative stress may involve DA itself, because oxidation of cytosolic levodopa/DA may be deleterious to neurons. Indeed, DA causes apoptotic cell death as shown by morphological nuclear changes and DNA fragmentation (Luo et al. 1998). DA and its metabolites seem to exert cytotoxicity mainly by generating highly reactive quinones through auto-oxidation (Martinez-Alvarado et al. 2001). On the other hand, the toxicity of DA may be due to the generation of reactive oxygen species (ROS) that could disrupt cellular integrity, causing cell death. Indeed, aberrant regulation of DA is accompanied by accumulation of DA metabolites that could induce toxicity (Arriagada et al. 2004; Hattori et al. 2009).

The possibility that environmental factors may play a role in sporadic PD was addressed over a decade ago (Tanner et al. 2009). Epidemiological risk factor analyses of typical PD cases have identified several neurotoxins, including the aforementioned active metabolite of MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium ion (MPP^+), paraquat, dieldrin, manganese, copper, and salsolinol (Chun et al. 2001; Paris et al. 2008). Regarding MPP^+, Chun et al. (2001), using a nigral dopaminergic cell line, SN4741, demonstrated that such neurotoxins can induce apoptotic cell death through a common molecular mechanism. These authors observed initial increases in H_2O_2-related ROS activity, followed by activation of JNK1/2 MAP kinases; activation of the PITSLRE kinase, p110, by both caspase-1- and caspase-3-like activities; and, finally, apoptotic cell death. Pharmacological intervention with combinations of the antioxidants significantly protected the cells against such damage.

The SN4741 model would then appear as an interesting model to study PD-relevant pathophysiology. Yet, this line was established from transgenic mouse embryos containing the targeted expression of the thermolabile SV40Tag in SN neurons. Cell lines generated in this fashion tend to show instability and loss of function (Frederiksen et al. 1996), a problem not reported for UCHT1-established cell lines. Further, SN4741 cells are embryonic in origin and require differentiation in vitro. In this regard, RCSN-3 cells express dopaminergic traits without need of lengthy or costly differentiation protocols, and it has retained such traits for years. Interestingly, they also undergo toxicity and death upon exposure to such the aforementioned neurotoxins (Paris et al. 2008). Also, the RCSN-3 cell line expresses the reuptake transporters for DA (DAT) and norepinephrine (NET) (Paris et al. 2005). These properties of RCSN-3 cells allowed the discovery of a novel mechanism involving heavy metal toxicity (Cu^{2+}, Fe^{2+}) related to formation of complexes with dopamine (Paris et al. 2001, 2005); and their subsequent entry into the cell specifically by these transporters has provided new

light on how such environmental factors can contribute to PD pathogenicity. Indeed, by using highly efficient transport systems, heavy metals can then enter rapidly, generating oxidative stress in submitochondrial compartments via the Fenton reaction (Thomas et al. 2009).

Inside the cell, DA can induce toxicity, in particular when its redox state is shifted toward more pro-oxidizing conditions. Indeed, DA-oxidized metabolites can inhibit the respiratory chain in mitochondria (Ben-Shachar et al. 2004), thus producing an energy crisis in the cell. In this regard, RCSN-3 cell can undergo toxicity phenomena with treatment of DA oxidation products such as aminochrome treatment, once reductases such as DT-diaphorase are inhibited (Arriagada et al. 2000, 2004). RCSN-3 cells also express the vesicular monoamine transporter-2 – VMAT-2 (Fuentes-Bravo et al. 2007). When this transporter was selectively inhibited with reserpine, exposure of cell to external DA induced clear apoptotic phenomena, such as blebbing and chromatin condensation. Also, mitochondrial damage was evident at the electron microscopy level (Fuentes-Bravo et al. 2007).

6 Cell Transplantation in PD: The Final Frontier?

Cell transplantation therapy was proposed several decades ago as a curative therapy for PD. The rationale for such an approach was based on the discrete anatomical location of the neuronal population compromised and the fact that dopaminergic output from the SN to the striatum has a rather tonic form of discharge. Hence, replacing such tissue with new, DA-secreting cells appeared as clinically promising experimental treatments, in particular in late-stage PD, where tissue replacement is essential. Efforts so far have reached the clinical setting in PD and other neuropathological conditions, using sources such as fetal tissue (Olanow et al. 2006), xenografts from porcine tissue (Borlongan and Sanberg 2002; Poncelet et al. 2009), and immortalized cell lines (Prasad et al. 1998; Paul et al. 2007; Miljan et al. 2009). Indeed, considering that PD patients develop symptoms when approximately 50–70 % of the dopaminergic neurons in the SN are lost (Lozano et al. 1998), the development of cell transplant models appears as a necessary step in the cure for the disease. This is also relevant in light of the lack of predictive markers of the disease; hence, diagnosis is clinical and essentially tardy. Further, the discrete anatomical localization of the degenerating tissue and the large size of the target nucleus (striatum) make it a likely candidate for cell transplant therapy with minimally invasive stereotaxic procedures. In this regard, dopaminergic cell lines can provide a model for testing such therapeutical approaches, considering the existence of relevant animals models such as the 6-OHDA rat and MPTP-intoxicated monkeys. Cell lines are easy to manipulate, and genetic modifications set forth to improve dopaminergic function and/or life expectancy of the graft (i.e., transfection with genes that encode for neurotrophic factors) may provide pertinent, useful information to determine conditions that optimize these functions in transplantable dopaminergic cells.

7 Cell Therapy: RCSN-3 Contribution and the Use of Induced Pluripotent Stem Cells (iPSCs)

RCSN-3 cells have also been used as in cell transplant approaches, using induced PD animal models. Indeed, implant of RCSN-3 in the striatum of 6-OHDA-intoxicated rats resulted in a stable reduction of around 50 % in apomorphine-induced rotations. Lately, the development of methodologies to generate pluripotent stem cells from somatic tissues (iPSCs) has provided researchers with an interesting source of stem cells, with the important added value of being patient specific. Hence, the first advantage of iPSC technology is the generation of relevant in vitro models for diseases and drug treatment assays (Soldner et al. 2009). Further, protocols to increase the yield of dopaminergic neuron generation from iPSC from either humans or mice are now available (Park et al. 2008; Soldner et al. 2009; Hargus et al. 2010; Devine et al. 2011). Indeed, neural precursor cells and dopaminergic neurons have been generated from healthy iPSC (Wernig et al. 2008), which when transplanted in the brain can integrate in the parenchyma, migrate, differentiate, and display neuronal function such as action potentials. Most interestingly, grafts derived from iPSC are capable of restoring motor function in animal models for PD (Wernig et al. 2008), suggesting that dopaminergic neurons derived from iPSC can survive implantation and remain functional in vivo.

In humans, iPSC-derived dopaminergic neurons have been obtained from healthy donors (Swistowski et al. 2010), sporadic PD patients (Soldner et al. 2009; Hargus et al. 2010), or PD patients bearing some of the known mutations (Devine et al. 2011; Martínez-Morales and Liste 2012). For instance, DA neurons can be generated from iPSCs that carry a mutation in *LRRK2* gene (p.G2019S), the most common PD-related mutation (Drolet et al. 2011). After reprogramming, and applying a culture differentiation protocol using feeders, iPSC-p.G2019S generated significant amounts of DA. This constitutes the most interesting application for such models, as "custom" therapies can potentially derive from such unique models.

8 Conclusion

Overcoming neurodegenerative diseases, of which PD represents an important fraction, is the most demanding challenge. Relevant therapies will come from the understanding of cellular and molecular mechanisms involved in cell damage, along with the development of predictive markers. The lack of the latter introduces yet another limitation in the quest for new therapies, where neuroprotective strategies are clearly at a loss.

Relevant cell models are a must in the undertaking of both the aforementioned tasks. Novel treatments require models such as RCSN-3 cells for first-level screening and may also play a part in the study of predictive markers. The development of iPSCs is the most exciting chapter in the field, as they can provide disease-specific models from patients. Eventually, they may constitute adequate

sources for cell transplant therapy. Yet, much is to be said about the long-term stability of iPSCs; hence, their routine application to cell transplant therapy may be further away than desired, as well as their application as in vitro models. Until that day, well-established models like RCSN-3 will have a role in addressing the problem at hand.

References

Allen, D. D., Martín, J., Arriagada, C., Cárdenas, A. M., Rapoport, S. I., Caviedes, R., & Caviedes, P. (2000). Impaired cholinergic function in cell lines derived from the cerebral cortex of normal and trisomy 16 mice. *European Journal of Neuroscience, 12*(9), 3259–3264.

Allen, D. D., Caviedes, R., Cárdenas, A. M., Shimahara, T., Segura-Aguilar, J., & Caviedes, P. (2005). Cell lines as in vitro models for drug screening and toxicity studies. *Drug Development and Industrial Pharmacy, 31*(8), 747–758.

Arriagada, C., Dagnino-Subiabre, A., Caviedes, P., Martín, J., Caviedes, R., & Segura-Aguilar, J. (2000). Studies of aminochrome toxicity in a mouse derived neuronal cell line: is this toxicity mediated via glutamate transmission? *Amino Acids, 18*(4), 363–373.

Arriagada, C., Salazar, J., Shimahara, T., Caviedes, R., & Caviedes, P. (2002). An immortalized neuronal cell line derived from the substantia nigra of an adult rat: application to cell transplant therapy. In E. Ronken & G. van Scharrenburg (Eds.), *Parkinson's disease* (pp. 120–132). Amsterdam: IOS Press. ISBN 1 58603 207 0.

Arriagada, C., Paris, I., Sánchez de las Matas, M. J., Martínez-Alvarado, P., Graumann, R., Cárdenas Castañeda, P., Pérez-Pastene, C., Olea Azar, C., Couve, E., Herrero, M. T., Caviedes, P., & Segura-Aguilar, J. (2004). Neurotoxic effects of leukoaminochrome o-semiquinone radical on RCSN-3 cells: Mitochondrial damage, necrosis, and hydroxyl radical formation. *Neurobiology of Disease, 16*, 468–477.

Ben-Shachar, D., Zuk, R., Gazawi, H., & Ljubuncic, P. (2004). Dopamine toxicity involves mitochondrial complex I inhibition: implications to dopamine-related neuropsychiatric disorders. *Biochemical Pharmacology, 67*(10), 1965–1974.

Blandini, F., & Armentero, M. T. (2012). Animal models of Parkinson's disease. *The FEBS Journal, 279*(7), 1156–1166.

Borlongan, C. V., & Sanberg, P. R. (2002). Neural transplantation for treatment of Parkinson's disease. *Drug Discovery Today, 7*(12), 674–682.

Cárdenas, A. M., Rodríguez, M. P., Cortés, M. P., Alvarez, R. M., Wei, W., Rapoport, S. I., Shimahara, T., Caviedes, R., & Caviedes, P. (1999). Intracellular calcium signals in immortal cell lines derived from the cerebral cortex of normal and trisomy 16 fetal mice, an animal model of human trisomy 21 (Down syndrome). *Neuroreport, 10*, 363–369.

Cárdenas, A. M., Allen, D. D., Arriagada, C., Olivares, A., Bennett, L. B., Caviedes, R., Dagnino-Subiabre, A., Mendoza, I. E., Segura-Aguilar, J., Rapoport, S. I., & Caviedes, P. (2002). Establishment and characterization of immortalized neuronal cell lines derived from the spinal cord of normal and trisomy 16 fetal mice, an animal model of Down Syndrome. *Journal of Neuroscience Research, 68*(2), 46–58.

Caviedes, P., Olivares, E., Salas, K., Caviedes, R., & Jaimovich, E. (1993). Calcium fluxes, ion currents and dihydropyridine receptors in a newly established cell line from rat heart muscle. *Journal of Molecular and Cell Cardiology, 25*, 829–845.

Caviedes, R., Caviedes, P., Liberona, J. L., & Jaimovich, E. (1994). Ion currents in a skeletal muscle cell line from a Duchenne muscular dystrophy patient. *Muscle & Nerve, 17*, 1021–1028.

Caviedes, P., Caviedes, R., Freeman, TB, Asenjo, J., Andrews, B., Sepúlveda, D, Arriagada, C., Salazar Rivera, J. 2012. Materials and methods for regulating process formation in cell culture. US Patent No 8,252,279.

Choi-Lundberg, D. L., Lin, Q., Chang, Y. N., Chiang, Y. L., Hay, C. M., Mohajeri, H., Davidson, B. L., & Bohn, M. C. (1997). Dopaminergic neurons protected from degeneration by GDNF gene therapy. *Science, 275*, 838–841.

Chun, H. S., Gibson, G. E., DeGiorgio, L. A., Zhang, H., Kidd, V. J., & Son, J. H. (2001). Dopaminergic cell death induced by MPP^+, oxidant and specific neurotoxicants shares the common molecular mechanism. *Journal of Neurochemistry, 76*, 1010–1021.

Dagnino-Subiabre, A., Marcelain, K., Arriagada, C., Paris, I., Caviedes, P., Caviedes, R., & Segura-Aguilar, J. (2000). Angiotensin receptor II is present in a dopaminergic cell line of rat substantia nigra and it is down regulated by aminochrome. *Molecular and Cellular Biochemistry, 212*(1–2), 131–134.

Davie, C. A. (2008). A review of Parkinson's disease. *British Medical Bulletin, 86*(1), 109–127.

de Lau, L. M. L., & Breteler, M. M. B. (2006). Epidemiology of Parkinson's disease. *Lancet Neurology, 5*(6), 525–535.

Deacon, T., Schumacher, J., Dinsmore, J., Thomas, C., Palmer, P., Kott, S., Edge, A., Penney, D., Kassissieh, S., Dempsey, P., & Isacson, O. (1997). Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. *Nature Medicine, 3*, 350–353.

Devine, M. J., Ryten, M., Vodicka, P., Thomson, A. J., Burdon, T., Houlden, H., Cavaleri, F., Nagano, M., Drummond, N. J., Taanman, J. W., Schapira, A. H., Gwinn, K., Hardy, J., Lewis, P. A., & Kunath, T. (2011). Parkinson's disease induced pluripotent stem cells with triplication of the α-synuclein locus. *Nature Communications, 2*, 440. 2011 Aug 23.

Drolet, R. E., Sanders, J. M., & Kern, J. T. (2011). Leucine-rich repeat kinase 2 (LRRK2) cellular biology: a review of recent advances in identifying physiological substrates and cellular functions. *Journal of Neurogenetics, 25*(4), 140–151.

Frederiksen, K., Thorpe, A., Richards, S. J., Waters, J., Dunnett, S. B., & Sandberg, B. E. (1996). Immortalized neural cells from trisomy 16 mice as models for Alzheimer's disease. *Annals of the New York Academy of Sciences, 777*, 415–420.

Freshney, R. I. (2000). Specialized cells. In R. I. Freshney (Ed.), *Culture of animal cells: A manual of basic technique* (4th ed., pp. 373–385). New York: Wiley-Liss.

Fuentes-Bravo, P., Paris, I., Nassif Caligaro, M., Caviedes, P., & Segura-Aguilar, J. (2007). Inhibition of VMAT-2 and DT diaphorase induce cell death in substantia nigra derived cell line- an experimental cell model for dopamine toxicity studies. *Chemical Research in Toxicology, 20*(5), 776–783.

Greene, L. A., & Tischler, A. S. (1976). Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proceedings of the National Academy of Sciences of the United States of America, 73*(7), 2424–2428. 1976 Jul.

Hargus, G., Cooper, O., Deleidi, M., Levy, A., Lee, K., Marlow, E., Yow, A., Soldner, F., Hockemeyer, D., Hallett, P. J., Osborn, T., Jaenisch, R., & Isacson, O. (2010). Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *Proceedings of the National Academy of Sciences of the United States of America, 107*(36), 15921–15926.

Hattori, N., Wang, M., Taka, H., Fujimura, T., Yoritaka, A., Kubo, S., & Mochizuki, H. (2009). Toxic effects of dopamine metabolism in Parkinson's disease. *Parkinsonism & Related Disorders, 15*(Supp 1), S35–S38.

Jankovic, J. (2008). Parkinson's disease: clinical features and diagnosis. *Journal of Neurology, Neurosurgery, and Psychiatry, 79*(4), 368–376. 2008 Apr.

Lesage, S., & Brice, A. (2009). Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Human Molecular Genetics, 18*(R1), R48–R59.

Levi-Montalcini, R. (1979). The nerve growth factor. *Scientific American, 240*, 68–77.

Liberona, J. L., Caviedes, P., Tascón, S., Giglio, J. R., Sampaio, S. V., Hidalgo, J., Caviedes, R., & Jaimovich, E. (1997). Expression of ion channels during differentiation of a human skeletal muscle cell line. *Journal of Muscle Research and Cell Motility, 18*(5), 586–598.

Lozano, A. M., Lang, A. E., Hutchison, W. D., & Dostrovsky, J. O. (1998). New developments in understanding the etiology of Parkinson's disease and in its treatment. *Current Opinion in Neurobiology, 8*, 783–790.

Luo, Y., Umegaki, H., Wang, X., Abe, R., & Roth, G. S. (1998). Dopamine induces apoptosis through an oxidation-involved SAPK/JNK activation pathway. *The Journal of Biological Chemistry, 273*(6), 3756–3764.

Marchionni, M., Goodearl, A. D., Chen, M. S., Bermingham-McDonough, O., Kirk, C., Hendricks, M., Danehy, F., Misunmi, D., Sudhalter, J., Kobayashi, K., Wroblewski, D., Lynch, C., Baldassare, M., Hiles, I., Davis, J. B., Hsuan, J. J., Totty, N. F., Otsu, M., McBurney, R., Waterfield, M. D., Strrobant, P., & Geynne, D. (1993). Glial growth factors are alternatively spliced erbB2 ligands expressed in the nervous system. *Nature, 254*, 515–516.

Martinez-Alvarado, P., Dagnino-Subiabre, A., Paris, I., Metodiewa, D., Welch, C. J., Olea-Azar, C., Caviedes, P., Caviedes, R., & Segura-Aguilar, J. (2001). Oxidation of the dopaminergic neurotoxin salsolinol to ortho-semiquinone radical and o-quinone tautomers and its one-electron reduction: A possible mechanism of neurotoxicity. *Biochemical and Biophysical Research Communications, 283*, 1069–1076.

Martínez-Morales, P. L., & Liste, I. (2012). Stem cells as in vitro model of Parkinson's disease. Stem Cells International, 2012, 980941, doi:10.1155/2012/980941

Miljan, E. A., Hines, S. J., Pande, P., Corteling, R. L., Hicks, C., Zbarsky, V., Umachandran, M., Sowinski, P., Richardson, S., Tang, E., Wieruszew, M., Patel, S., Stroemer, P., & Sinden, J. D. (2009). Implantation of c-mycER TAM immortalized human mesencephalic-derived clonal cell lines ameliorates behavior dysfunction in a rat model of Parkinson's disease. *Stem Cells and Development, 18*(2), 307–319.

Olanow, C. W., Goetz, C. G., Kordower, J. H., Stoessl, A. J., Sossi, V., Brin, M. F., Shannon, K. M., Nauert, G. M., Perl, D. P., Godbold, J., & Freeman, T. B. (2003). A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol, 54*(3), 403–414.

Oyarce, A. M., & Fleming, P. J. (1991). Multiple forms of human dopamine beta-hydroxylase in SH-SY5Y neuroblastoma cells. *Archives of Biochemistry and Biophysics, 290*(2), 503–510.

Paris, I., Dagnino, A., Marcelaine, K., Bennet, L., Caviedes, P., Caviedes, R., Olea Azar, C., & Segura-Aguilar, J. (2001). Copper neurotoxicity is dependent on dopamine-mediated copper uptake, oxidation of dopamine to aminochrome and its one-electron reduction in rat substantia nigra neurons. *Journal of Neurochemistry, 77*, 519–529.

Paris, I., Martinez-Alvarado, P., Perez-Pastene, C., Vieira, M. N. N., Olea-Azar, C., Raisman-Vozari, R., Cardenas, S., Graumann, R., Caviedes, P., & Segura-Aguilar, J. (2005). Monoamine transporter inhibitors and norepinephrine reduce dopamine-dependent iron toxicity in cells derived from the Substantia Nigra. *Journal of Neurochemistry, 92*(5), 1021–1032.

Paris, I., Lozano, J., Cardenas, S., Perez-Pastene, C., Saud, K., Fuentes, P., Caviedes, P., Dagnino-Subiabre, A., Raisman-Vozari, R., Shimahara, T., Kostrzewa, J. P., Chi, D., Kostrzewa, R. M., Caviedes, R., & Segura-Aguilar, J. (2008). The catecholaminergic RCSN-3 cell line: a model to study dopamine metabolism. *Neurotoxicity Research, 13*(3–4), 221–230.

Park, I. H., Arora, N., Huo, H., Maherali, N., Ahfeldt, T., Shimamura, A., Lensch, M. W., Cowan, C., Hochedlinger, K., & Daley, G. Q. (2008). Disease-specific induced pluripotent stem cells. *Cell, 134*(5), 877–886.

Paul, G., Christophersen, N. S., Raymon, H., Kiaer, C., Smith, R., & Brundin, P. (2007). Tyrosine hydroxylase expression is unstable in a human immortalized mesencephalic cell line–studies in vitro and after intracerebral grafting in vivo. *Molecular and Cellular Neurosciences, 34*(3), 390–399.

Poncelet, A. J., Denis, D., & Gianello, P. (2009). Cellular xenotransplantation. *Current Opinion in Organ Transplantation, 14*(2), 168–174.

Prasad, K. N., Clarkson, E. D., La Rosa, F. G., Edwards-Prasad, J., & Freed, C. R. (1998). Efficacy of grafted immortalized dopamine neurons in an animal model of parkinsonism: a review. *Molecular Genetics and Metabolism, 65*(1), 1–9.

Sian, J., Youdim, M. B. H., Riederer, P., & Gerlach, M. (1999). Neurotransmitters and disorders of the Basal Ganglia. In G. J. Siegel (Ed.), *Basic neurochemistry: Molecular, cellular and medical aspects* (6th ed.). Philadelphia: Lippincott Raven.

Soldner, F., Hockemeyer, D., Beard, C., Gao, Q., Bell, G. W., Cook, E. G., Hargus, G., Blak, A., Cooper, O., Mitalipova, M., Isacson, O., & Jaenisch, R. (2009). Parkinson's disease patient-derived induced Pluripotent stem cells free of viral reprogramming factors. *Cell, 136*(5), 964–977.

Swistowski, A., Peng, J., Liu, Q., Mali, P., Rao, M. S., Cheng, L., & Zenga, X. (2010). Efficient generation of functional dopaminergic neurons from human induced Pluripotent stem cells under defined conditions. *Stem Cells, 28*(10), 1893–1904.

Takayama, H., Ray, J., Raymon, H. K., Baird, A., Hogg, J., Fisher, L. J., & Gage, F. H. (1995). Basic fibroblast growth factor increases dopaminergic graft survival and function in a rat model of Parkinson's disease. *Nature Medicine, 1*, 53–58.

Tanner, C. M., Ross, G. W., Jewell, S. A., Hauser, R. A., Jankovic, J., Factor, S. A., Bressman, S., Deligtisch, A., Marras, C., Lyons, K. E., Bhudhikanok, G. S., Roucoux, D. F., Meng, C., Abbott, R. D., & Langston, J. W. (2009). Occupation and risk of parkinsonism: a multicenter case–control study. *Archives of Neurology, 66*(9), 1106–1113.

Thomas, C., Mackey, M. M., Diaz, A. A., & Cox, D. P. (2009). Hydroxyl radical is produced via the Fenton reaction in submitochondrial particles under oxidative stress: implications for diseases associated with iron accumulation. *Redox Report, 14*(3), 102–108.

Wernig, M., Zhao, J. P., Pruszak, J., Hedlund, E., Fu, D., Soldner, F., Broccoli, V., Constantine-Paton, M., Isacson, O., & Jaenisch, R. (2008). Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 105*(15), 5856–5861.

Microglia: Neuroprotective and Neurodestructive Properties

G. Jean Harry and Christopher A. McPherson

Contents

Abstract

In the central nervous system, resident macrophages, microglia, and perivascular macrophages perform similar functions to peripheral macrophages yet display highly specialized features. They maintain some phenotypic characteristics and lineage-related properties common to their cells of origin and their rapid response in areas of neuronal death led to the contention that microglia serve as brain macrophages. While microglia are often considered the immune cell of the brain, they also show distinct features that make them unique from other tissue macrophages. They are maintained in a relatively quiescent and monitoring state by regulatory factors released by neurons and astrocytes. Via contact-dependent and receptor-dependent signaling, microglia rapidly respond to various events in the brain in an effort to return the microenvironment to homeostasis, to assist in refining the neural network

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during development and with repair, and to remove excess and aberrant proteins. Depending on the nature of the response and the physiological function of the microglia, a release of immune-related signaling factors can accompany a morphological change. It was initially thought that the immune-related response of microglia mirrors that of peripheral macrophages and considered to be adverse to the nervous system. However, further evaluation of these cells suggests that overall their responses are ones critical to the maintenance and function of the nervous system. This chapter will serve to set the framework for evaluating the microglia within the context of data available on their varied functions within the nervous system.

Keywords

Cytokines • Microglia • Neurodegeneration • Neuroinflammation • Pattern recognition receptors

List of Abbreviations

AD	Alzheimer's disease
AMPA	Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
ApoE	Apolipoprotein
ATP	Adenosine triphosphate
$A\beta_{42}$	Amyloid-beta 42
BBB	Blood–brain barrier
Ca^{2+}	Calcium
CNS	Central nervous system
COX	Cyclooxygenase
CR	Complement receptor
CSF	Colony-stimulating factor
CX3CL1	Fractalkine
DAMPs	Danger-associated molecular patterns
Fc	Fragment, crystallizable region
HO-1	Heme oxygenase-1
HSP	Heat shock protein
IFN	Interferon
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
mGluR	Metabotropic glutamate receptor
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NF-κB	Nuclear factor kappa B
NLRs	NOD-like receptors
NO	Nitric oxide

NOS	Nitric oxide synthase
NOX2	NADPH oxidase
P2	Purinergic
PAMPs	Pathogen-associated molecular patterns
PD	Parkinson's disease
PRRs	Pattern recognition receptors
RAGE	Receptor for advanced glycation end products
RNS	Nitric oxide-dependent reactive nitrogen species
ROS	Reactive oxygen species
SN	Substantia nigra
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TREM-2	Triggering receptor expressed on myeloid cells-2

1 Introduction

Macrophages are phagocytic cells originating from precursor cells in the bone marrow that are transported via the peripheral blood as monocytes to become tissue-specific macrophages (Davoust et al. 2008; Ginhoux et al. 2010; Morris et al. 1991). These cells maintain a first line of defense against injury and infection to return the system back to homeostasis by recruiting various effector molecules and other immune cells to assist in the task of killing pathogens and restoring tissue integrity (Gordon and Martinez 2010; Mosser and Edwards 2008). Two arms of the immune system, a rapid innate immune response and a slow adaptive immune response, are responsible for these functions (Lo et al. 1999). Innate immune receptors are preformed and stably encoded in the genome, while those of the adaptive immune system are both stochastically generated and shaped by the types and frequency of pathogens the cell encounters. First-responder cells rapidly recognize and respond to cell damage or infection-related danger-associated molecular patterns (DAMPs) increasing phagocytic activity. They also respond to pathogen-associated molecular patterns (PAMPs) expressed by pathogens (e.g., lipopolysaccharide (LPS)) but not by host cells. In responding, there is a production of cytotoxic reactive oxygen species (ROS), nitric oxide (NO), and proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, and IL-12. The second arm provides adaptive immunity to detect and respond to novel as well as evolutionarily conserved pathogenic molecules, i.e., antigens (Lo et al. 1999; Schwartz and Kipnis 2011). The adaptive immune system is comprised of long-lived lymphocytes (T cells and B cells) and antigen-presenting cells. The antigen-driven expansion of clonal populations of lymphocytes allows for experience to shape the response and for establishment of memory lymphocytes that are able to more rapidly attack a reencountered antigen.

Under such conditions, antigen-presenting cells play a critical role by phagocytizing material, loading the captured antigen into the pocket of the major histocompatibility complex (MHC) molecule for cell-surface antigen presentation on B cells, macrophages, and dendritic cells to facilitate recognition and clearance (Lo et al. 1999).

In the central nervous system (CNS), resident macrophages, microglia, and perivascular macrophages perform similar functions to peripheral macrophages yet display highly specialized features. In a temporal and spatial manner, monocyte colonization of the brain occurs with tissue vascularization and contact with vascular sprouts prior to birth (Cuadros and Navascues 1998; Marin-Padilla 1985; Rymo et al. 2011). The resulting microglia are yolk-sac derived cells maintained independently of blood-borne hematopoietic progenitors (Ajami et al. 2007; Ginhoux et al. 2010). They maintain some phenotypic characteristics and lineage-related properties common to macrophages, e.g., phagocytosis, expression of Fc (fragment, crystallizable region) receptors, and complement receptor (CR)3. The rapid response of microglia to areas of neuronal death and shift in morphology to an amoeboid phagocytic phenotype support early contentions that microglia served as brain macrophages. While T cells actively monitor the brain and can be recruited to sites of injury or infection, they do not remain or become functionally active unless a stimulating antigen is present. In the CNS, infiltrating and resident myeloid cells express MHC class II (Carson et al. 2006; Siffrin et al. 2007), while neurons, astrocytes, and oligodendrocytes are not players in antigen-mediated activation or retention of CD4+ T cells. Similarities between peripheral and central macrophages have led to assumptions that the innate immune system in the CNS equates to the parallel peripheral system and that expression of various factors such as MHC II or cytokines by resident cells of the brain represents neuroinflammation that can occur in the absence of peripheral immune cells. However, this independent capability of CNS immune cells comes under question with regard to whether the cellular response of microglia demonstrates neuroinflammation or simply the diverse nature of the cell (Graber et al. 2011).

Microglia are distinct from other tissue macrophages by their relatively quiescent phenotype and tight regulation by the microenvironment. Microglia display a small amount of perinuclear cytoplasm; small, dense, and heterochromatic nucleus; and several long processes with multiple branches covered with very fine protrusions. They are located outside of the vascular basement membrane; however, the cytoplasmic processes intermingle with astrocytic foot processes in the perivascular glia limitans (Lassmann et al. 1991). Perivascular microglia are limited along vascular walls and distinct from perivascular cells (Graeber et al. 1990). Microglia comprise approximately 15–20 % of the total number of cells in the mature brain. Occupying defined spatial territories, they actively survey the surrounding parenchyma via dynamic movement of processes, while the soma remains static, allowing each cell to sample its environment over the course of a few hours (Davalos et al. 2005; Nimmerjahn et al. 2005). The physical extent of this surveillance depends upon distribution, individual cell ramification, regional location, and rate of process remodeling.

2 Distribution of Microglia

Differences in microglia localization and morphology (Fig. 1) are suggested to be associated with functional differences and the phenotypic heterogeneity, including receptor expression patterns (Olah et al. 2011). Early evaluations by Lawson et al. (1990) showed a relatively uniform distribution of microglia in the adult rodent brain, with the exception of a higher relative number of microglia with similar phenotype in the dentate gyrus of the hippocampus, the substantia nigra (SN), and portions of the basal ganglia and the highest number of microglia in the olfactory telencephalon. Microglia modify their morphology and expression of cell-surface antigens depending upon their environment (Kaur and Ling 1991). Ramified microglia in gray matter show processes extending in multiple directions, while in the white matter, cells align cytoplasmic extensions in parallel or at right angles to nerve processes. In regions lacking a blood–brain barrier (BBB) such as circumventricular organs, a less ramified morphology is observed (Perry and Gordon 1987).

3 Microglia Network and Response

Microglia are in constant contact with other cells of the brain maintaining interactions with astrocytes, oligodendrocytes, and neurons, as well as brain vasculature. However, the cells do not normally demonstrate a direct intercellular coupling such as through gap junctions (Eugenin et al. 2001); therefore, communication is considered to rely heavily on auto- and paracrine signaling. It is thought that microglia are maintained in a relatively quiescent state due to signals derived from neuronal and astrocyte-derived factors specifically targeted to this regulatory task (Biber et al. 2007; Cardona et al. 2006; Simard et al. 2006). Healthy neurons are able to maintain microglia in an inactive state via secreted and membrane-bound signals including CD200, CX3CL1 (fractalkine), neurotransmitters, and neurotrophins (Biber et al. 2007; Neumann 2001; Pocock and Kettenmann 2007). In the absence of these signals or in the presence of stimulatory signals, the dynamic surveillance feature allows microglia to rapidly respond to changes in the environment. This is demonstrated in mice deficient of CD200 in which microglia exhibit a less ramified and shorter processes morphological phenotype, increased expression of CD11b and CD45, and elevated production of inflammatory mediators following immune challenge (Hoek et al. 2000).

With stimulation, microglial response patterns are highly varied across morphological changes. Often the initial response of microglia is to monitor and control the extracellular environment. These ever-changing phenotypes represent the unique nature of the insult as well as the ability of the cells to respond in a graded manner to changes in their environment (Graeber and Streit 2010) and to shift within any specific stage of the response (Stout et al. 2005). The phagocytic feature of these cells serves to remove excess cellular debris and aberrant proteins such as apolipoprotein E (ApoE), mutant huntingtin, or amyloid beta 42 ($A\beta_{42}$).

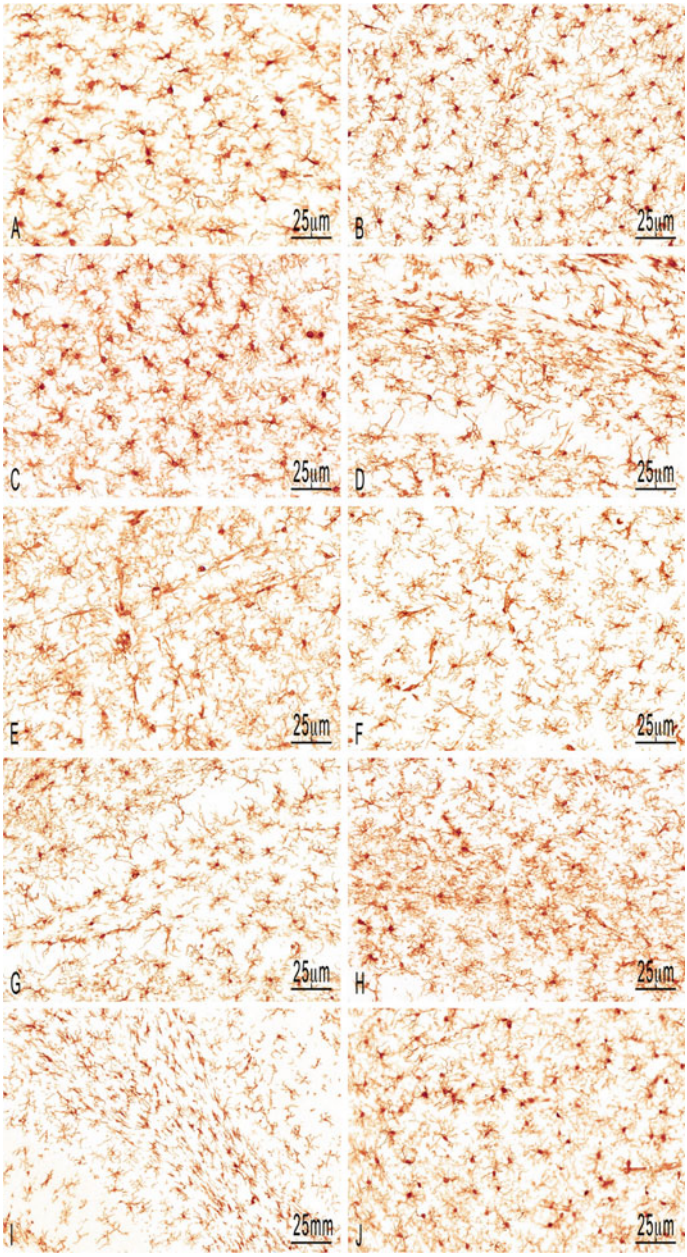

Fig. 1 Representative Iba-1+ microglia in various regions of the rat brain. (**a**) Frontal cortex, (**b**) motor cortex, (**c**) retrosplenial agranular cortex, (**d**) corpus callosum, (**e**) striatum, (**f**) thalamus, (**g**) hippocampal dentate hilus, (**h**) hippocampus molecular layer, (**i**) cerebellum, (**j**) olfactory bulb. Images from 40 m sections were scanned at 20× magnification using an Aperio Scanscope T2 Scanner (Aperio Technologies, Inc., Vista, CA) and viewed using Aperio Imagescope v. 6.25.0.1117

Microglia activities observed in neuroimmunological disorders, such as multiple sclerosis, and their related function to remove myelin debris are a classical representation of the cell's macrophage and antigen-presentation role. This is characterized by prototypical pathology dominated by perivascular mononuclear infiltrates, residual fibrosis of blood vessels, and demyelinating lesions (Barnett et al. 2009; Stadelmann 2011). In an autoimmune disease such as MS, microglia serve in reactivation of infiltrating T cells (Coyle 2011). In contrast, microglial activation observed with a sterile insult does not involve antigen-based interactions with T cells or a predominant infiltration of peripheral immune cells. A strong microglia reaction occurs at the primary site of injured neurons progressing temporally and spatially to recruit responses in areas that are projection sites to and from the area of damage (Graeber and Kreutzberg 1988; Jensen et al. 1999; Sanders and Jones 2006). While anterograde and retrograde degeneration of neuronal projections most likely causes a microglial reaction in the first-order projection areas, other mechanisms, like neuronal ionic balance, likely drive the microglia response in more distant and remote areas. Even within a focal injury site, a graded level of response can be observed. For example, with an ischemic or physical lesion to the brain, the microglia rapidly wall off areas of the CNS from non-CNS tissue and remove dead, damaged, or dysfunctional cells, while in the periphery of the injury, the cells likely perform a survival function.

4 Cell-Responsive Interactions

While a number of mechanisms have been proposed for cell-responsive interactions of microglia, many associated with ionic balance are related to the presence of inwardly rectifying potassium channels (Kettenmann et al. 1993) and multiple neurotransmitter receptors (Pocock and Kettenmann 2007). Microglia can be induced to produce several agonists of excitatory amino acid receptors including glutamate, quinolinate, and D-serine (Barger 2004). Several types of glutamate receptors are expressed, including the mGluR (metabotropic glutamate receptor) and AMPA (alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) classes (D'Antoni et al. 2008) and individual receptor subunits of the metabotropic groups I, II, and III (Byrnes et al. 2009; Noda et al. 2000). Activation of mGluR from group I or III inhibits microglia activation; group II evokes mal-activation, and activation of mGluR3 inhibits toxicity of microglia toward oligodendrocytes (Pinteaux-Jones et al. 2008). Recent work demonstrated that activation of Gai-coupled receptors, including group II and group III mGLuRs, suppresses the export of glutamate from activated microglia (McMullan et al. 2012).

A multitude of signals, both physical and secreted factors, posing a potential threat to brain homeostasis are sensed by microglial receptors (Hanisch and Kettenmann 2007). Microglial activation, in addition to being stimulus dependent, is also likely to be a multistep process (Ponomarev et al. 2006). Specific factors released by stressed or damaged neurons can stimulate the production of proinflammatory cytokines by microglia including matrix metalloproteinase-3 (MMP-3), α-synuclein, neuromelanin, and adenosine triphosphate (ATP). In an in vitro transmigration assay, conditioned media from mesencephalic cultures

treated with MPP+ (1-methyl-4-phenylpyridinium) were sufficient to trigger microglia attraction. This response occurred even at early stages of the neuronal injury, suggesting signaling from the neuron to trigger microglia activation. The rapid response to injury of directed process extension relies on purinergic signaling mediated by ATP and P2 (purinergic) receptors. In addition to local process extension, microglia can sense a chemical gradient of ATP and exhibit chemotaxis to the source. The P2Y12 receptors mediate chemotaxis and the P2Y6 receptors stimulate phagocytosis (Koizumi et al. 2007; Langosch et al. 1994).

Depending upon the stimulus, inflammatory responses by microglia can be initiated by pattern recognition receptors (PRRs) that include Toll-like receptors (TLRs), the receptor for advanced glycation end products (RAGE), and scavenger receptors to detect the aberrant expression of phosphatidylserine on the extracellular surface of dying cells. Microglia detect ligands for CD40, CD91, and the intracellular NOD-like receptors (NLRs) that initiate the signaling process by binding to PAMPs. Ligation of PRRs leads to the activation of signal transduction pathways and regulation of diverse transcriptional and posttranscriptional molecules including members of the nuclear factor kappa B (NF-κB), activator protein 1, and interferon regulator factor families, which modulate proinflammatory target genes encoding cytokines, chemokines, enzymes, and other molecules essential for pathogen elimination (Akira et al. 2006).

TLRs are a major family of PRRs for a diverse set of novel pathogen-associated molecules (Takeuchi and Akira 2001) that, with their related signaling proteins, are expressed in the CNS (Bowman et al. 2003; Kielian et al. 2002) with microglia expressing TLRs 1–9 (Farina et al. 2005). In vivo, TLR2 expression is exclusively in microglia activated with cerebral ischemia (Lehnardt et al. 2007) or entorhinal cortex lesion (Babcock et al. 2006). Microglia derived from human white matter and in primary rat cell cultures express TLR4 (Lehnardt et al. 2003; Visintin et al. 2001) that recognizes the fragment of gram-negative bacteria and LPS and can initiate innate immune responses to infection in mammals. A large proportion of the data suggests that TLR signaling mediates beneficial effects essential for pathogen elimination, but additional data suggests that TLR-induced microglia activation and release of proinflammatory molecules can contribute to neurotoxicity. Based upon studies examining the various TLRs, it has been suggested that activation of innate immune responses in the brain is tailored according to the cell type and environmental signal. As an example, TLR3 signaling induces a strong proinflammatory response in microglia as characterized by the secretion of IL-12, TNF-α, IL-6, CXCL-10, and interferon (IFN)-β. TLR2-mediated responses are primarily associated with secretion of IL-6 and IL-1β (Jack et al. 2005).

More recently, PRRs have been found to respond to factors released from necrotic cells and by molecules that may be secondary to a pathogenic process and may facilitate neuronal damage. TLRs, especially TLR2 and TLR4, are capable of sensing damage induced by ischemia and, as such, boost the proinflammatory response such that the infarct size is increased (Caso et al. 2008; Kilic et al. 2008; Lehnardt et al. 2007). There are several host-derived ligands for TLR. One of these, heat shock protein (HSP)60, is released from dying CNS cells and, upon binding to

microglia, TLR4- and MyD88-dependent secretion of NO is induced (Lehnardt et al. 2008). Similarly, necrotic neurons have been shown to activate microglia in an MyD88-dependent manner with the subsequent proinflammatory response leading to an increased neurotoxic activity through the induction of the glutamate enzyme, glutaminase (Pais et al. 2008). While TLR activation can contribute to neurotoxicity during CNS infection, there is evidence that TLR signaling can mediate beneficial effects (Glezer et al. 2006). Microglia appear to be the major initial sensors of danger or stranger signals recognized by TLR4, and they secrete inflammatory mediators such as TNF-α and IL-1β. These cytokines can then act on astrocytes to induce a secondary inflammatory or growth factor repair response (Saijo et al. 2009). With an acute injury, the release of TNF from microglia may serve to counter any secondary injury (Nawashiro et al. 1997), while activation of microglia to remove cellular debris following acute damage may serve to prevent subsequent tissue inflammation (Glezer et al. 2007; McPherson et al. 2011). TLR4 is also used by microglia and astrocytes to detect Aβ (Landreth and Reed-Geaghan 2009), leading to activation of signal-dependent transcription factors for downstream inflammatory response genes and clearance of aberrant proteins (Reed-Geaghan et al. 2009; Walter et al. 2007). Supporting data suggests that stimulation of the system with TLR ligand encourages the appropriate phenotype of resident or invading monocytes to promote the clearance of Aβ (Yong and Rivest 2009).

RAGE, a cell-surface receptor belonging to the immunoglobulin superfamily (Neeper et al. 1992; Schmidt et al. 1992), is present on the surface of microglia, astrocytes, vascular endothelial cells, and neurons. Activation occurs with production of advanced glycation end products in prooxidant and inflammatory environments. RAGE contributes in the clearance of Aβ and is involved in apolipoprotein E (ApoE)-mediated cellular processing and signaling. It recognizes other ligands, including serum amyloid A, S100 protein, and high-mobility group box 1. NLRs are soluble, cytoplasmic PRRs that act as sensors of cellular damage. In Alzheimer's disease (AD), Aβ oligomers and fibrils induce lysosomal damage, triggering the NOD-like receptor, NALP3, in microglia (Halle et al. 2008). NALPs activate downstream signaling proteins, such as apoptosis-associated speck-like protein containing a caspase recruitment domain. This induces apoptosis, but it also contributes to the maturation of proinflammatory mediators like IL-1β and IL-18. In addition to peptide fragments, a decrease in cellular potassium concentration can activate NALP1 in neurons, leading to a similar activation of ASC, apoptosis, and IL-1β and IL-18 maturation.

Additional receptors that recognize apoptotic cellular material, such as phosphatidylserine on the inner membrane leaflet, are important for phagocyte clearance processes and may stimulate an anti-inflammatory response (Ravichandran 2003). Recent identification of such receptors includes T-cell immunoglobulin- and mucin-domain-containing molecule-4 (TIM4) (Miyanishi et al. 2007), the metabotropic P2Y6 receptor that recognizes the nucleotide UDP released from injured neurons (Koizumi et al. 2007), and the triggering receptor expressed on myeloid cells-2 (TREM-2; Schmid et al. 2002) to facilitate debris clearance in the absence of inflammation (Takahashi et al. 2005). A critical role for TREM-2 signaling has been demonstrated in polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy,

or Nasu-Hakola disease, with early-onset dementia possibly due to the inability of microglia to clear tissue debris via TREM-2 signaling (Neumann and Takahashi 2007). The differential expression of these receptors in response to the intensity or stage of tissue injury, type of injurious stimuli, and presence of other soluble signals can exert significant control over the potency of the microglial response.

5 Microglia Cytokine Expression and Regulation

Microglia are a source of a number of cytokines such as IL-1, IL-6, and TNF and are responsive to growth factors such as granulocyte-macrophage-CSF and CSF-1. Cytokines can induce or modulate a broad spectrum of cellular responses including cell adhesion, migration, survival, differentiation, replication, secretory function, and cell death. In the same cell, a cytokine can either induce death or promote survival or proliferation depending upon the function of the context in which it acts. Cell death can be actively triggered by a cytokine as a physiologically necessary role during development, differentiation, and in host defense responses to injury and subsequent repair. However, the same cytokine can also provide critical survival factors, and thus, its absence can result in cell death at a specific point in the cell cycle.

A specific phenotype or function has yet to be directly linked to specific altered or activated microglia morphology. A specific classification of microglia phenotypes has been proposed for microglia cells based upon peripheral myeloid cell responses and cytokine release (Martinez et al. 2008; Michelucci et al. 2009; Mosser and Edwards 2008). For example, proinflammatory (Th1, Th17) CD4+ T cells promote myeloid cells toward a classical activation state (M1 state) characterized by high expression levels of proinflammatory cytokines (IL-1β, IL-12, IL-23, IL-6, TNF-α) (Colton and Wilcock 2010; Gordon and Martinez 2010; Graeber and Streit 2010). In contrast, Th2 CD4 + T cells promote myeloid cells toward alternative activation states (M2a and M2b) characterized by expression of macrophage mannose receptor (CD206), YM-1, stabilin-1, and arginase I. Under this alternative activation, cells are functionally associated with enhanced phagocytosis, tissue repair, and parasite elimination. A regulatory activation state (M2c) is promoted by IL-10 and TGFβ characterized by expression of molecules related to tissue repair and immunosuppression. Each state may have its own particular phenotype with likely transitional phenotypes between wound healing classical and regulatory macrophages (Mosser and Edwards 2008). While this stage distinction has been established for peripheral cells, whether this will translate to the CNS has yet to be clearly established, as neither a temporal nor a spatial transition from an M1 to an M2 phenotype has been clearly described in models of neuroinflammation or neurodegeneration (Ajami et al. 2007; Colton and Wilcock 2010; Hume et al. 1983). Rather, as a general pattern, data suggest heterogenous populations of M1 and M2 microglia throughout an injury response or neurodegenerative disease process.

To understand the impact of a cytokine response, the basal level and regulatory mechanisms need to be considered. Under normal homeostatic conditions, the majority of cytokines are either not expressed or are expressed at low levels.

In the early phase, host defense responses to injury are triggered by microbial antigens, extracellular matrix antigens, or by activation products or preformed components of humoral defense systems. This process consists of cell recruitment and induction of cytokines that contribute to activation of proinflammatory, immunostimulatory, and catabolic responses. Cytokine mRNA transcripts have a short half-life of 10–30 min; thus, cytokines are rapidly translated and secreted into the extracellular space, producing a burst of cytokine release. Some cytokines are secreted upon complete intracellular processing, while others are stored intracellularly, and additional stimuli are required to trigger secretion. For example, transcription without translation can be observed for IL-1 following such things as adherence of blood monocytes to surfaces or exposure to calcium ionophore. In these cases, steady-state mRNA levels for IL-1β are comparable with those following induction by endotoxin; however, there is no protein translation. Alternatively, the system may effectively regulate the downstream effects of IL-1 by the induction of IL-1 receptor antagonist to bind the protein and prevent receptor activation.

Most cytokines are biologically active when secreted; however, some, such as TNF-α and transforming growth factor (TGF)β, are secreted in a biologically inactive or latent form and require further extracellular processing by proteases. Cytokines regulate the expression of cytokine receptors involved in the modulation of activity. For IL-1 and TNF-α, these effects are mediated primarily by IL-1 type I receptor and TNF receptor TNFR1 (p55) or TNFR2 (p75), respectively. IL-6 and other members of this family, oncostatin M, leukemia inhibitory factor, IL-11, and ciliary neurotrophic factor, are induced by IL-1 and TNF and protect against the proinflammatory and catabolic effects. As one primary example, microglial functions related to an innate immune response are associated with TNF signaling. TNF can induce apoptosis via activation of receptors containing an intracellular death domain such as TNFR1 and CD95 (APO-1/Fas) with their corresponding death ligands, TNF, and the structurally related type II transmembrane protein, FasL. Release of TNF-α and FasL shedding by microglia are implicated in neurotoxicity. Signaling by TNF is dependent upon constitutive and induced target cell expression of TNFRs (Tartaglia and Goeddel 1992). Membrane receptor mechanisms of apoptosis are implicated in neuronal death, while soluble TNFR1 can offer protection by binding TNF and preventing receptor signaling. TNFR1 activation has been proposed as a molecular mechanism for rapid apoptosis of injured or sick neurons through a caspase 3-mediated pathway (Yang et al. 2002). Recent studies examining cell death and survival following an ischemic insult or induced by a systemic injection of the organometal, trimethyltin, suggest that the level of TNF-α produced by microglia and the neuronal expression pattern of TNF receptors serve as critical determining factors for neuronal death (Harry et al. 2008).

6 Release of Factors Associated with Oxidative Stress

In vitro, microglia are capable of releasing several potentially cytotoxic substances, such as free oxygen intermediates, NO, proteases, arachidonic acid derivates,

excitatory amino acids, quinolinic acid, prostaglandins, cytokines, and other factors that, if occurring in vivo, may contribute to the exacerbation of neurons already compromised by disease or injury. In parallel with the production of proinflammatory cytokines, microglial neuroinflammation is commonly associated with the production of ROS and NO-dependent reactive nitrogen species (RNS). In excess concentrations, NO forms peroxynitrite and, through nitrosylation of cell signaling messengers, functions in immune regulation (Guix et al. 2005). ROS are oxygen-containing molecules that react with and oxidize vulnerable cellular constituents, including proteins, nucleic acids, and lipids. In neural tissues, oxidative stress can result in disrupted signaling processes and ion homeostasis and is held accountable for events ranging from protein mis-folding to death of newly generated neurons (Lipton et al. 2007; Taupin 2010). Superoxide, in particular, is implicated in microglial activation, cellular redox imbalance, and associated neurodegeneration. The translation of these processes to humans is hindered by the fact that levels induced by activating stimuli significantly differ across species and are relatively low in humans (Colton et al. 1996).

Endogenously produced ROS and RNS are essential components of brain homeostatic processes. ROS are commonly released by microglia to eliminate pathogens and elicit the controlled destruction of neuronal debris. In general, superoxide production by myeloid cells is mediated by the phagocytic NADPH oxidase (NOX2), consisting of membrane-bound (gp91 and p22) and cytosolic (p47, p67, and p40) subunits, as well as a requirement for the GTPase, Rac, for full activity (Lambeth 2004; Sumimoto et al. 2004). The microglial respiratory burst in response to certain tissue-disrupting stimuli involves Ca^{2+} and K^{+} channels and NADPH-dependent signaling to induce the release of superoxide (Colton et al. 1994; Khanna et al. 2001). Opening of cell-surface ion channels on microglia specific for induction of ROS as opposed to NO, including P2X7, Kv1.3, TRPV1, and KCa3.1, causes an "acute phase" of activation, often involving protein kinase C (PKC)-dependent signaling to NADPH oxidase and downstream NF-κB prior to superoxide release and gene induction (Fordyce et al. 2005; Infanger et al. 2006; Parvathenani et al. 2003; Schilling and Eder 2010).

NOX2 can be activated in mononuclear cells by TNF-α, IFN-γ, IL-1β, prion protein, ATP, and fibrillar Aβ. In addition, although phagocytosis can cause activation of NOX2, microglia do not uniformly undergo a respiratory burst when they initiate a phagocytic action (Savill et al. 2003). Notably, activation of NOX2 alone is not always sufficient to cause neurotoxicity (Mander and Brown 2005), suggesting that microglial superoxide production may have to occur in connection with other stressors to induce neurodegeneration. Some of these other factors that may contribute to neurodegeneration include free radicals (i.e., hypochlorous acid and reactive nitrites) produced via myeloperoxidase activity in astrocytes or peripheral leukocytes (Choi et al. 2003; Chung et al. 2010), and ROS-stimulated microglial proliferation and cytokine production (Jekabsone et al. 2006; Mander et al. 2006). The work of Barger et al. (2007) suggested that glutathione (GSH) depletion and oxidative stress resulting from the NADPH-dependent respiratory burst induce glutamate release from microglia, resulting in neuronal loss. Induction

of NADPH oxidases has been shown to be involved in the neurotoxic response in various Parkinson's disease (PD)-type models, including LPS, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat, with a possible requirement for protein kinase C (PKC) delta phosphorylation of p67 and p47 (Miller et al. 2009; Qin et al. 2004; Tieu et al. 2003).

A key event in a ROS stress response is the induction of heme oxygenase-1 (HO-1) in microglia and other cells that can help protect against subsequent insults (Lee and Suk 2007). Although increased HO-1 activity and CO production can have neurotoxic effects in response to select insults, the regulated activation of this system in microglia is hypothesized to be involved in resolving neuroinflammation and preventing accessory tissue damage (Syapin 2008). While NO-mediated stress is implicated in the progression of multiple brain insults, including AD, PD, and stroke (Guix et al. 2005), it can also induce neuroprotective signaling events such as inactivation of caspases, modulate release of neurotransmitters (e.g., dopamine, acetylcholine, GABA, and glutamate), and regulate synaptic plasticity by facilitating the induction of long-term potentiation via soluble guanylyl cyclase (Bon and Garthwaite 2003; Calabrese et al. 2007). Inducible NOS (iNOS; NOS2) present in microglia is Ca^{2+} independent and can be upregulated in response to TNF-α and IFN-γ or downregulated in response to TGF-β and IL-1β (Tichauer et al. 2007). Microglial NO can be cytoprotective and neuroprotective against select insults, such as ischemia. At high levels, iNOS can be cytotoxic to oligodendrocytes and can lead to excitotoxicity and/or synapse strengthening and influence BBB permeability. Thus, the induction of an oxidative stress response in microglia may likely underlie much of the neurodestructive effects attributed to microglia activation.

7 Microglia Aging and Senescence

The progressive, age-related change in MHC II antigen expression by microglia led to the assumption that microglial activation occurs with aging. The expression pattern was reported as similar to what occurs in experimental animal models of injury (Letiembre et al. 2007; Perry et al. 2002). Systemic challenge of aged mice with LPS suggested a primed phenotype for the microglia based upon the elevated basal level of cytokine synthesis as compared to young animals (Dilger and Johnson 2008). Further investigation showed morphological changes of microglia cytoplasmic structure in the aged human brain reflective of dystrophy and senescence but no evidence of priming (Sheng et al. 1998). Thus, while the cells change as a function of age, the morphological phenotype is suggestive of a decreased ability to mount a normal host-resistance response rather than a transformation to an activated cell. It has been suggested that age-related neurodegeneration might be due to not only a loss of neuroprotective properties but also the actual loss of microglia (Streit 2006; Streit and Xue 2012). One of the more prominent features of microglia is their function to clear aberrant proteins and debris from the CNS. Thus, the loss of efficiency in this function would result in a more toxic microenvironment.

A recent hypothesis put forth to explain phenotypic changes in aging microglia suggests that microglial senescence is triggered by an intracellular oxidative stress response due to enhanced intracellular accumulation of iron (Streit 2006). Under this hypothesis, a prolonged oxidative stress response would lead to senescence rather than activation. Additional support for microglial replicative senescence in normal and pathological aging comes from the demonstration of telomere shortening and decreased telomerase activity with aging. Whether this occurs with age-related diseases remains in question; however, data suggest that microglia from AD brains have shorter telomerase, as compared to control brains (Streit 2006). Using Aβ clearance as a phagocytic function of microglia that may be compromised with aging, a number of studies have examined a possible cellular process that could contribute to plaque accumulation in aging brains. Microglia cultured from aged mice showed a diminished ability to internalize Aβ peptide. CD47-dependent ability to phagocytize Aβ fibrils seen in postnatal day 0 microglia was lost in microglia isolated from 6-month-old mice and appeared to manifest between the ages of 2 and 6 months. This effect was not due to a generalized loss of phagocytic capability as no age-related difference was observed in clearance of bacteria but rather appeared specific for Aβ. In mouse models of AD, the increased plaque deposition that can occur as early as 6 months of age may be related to the loss of selective phagocytic function of microglia (Westerman et al. 2002). It has also been reported that between 6 and 18 months of age, microglia show an increase in mRNA levels for the alternative activation marker, YM-1. By comparison in a transgenic AD mouse line expressing human mutant amyloid precursor protein (APP) and presenilin 1 (PS1), significantly higher levels of YM-1 mRNA were demonstrated at 6 months of age (Jimenez et al. 2008). In contrast to previous reports suggesting that mRNA levels for proinflammatory cytokines increased with age, AD transgenic but not wild-type microglia displayed decreased Aβ phagocytic ability and increased mRNA levels of classic activation markers such as TNF-α at 18 months in non-plaque areas of the brain. One hypothesis put forth is that interactions of Aβ peptide with other inflammatory stimuli in the diseased brain drive the cells to an early and maintained alternative activation state that shifts the ability of the cells to respond in a normal manner (Floden and Combs 2011). In support of an age-related decrease in responsiveness of microglia, ApoE4 transgenic mice displayed a deficit in the resident microglia host-resistance and proinflammatory cytokine response to injury as a function of age (Harry et al. 2000). The deficit was not observed at 2 months of age but only became apparent at 8 months of age.

8 Acute Injury

Several studies demonstrate that, with acute injury, microglia can be destructive or protective (Butovsky et al. 2005; Nelson et al. 2002). With an acute physical disruption of the brain, the cellular response of the brain macrophage is primarily limited to the immediate focal site and the surrounding parenchyma. With a slightly

deeper penetration to include the corpus callosum, responses along the myelinated tracts can be observed. The nature of a puncture wound injury involves recruitment of blood-borne cells to the reaction including leukocytes, granulocytes, and monocytes. Within the lesion itself, round hematogenous macrophages are primarily found. At the edge of the lesion, process-bearing, hypertrophic, resident microglia are evident. This initial response occurs within the first 12 h or so, peaks between days 2 and 3, and by day 5, hypertrophied microglia express MHC class II and the CD4 antigen. This response then declines over the next 5 days. With the injection of IFN-γ/LPS, the response is slightly modified given the more active recruitment of peripheral immune cells. Interactions with CD4+ T cells with influence microglial phenotype and effects on neurons with fewer neurotoxic factors produced and an increase in anti-inflammatory and growth mediators and a reverse of glutamate transporters resulting in a buffering rather than a release of glutamine (Shaked et al. 2004, 2005). Thus, acute injury models offer critical information on the interaction between peripheral and central immune cells for consideration in more delayed neuronal death or progressive neurodegenerative models.

9 Progressive Degenerative Disease States

While the presence of reactive and activated microglia has been observed in numerous animal models of human neurodegenerative disease and in patients, the overall evidence suggests that, in the context of a pathogen-free environment, a relatively benign innate immune response is evoked. However, how, and if, this environment can be altered or stressed in a manner to change this balance remains a critical question. In murine prion disease models, particular prion agents can induce pathology in specific regions of the brain. The pathology is often characterized by protease-resistant protein deposition, spreading through the brain, accompanied by an increase in morphologically activated microglia with hypertrophied cell bodies. In the ME7 model in C57BL mice, at the time of first detectable synaptic loss, microglia in the hippocampus show an activated morphology and elevated expression of antigens such as CR3, ERM1, and CD68 (Betmouni et al. 1996). With further activation and during the progression to terminal stages of the disease, an increase is observed in TGFβ, cyclooxygenase (COX)-2, and its product PGE2 and the chemokine CCL2/MCP-1 (Perry et al. 2002), which often function in an anti-inflammatory manner. While the expression of TGFβ and PGE2 often represents macrophages that have phagocytosed apoptotic cells (Savill et al. 2002), there is no evidence of neuronal apoptosis in these early stages. It is possible that the activation occurring with synapse loss reflects a microglia phenotype as a consequence of clearing degenerating synapses; however, in this model, it is the dendritic spine that envelops the degenerating synapse and not the microglia (Siskova et al. 2009).

A similar benign role has been suggested in models of AD. In postmortem brain tissue of AD patients, an atypical inflammation occurs with both activation of the resident microglia and infiltrating monocytes surrounding dense-core

amyloid-β1-40/42 plaques showing increased expression of cell-surface antigens (D'Andrea et al. 2004; McGeer and McGeer 2003; Simard et al. 2006). In transgenic mice expressing human APP, amyloid plaque formation and amyloid-associated neuritic dystrophy occur independent of microglia presence (Grathwohl et al. 2009). In many of these animal models, microglia are a major component of the dense-cored amyloid plaques but not diffuse plaques, and it has been hypothesized that in the absence of effective phagocytosis, microglia potentiate the toxicity of Aβ peptide (Streit et al. 2005). Current data support the idea that inflammatory mediators do not promote Aβ accumulation but limit Aβ deposition (Chakrabarty et al. 2010). While the phagocytic activation serves in a beneficial manner to eliminate aggregated and toxic proteins from the brain, it has been proposed that an extended activation or dysregulation of this system can lead to an adverse environment and toxicity to surrounding cells. A recent study using the Tg-SwDI mice as a model for AD demonstrated that activated microglia localized around microvascular amyloid deposits increased synthesis of native complement proteins C1q, C3, and C4 (Fan et al. 2007). In transgenic mouse models of AD, less neuropathology is demonstrated in the absence of C1q; however, inhibition of complement activation diminishes microglial activity, increases amyloid load, and enhances neuronal degeneration (Fonseca et al. 2004; Wyss-Coray et al. 2002).

In PD, dopaminergic and non-dopaminergic neurons in the nigrostriatal pathway are compromised, contributing to the motor-related symptoms. A number of mechanisms have been proposed for the death of dopamine-containing neurons in the SN pars compacta including mitochondrial dysfunction, oxidative stress, and impairment of protein degradation. The detection of elevated levels of proinflammatory cytokines and evidence of oxidative stress-mediated damage in postmortem PD brains gave rise to the hypothesis of a microglia involvement. However, observations of the presence of cytotoxic T cells in the SN of a patient with PD and an increased density in insulin-like growth factor γ+ cells in the brains of PD patients suggest a role for the cellular arm of the adaptive immune system. A critical role for T cells was demonstrated in two different strains of immunodeficient mice lacking mature T lymphocytes (*Tcrb*−/− and *Rag1*−/− mice) by a reduction in MPTP-induced dopaminergic cell death. Further work demonstrated CD4+ T cell-mediated cytotoxicity of MPTP (Brochard et al. 2009) possibly via microglia activation and release of free radicals and proinflammatory cytokines. However, the recent description of an IL-17 secreting Th17 lymphocyte suggests an alternative cellular source for proinflammatory cytokines. One could envision that with progressive changes in the neuron, a microglia response to α-synuclein occurs, recruiting CD4+ T cells, leading to an induction of proinflammatory cytokines and NO secretion by microglia alternatively or in conjunction with the infiltration of TH17 lymphocytes. When a progressive model of PD was examined with reference to the adverse impact of microglial activation and inflammation on dopaminergic neurons, Wright and colleagues (2008) demonstrated in vivo that while the immunosuppressant drug, tacrolimus (FK506), reduced the initial stage of neuronal death in the SN, it did not alter the morphological response of microglia, suggesting a lack of direct correlation between the two cellular responses.

10 Conclusion

The fact that microglial activation correlates with neurodegenerative disease progression has led to the hypothesis of a causal link; however, the rapid nature of microglia response to neuronal injury, both physical and chemical signals, and death makes it difficult to determine an initiating role for microglia. There is a critical need to gain a better understanding of the plethora of responses representing the dichotomy of microglial reactivity for beneficial versus destructive effects. Identifying what can trigger a microglia response and identifying characteristics of the various types of responses will further advance the ability to interpret the role being played by microglia under normal and aberrant brain conditions.

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References

Ajami, B., Bennett, J. L., Krieger, C., Tetzlaff, W., & Rossi, F. M. (2007). Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nature Neuroscience, 10*(12), 1538–1543.

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell, 124*(4), 783–801.

Babcock, A. A., Wirenfeldt, M., Holm, T., Nielsen, H. H., Dissing-Olesen, L., Toft-Hansen, H., Millward, J. M., Landmann, R., Rivest, S., Finsen, B., & Owens, T. (2006). Toll-like receptor 2 signaling in response to brain injury: An innate bridge to neuroinflammation. *Journal of Neuroscience, 26*(49), 12826–12837.

Barger, S. W. (2004). An unconventional hypothesis of oxidation in Alzheimer's disease: Intersections with excitotoxicity. *Frontiers in Bioscience, 9*, 3286–3295.

Barger, S. W., Goodwin, M. E., Porter, M. M., & Beggs, M. L. (2007). Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *Journal of Neurochemistry, 101*(5), 1205–1213.

Barnett, M. H., Parratt, J. D., Pollard, J. D., & Prineas, J. W. (2009). MS: Is it one disease? *International MS journal//MS Forum, 16*(2), 57–65.

Betmouni, S., Perry, V. H., & Gordon, J. L. (1996). Evidence for an early inflammatory response in the central nervous system of mice with scrapie. *Neuroscience, 74*(1), 1–5.

Biber, K., Neumann, H., Inoue, K., & Boddeke, H. W. (2007). Neuronal 'On' and 'Off' signals control microglia. *Trends in Neurosciences, 30*(11), 596–602.

Bon, C. L., & Garthwaite, J. (2003). On the role of nitric oxide in hippocampal long-term potentiation. *Journal of Neuroscience, 23*(5), 1941–1948.

Bowman, C. C., Rasley, A., Tranguch, S. L., & Marriott, I. (2003). Cultured astrocytes express toll-like receptors for bacterial products. *Glia, 43*(3), 281–291.

Brochard, V., Combadiere, B., Prigent, A., Laouar, Y., Perrin, A., Beray-Berthat, V., Bonduelle, O., Alvarez-Fischer, D., Callebert, J., Launay, J. M., Duyckaerts, C., Flavell, R. A., Hirsch, E. C., & Hunot, S. (2009). Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *The Journal of Clinical Investigation, 119*(1), 182–192.

Butovsky, O., Talpalar, A. E., Ben-Yaakov, K., & Schwartz, M. (2005). Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. *Molecular and Cellular Neurosciences, 29*(3), 381–393.

Byrnes, K. R., Loane, D. J., & Faden, A. I. (2009). Metabotropic glutamate receptors as targets for multipotential treatment of neurological disorders. *Neurotherapeutics, 6*(1), 94–107.

Calabrese, V., Mancuso, C., Calvani, M., Rizzarelli, E., Butterfield, D. A., & Stella, A. M. (2007). Nitric oxide in the central nervous system: Neuroprotection versus neurotoxicity. *Nature Reviews Neuroscience, 8*(10), 766–775.

Cardona, A. E., Pioro, E. P., Sasse, M. E., Kostenko, V., Cardona, S. M., Dijkstra, I. M., Huang, D., Kidd, G., Dombrowski, S., Dutta, R., Lee, J. C., Cook, D. N., Jung, S., Lira, S. A., Littman, D. R., & Ransohoff, R. M. (2006). Control of microglial neurotoxicity by the fractalkine receptor. *Nature Neuroscience, 9*(7), 917–924.

Carson, M. J., Doose, J. M., Melchior, B., Schmid, C. D., & Ploix, C. C. (2006). CNS immune privilege: Hiding in plain sight. *Immunological Reviews, 213*, 48–65.

Caso, J. R., Pradillo, J. M., Hurtado, O., Leza, J. C., Moro, M. A., & Lizasoain, I. (2008). Toll-like receptor 4 is involved in subacute stress-induced neuroinflammation and in the worsening of experimental stroke. *Stroke, 39*(4), 1314–1320.

Chakrabarty, P., Jansen-West, K., Beccard, A., Ceballos-Diaz, C., Levites, Y., Verbeeck, C., Zubair, A. C., Dickson, D., Golde, T. E., & Das, P. (2010). Massive gliosis induced by interleukin-6 suppresses Abeta deposition in vivo: Evidence against inflammation as a driving force for amyloid deposition. *The FASEB Journal, 24*(2), 548–559.

Choi, S. H., Lee, D. Y., Ryu, J. K., Kim, J., Joe, E. H., & Jin, B. K. (2003). Thrombin induces nigral dopaminergic neurodegeneration in vivo by altering expression of death-related proteins. *Neurobiology of Disease, 14*(2), 181–193.

Chung, Y. C., Kim, S. R., & Jin, B. K. (2010). Paroxetine prevents loss of nigrostriatal dopaminergic neurons by inhibiting brain inflammation and oxidative stress in an experimental model of Parkinson's disease. *Journal of Immunology, 185*(2), 1230–1237.

Colton, C., Wilt, S., Gilbert, D., Chernyshev, O., Snell, J., & Dubois-Dalcq, M. (1996). Species differences in the generation of reactive oxygen species by microglia. *Molecular and Chemical Neuropathology, 28*(1–3), 15–20.

Colton, C. A., Jia, M., Li, M. X., & Gilbert, D. L. (1994). K^+ modulation of microglial superoxide production: Involvement of voltage-gated Ca^{2+} channels. *American Journal of Physiology, 266* (6 Pt 1), C1650–C1655.

Colton, C. A., & Wilcock, D. M. (2010). Assessing activation states in microglia. *CNS & Neurological Disorders Drug Targets, 9*(2), 174–191.

Coyle, P. K. (2011). Dissecting the immune component of neurologic disorders: A grand challenge for the 21st century. *Frontiers in Neurology, 2*, 37.

Cuadros, M. A., & Navascues, J. (1998). The origin and differentiation of microglial cells during development. *Progress in Neurobiology, 56*(2), 173–189.

D'Andrea, M. R., Cole, G. M., & Ard, M. D. (2004). The microglial phagocytic role with specific plaque types in the Alzheimer disease brain. *Neurobiology of Aging, 25*(5), 675–683.

D'Antoni, S., Berretta, A., Bonaccorso, C. M., Bruno, V., Aronica, E., Nicoletti, F., & Catania, M. V. (2008). Metabotropic glutamate receptors in glial cells. *Neurochemical Research, 33*(12), 2436–2443.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., Littman, D. R., Dustin, M. L., & Gan, W. B. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nature Neuroscience, 8*(6), 752–758.

Davoust, N., Vuaillat, C., Androdias, G., & Nataf, S. (2008). From bone marrow to microglia: Barriers and avenues. *Trends in Immunology, 29*(5), 227–234.

Dilger, R. N., & Johnson, R. W. (2008). Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system. *Journal of Leukocyte Biology, 84*(4), 932–939.

Eugenin, E. A., Eckardt, D., Theis, M., Willecke, K., Bennett, M. V., & Saez, J. C. (2001). Microglia at brain stab wounds express connexin 43 and in vitro form functional gap junctions after treatment with interferon-gamma and tumor necrosis factor-alpha. *Proceedings of the National Academy of Sciences of the United States of America, 98*(7), 4190–4195.

Fan, R., DeFilippis, K., & Van Nostrand, W. E. (2007). Induction of complement proteins in a mouse model for cerebral microvascular A beta deposition. *Journal of Neuroinflammation, 4*, 22.

Farina, C., Krumbholz, M., Giese, T., Hartmann, G., Aloisi, F., & Meinl, E. (2005). Preferential expression and function of Toll-like receptor 3 in human astrocytes. *Journal of Neuroimmunology, 159*(1–2), 12–19.

Floden, A. M., & Combs, C. K. (2011). Microglia demonstrate age-dependent interaction with amyloid-beta fibrils. *Journal of Alzheimer's Disease, 25*(2), 279–293.

Fonseca, M. I., Zhou, J., Botto, M., & Tenner, A. J. (2004). Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimer's disease. *Journal of Neuroscience, 24*(29), 6457–6465.

Fordyce, C. B., Jagasia, R., Zhu, X., & Schlichter, L. C. (2005). Microglia Kv1.3 channels contribute to their ability to kill neurons. *Journal of Neuroscience, 25*(31), 7139–7149.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M. F., Conway, S. J., Ng, L. G., Stanley, E. R., Samokhvalov, I. M., & Merad, M. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science, 330*(6005), 841–845.

Glezer, I., Lapointe, A., & Rivest, S. (2006). Innate immunity triggers oligodendrocyte progenitor reactivity and confines damages to brain injuries. *The FASEB Journal, 20*(6), 750–752.

Glezer, I., Simard, A. R., & Rivest, S. (2007). Neuroprotective role of the innate immune system by microglia. *Neuroscience, 147*(4), 867–883.

Gordon, S., & Martinez, F. O. (2010). Alternative activation of macrophages: Mechanism and functions. *Immunity, 32*(5), 593–604.

Graber, D. J., Park, P. J., Hickey, W. F., & Harris, B. T. (2011). Synthetic triterpenoid CDDO derivatives modulate cytoprotective or immunological properties in astrocytes, neurons, and microglia. *Journal of Neuroimmune Pharmacology, 6*(1), 107–120.

Graeber, M. B., & Kreutzberg, G. W. (1988). Delayed astrocyte reaction following facial nerve axotomy. *Journal of Neurocytology, 17*(2), 209–220.

Graeber, M. B., & Streit, W. J. (2010). Microglia: Biology and pathology. *Acta Neuropathologica, 119*(1), 89–105.

Graeber, M. B., Streit, W. J., & Kreutzberg, G. W. (1990). The third glial cell type, the microglia: Cellular markers of activation in situ. *Acta Histochemica. Supplementband, 38*, 157–160.

Grathwohl, S. A., Kalin, R. E., Bolmont, T., Prokop, S., Winkelmann, G., Kaeser, S. A., Odenthal, J., Radde, R., Eldh, T., Gandy, S., Aguzzi, A., Staufenbiel, M., Mathews, P. M., Wolburg, H., Heppner, F. L., & Jucker, M. (2009). Formation and maintenance of Alzheimer's disease beta-amyloid plaques in the absence of microglia. *Nature Neuroscience, 12*(11), 1361–1363.

Guix, F. X., Uribesalgo, I., Coma, M., & Munoz, F. J. (2005). The physiology and pathophysiology of nitric oxide in the brain. *Progress in Neurobiology, 76*(2), 126–152.

Halle, A., Hornung, V., Petzold, G. C., Stewart, C. R., Monks, B. G., Reinheckel, T., Fitzgerald, K. A., Latz, E., Moore, K. J., & Golenbock, D. T. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nature Immunology, 9*(8), 857–865.

Hanisch, U. K., & Kettenmann, H. (2007). Microglia: Active sensor and versatile effector cells in the normal and pathologic brain. *Nature Neuroscience, 10*(11), 1387–1394.

Harry, G. J., d'Hellencourt, C. L., Bruccoleri, A., & Schmechel, D. (2000). Age-dependent cytokine responses: Trimethyltin hippocampal injury in wild-type, APOE knockout, and APOE4 mice. *Brain, Behavior, and Immunity, 14*(4), 288–304.

Harry, G. J., d'Hellencourt, C. L., McPherson, C. A., Funk, J. A., Aoyama, M., & Wine, R. N. (2008). Tumor necrosis factor p55 and p75 receptors are involved in chemical-induced apoptosis of dentate granule neurons. *Journal of Neurochemistry, 106*(1), 281–298.

Hoek, R. M., Ruuls, S. R., Murphy, C. A., Wright, G. J., Goddard, R., Zurawski, S. M., Blom, B., Homola, M. E., Streit, W. J., Brown, M. H., Barclay, A. N., & Sedgwick, J. D. (2000).

Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science, 290*(5497), 1768–1771.

Hume, D. A., Perry, V. H., & Gordon, S. (1983). Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: Phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. *The Journal of Cell Biology, 97*(1), 253–257.

Infanger, S. W., Sharma, R. V., & Davisson, R. L. (2006). NADPH oxidases of the brain: Distribution, regulation, and function. *Antioxidants & Redox Signaling, 8*(9–10), 1583–1596.

Jack, C. S., Arbour, N., Manusow, J., Montgrain, V., Blain, M., McCrea, E., Shapiro, A., & Antel, J. P. (2005). TLR signaling tailors innate immune responses in human microglia and astrocytes. *Journal of Immunology, 175*(7), 4320–4330.

Jekabsone, A., Mander, P. K., Tickler, A., Sharpe, M., & Brown, G. C. (2006). Fibrillar beta-amyloid peptide $Abeta_{1-40}$ activates microglial proliferation via stimulating TNF-alpha release and H_2O_2 derived from NADPH oxidase: A cell culture study. *Journal of Neuroinflammation, 3*, 24.

Jensen, M. B., Hegelund, I. V., Poulsen, F. R., Owens, T., Zimmer, J., & Finsen, B. (1999). Microglial reactivity correlates to the density and the myelination of the anterogradely degenerating axons and terminals following perforant path denervation of the mouse fascia dentata. *Neuroscience, 93*(2), 507–518.

Jimenez, S., Baglietto-Vargas, D., Caballero, C., Moreno-Gonzalez, I., Torres, M., Sanchez-Varo, R., Ruano, D., Vizuete, M., Gutierrez, A., & Vitorica, J. (2008). Inflammatory response in the hippocampus of PS1M146L/APP751SL mouse model of Alzheimer's disease: Age-dependent switch in the microglial phenotype from alternative to classic. *Journal of Neuroscience, 28*(45), 11650–11661.

Kaur, C., & Ling, E. A. (1991). Study of the transformation of amoeboid microglial cells into microglia labelled with the isolectin *Griffonia simplicifolia* in postnatal rats. *Acta Anatomica (Basel), 142*(2), 118–125.

Kettenmann, H., Banati, R., & Walz, W. (1993). Electrophysiological behavior of microglia. *Glia, 7*(1), 93–101.

Khanna, R., Roy, L., Zhu, X., & Schlichter, L. C. (2001). K+ channels and the microglial respiratory burst. *American Journal of Physiology. Cell Physiology, 280*(4), C796–C806.

Kielian, T., Mayes, P., & Kielian, M. (2002). Characterization of microglial responses to Staphylococcus aureus: Effects on cytokine, costimulatory molecule, and Toll-like receptor expression. *Journal of Neuroimmunology, 130*(1–2), 86–99.

Kilic, U., Kilic, E., Matter, C. M., Bassetti, C. L., & Hermann, D. M. (2008). TLR-4 deficiency protects against focal cerebral ischemia and axotomy-induced neurodegeneration. *Neurobiology of Disease, 31*(1), 33–40.

Koizumi, S., Shigemoto-Mogami, Y., Nasu-Tada, K., Shinozaki, Y., Ohsawa, K., Tsuda, M., Joshi, B. V., Jacobson, K. A., Kohsaka, S., & Inoue, K. (2007). UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. *Nature, 446*(7139), 1091–1095.

Lambeth, J. D. (2004). NOX enzymes and the biology of reactive oxygen. *Nature Reviews Immunology, 4*(3), 181–189.

Landreth, G. E., & Reed-Geaghan, E. G. (2009). Toll-like receptors in Alzheimer's disease. *Current Topics in Microbiology and Immunology, 336*, 137–153.

Langosch, J. M., Gebicke-Haerter, P. J., Norenberg, W., & Illes, P. (1994). Characterization and transduction mechanisms of purinoceptors in activated rat microglia. *British Journal of Pharmacology, 113*(1), 29–34.

Lassmann, H., Zimprich, F., Vass, K., & Hickey, W. F. (1991). Microglial cells are a component of the perivascular glia limitans. *Journal of Neuroscience Research, 28*(2), 236–243.

Lawson, L. J., Perry, V. H., Dri, P., & Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience, 39*(1), 151–170.

Lee, S., & Suk, K. (2007). Heme oxygenase-1 mediates cytoprotective effects of immunostimulation in microglia. *Biochemical Pharmacology, 74*(5), 723–729.

Lehnardt, S., Lehmann, S., Kaul, D., Tschimmel, K., Hoffmann, O., Cho, S., Krueger, C., Nitsch, R., Meisel, A., & Weber, J. R. (2007). Toll-like receptor 2 mediates CNS injury in focal cerebral ischemia. *Journal of Neuroimmunology, 190*(1–2), 28–33.

Lehnardt, S., Massillon, L., Follett, P., Jensen, F. E., Ratan, R., Rosenberg, P. A., Volpe, J. J., & Vartanian, T. (2003). Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. *Proceedings of the National Academy of Sciences of the United States of America, 100*(14), 8514–8519.

Lehnardt, S., Schott, E., Trimbuch, T., Laubisch, D., Krueger, C., Wulczyn, G., Nitsch, R., & Weber, J. R. (2008). A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. *Journal of Neuroscience, 28*(10), 2320–2331.

Letiembre, M., Hao, W., Liu, Y., Walter, S., Mihaljevic, I., Rivest, S., Hartmann, T., & Fassbender, K. (2007). Innate immune receptor expression in normal brain aging. *Neuroscience, 146*(1), 248–254.

Lipton, S. A., Gu, Z., & Nakamura, T. (2007). Inflammatory mediators leading to protein misfolding and uncompetitive/fast off-rate drug therapy for neurodegenerative disorders. *International Review of Neurobiology, 82*, 1–27.

Lo, D., Feng, L., Li, L., Carson, M. J., Crowley, M., Pauza, M., Nguyen, A., & Reilly, C. R. (1999). Integrating innate and adaptive immunity in the whole animal. *Immunological Reviews, 169*, 225–239.

Mander, P., & Brown, G. C. (2005). Activation of microglial NADPH oxidase is synergistic with glial iNOS expression in inducing neuronal death: A dual-key mechanism of inflammatory neurodegeneration. *Journal of Neuroinflammation, 2*, 20.

Mander, P. K., Jekabsone, A., & Brown, G. C. (2006). Microglia proliferation is regulated by hydrogen peroxide from NADPH oxidase. *Journal of Immunology, 176*(2), 1046–1052.

Marin-Padilla, M. (1985). Early vascularization of the embryonic cerebral cortex: Golgi and electron microscopic studies. *The Journal of Comparative Neurology, 241*(2), 237–249.

Martinez, F. O., Sica, A., Mantovani, A., & Locati, M. (2008). Macrophage activation and polarization. *Frontiers in Bioscience, 13*, 453–461.

McGeer, E. G., & McGeer, P. L. (2003). Inflammatory processes in Alzheimer's disease. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27*(5), 741–749.

McMullan, S. M., Phanavanh, B., Li, G. G., & Barger, S. W. (2012). Metabotropic glutamate receptors inhibit microglial glutamate release. *ASN NEURO*, *4*(5):art:e00094.doi:10.1042/AN20120044

McPherson, C. A., Kraft, A. D., & Harry, G. J. (2011). Injury-induced neurogenesis: Consideration of resident microglia as supportive of neural progenitor cells. *Neurotoxicity Research, 19*(2), 341–352.

Michelucci, A., Heurtaux, T., Grandbarbe, L., Morga, E., & Heuschling, P. (2009). Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-beta. *Journal of Neuroimmunology, 210*(1–2), 3–12.

Miller, R. L., James-Kracke, M., Sun, G. Y., & Sun, A. Y. (2009). Oxidative and inflammatory pathways in Parkinson's disease. *Neurochemical Research, 34*(1), 55–65.

Miyanishi, M., Tada, K., Koike, M., Uchiyama, Y., Kitamura, T., & Nagata, S. (2007). Identification of Tim4 as a phosphatidylserine receptor. *Nature, 450*(7168), 435–439.

Morris, L., Graham, C. F., & Gordon, S. (1991). Macrophages in haemopoietic and other tissues of the developing mouse detected by the monoclonal antibody F4/80. *Development, 112*(2), 517–526.

Mosser, D. M., & Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nature Reviews Immunology, 8*(12), 958–969.

Nawashiro, H., Tasaki, K., Ruetzler, C. A., & Hallenbeck, J. M. (1997). TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. *Journal of Cerebral Blood Flow and Metabolism, 17*(5), 483–490.

Neeper, M., Schmidt, A. M., Brett, J., Yan, S. D., Wang, F., Pan, Y. C., Elliston, K., Stern, D., & Shaw, A. (1992). Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *Journal of Biological Chemistry, 267*(21), 14998–15004.

Nelson, P. T., Soma, L. A., & Lavi, E. (2002). Microglia in diseases of the central nervous system. *Annals of Medicine, 34*(7–8), 491–500.

Neumann, H. (2001). Control of glial immune function by neurons. *Glia, 36*(2), 191–199.

Neumann, H., & Takahashi, K. (2007). Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis. *Journal of Neuroimmunology, 184*(1–2), 92–99.

Nimmerjahn, A., Kirchhoff, F., & Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science, 308*(5726), 1314–1318.

Noda, M., Nakanishi, H., Nabekura, J., & Akaike, N. (2000). AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. *Journal of Neuroscience, 20*(1), 251–258.

Olah, M., Biber, K., Vinet, J., & Boddeke, H. W. (2011). Microglia phenotype diversity. *CNS & Neurological Disorders Drug Targets, 10*(1), 108–118.

Pais, T. F., Figueiredo, C., Peixoto, R., Braz, M. H., & Chatterjee, S. (2008). Necrotic neurons enhance microglial neurotoxicity through induction of glutaminase by a MyD88-dependent pathway. *Journal of Neuroinflammation, 5*, 43.

Parvathenani, L. K., Tertyshnikova, S., Greco, C. R., Roberts, S. B., Robertson, B., & Posmantur, R. (2003). P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. *Journal of Biological Chemistry, 278*(15), 13309–13317.

Perry, V. H., Cunningham, C., & Boche, D. (2002). Atypical inflammation in the central nervous system in prion disease. *Current Opinion in Neurology, 15*(3), 349–354.

Perry, V. H., & Gordon, S. (1987). Modulation of CD4 antigen on macrophages and microglia in rat brain. *The Journal of Experimental Medicine, 166*(4), 1138–1143.

Pinteaux-Jones, F., Sevastou, I. G., Fry, V. A., Heales, S., Baker, D., & Pocock, J. M. (2008). Myelin-induced microglial neurotoxicity can be controlled by microglial metabotropic glutamate receptors. *Journal of Neurochemistry, 106*(1), 442–454.

Pocock, J. M., & Kettenmann, H. (2007). Neurotransmitter receptors on microglia. *Trends in Neurosciences, 30*(10), 527–535.

Ponomarev, E. D., Shriver, L. P., & Dittel, B. N. (2006). CD40 expression by microglial cells is required for their completion of a two-step activation process during central nervous system autoimmune inflammation. *Journal of Immunology, 176*(3), 1402–1410.

Qin, L., Liu, Y., Wang, T., Wei, S. J., Block, M. L., Wilson, B., Liu, B., & Hong, J. S. (2004). NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. *Journal of Biological Chemistry, 279*(2), 1415–1421.

Ravichandran, K. S. (2003). "Recruitment signals" from apoptotic cells: Invitation to a quiet meal. *Cell, 113*(7), 817–820.

Reed-Geaghan, E. G., Savage, J. C., Hise, A. G., & Landreth, G. E. (2009). CD14 and toll-like receptors 2 and 4 are required for fibrillar A{beta}-stimulated microglial activation. *Journal of Neuroscience, 29*(38), 11982–11992.

Rymo, S. F., Gerhardt, H., Wolfhagen Sand, F., Lang, R., Uv, A., & Betsholtz, C. (2011). A two-way communication between microglial cells and angiogenic sprouts regulates angiogenesis in aortic ring cultures. *PloS One, 6*(1), e15846.

Saijo, K., Winner, B., Carson, C. T., Collier, J. G., Boyer, L., Rosenfeld, M. G., Gage, F. H., & Glass, C. K. (2009). A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell, 137*(1), 47–59.

Sanders, V. M., & Jones, K. J. (2006). Role of immunity in recovery from a peripheral nerve injury. *Journal of Neuroimmune Pharmacology, 1*(1), 11–19.

Savill, J., Dransfield, I., Gregory, C., & Haslett, C. (2002). A blast from the past: Clearance of apoptotic cells regulates immune responses. *Nature Reviews Immunology, 2*(12), 965–975.

Savill, J., Gregory, C., & Haslett, C. (2003). Cell biology. Eat me or die. *Science, 302*(5650), 1516–1517.

Schilling, T., & Eder, C. (2010). Stimulus-dependent requirement of ion channels for microglial NADPH oxidase-mediated production of reactive oxygen species. *Journal of Neuroimmunology, 225*(1–2), 190–194.

Schmidt, A. M., Vianna, M., Gerlach, M., Brett, J., Ryan, J., Kao, J., Esposito, C., Hegarty, H., Hurley, W., Clauss, M., Wang, F., Pan, Y.-C. E., Tsang, T. C., & Stern, D. (1992). Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. *Journal of Biological Chemistry, 267*(21), 14987–14997.

Schmid, C. D., Sautkulis, L. N., Danielson, P. E., Cooper, J., Hasel, K. W., Hilbush, B. S., Sutcliffe, J. G., & Carson, M. J. (2002). Heterogeneous expression of the triggering receptor expressed on myeloid cells-2 on adult murine microglia. *Journal of Neurochemistry, 83*(6), 1309–1320.

Schwartz, M., & Kipnis, J. (2011). A conceptual revolution in the relationships between the brain and immunity. *Brain, Behavior, and Immunity, 25*(5), 817–819.

Shaked, I., Porat, Z., Gersner, R., Kipnis, J., & Schwartz, M. (2004). Early activation of microglia as antigen-presenting cells correlates with T cell-mediated protection and repair of the injured central nervous system. *Journal of Neuroimmunology, 146*(1–2), 84–93.

Shaked, I., Tchoresh, D., Gersner, R., Meiri, G., Mordechai, S., Xiao, X., Hart, R. P., & Schwartz, M. (2005). Protective autoimmunity: Interferon-gamma enables microglia to remove glutamate without evoking inflammatory mediators. *Journal of Neurochemistry, 92*(5), 997–1009.

Sheng, J. G., Mrak, R. E., & Griffin, W. S. (1998). Enlarged and phagocytic, but not primed, interleukin-1 alpha-immunoreactive microglia increase with age in normal human brain. *Acta Neuropathologica, 95*(3), 229–234.

Siffrin, V., Brandt, A. U., Herz, J., & Zipp, F. (2007). New insights into adaptive immunity in chronic neuroinflammation. *Advances in Immunology, 96*, 1–40.

Simard, A. R., Soulet, D., Gowing, G., Julien, J. P., & Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron, 49*(4), 489–502.

Siskova, Z., Page, A., O'Connor, V., & Perry, V. H. (2009). Degenerating synaptic boutons in prion disease: Microglia activation without synaptic stripping. *American Journal of Pathology, 175*(4), 1610–1621.

Stadelmann, C. (2011). Multiple sclerosis as a neurodegenerative disease: Pathology, mechanisms and therapeutic implications. *Current Opinion in Neurology, 24*(3), 224–229.

Stout, R. D., Jiang, C., Matta, B., Tietzel, I., Watkins, S. K., & Suttles, J. (2005). Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *Journal of Immunology, 175*(1), 342–349.

Streit, W. J. (2006). Microglial senescence: Does the brain's immune system have an expiration date? *Trends in Neurosciences, 29*(9), 506–510.

Streit, W. J., Conde, J. R., Fendrick, S. E., Flanary, B. E., & Mariani, C. L. (2005). Role of microglia in the central nervous system's immune response. *Neurological Research, 27*(7), 685–691.

Streit, W. J., & Xue, Q. S. (2012). Alzheimer's disease, neuroprotection, and CNS immunosenescence. *Frontiers in Pharmacology, 3*, 138.

Sumimoto, H., Ueno, N., Yamasaki, T., Taura, M., & Takeya, R. (2004). Molecular mechanism underlying activation of superoxide-producing NADPH oxidases: Roles for their regulatory proteins. *Japanese Journal of Infectious Disease, 57*(5), S24–S25.

Syapin, P. J. (2008). Regulation of haeme oxygenase-1 for treatment of neuroinflammation and brain disorders. *British Journal of Pharmacology, 155*(5), 623–640.

Takahashi, K., Rochford, C. D., & Neumann, H. (2005). Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *The Journal of Experimental Medicine, 201*(4), 647–657.

Takeuchi, O., & Akira, S. (2001). Toll-like receptors; their physiological role and signal transduction system. *International Immunopharmacology, 1*(4), 625–635.

Tartaglia, L. A., & Goeddel, D. V. (1992). Two TNF receptors. *Immunology Today, 13*(5), 151–153.

Taupin, P. (2010). A dual activity of ROS and oxidative stress on adult neurogenesis and Alzheimer's disease. *Central Nervous System Agents in Medicinal Chemistry, 10*(1), 16–21.

Tichauer, J., Saud, K., & von Bernhardi, R. (2007). Modulation by astrocytes of microglial cell-mediated neuroinflammation: Effect on the activation of microglial signaling pathways. *Neuroimmunomodulation, 14*(3–4), 168–174.

Tieu, K., Ischiropoulos, H., & Przedborski, S. (2003). Nitric oxide and reactive oxygen species in Parkinson's disease. *IUBMB Life, 55*(6), 329–335.

Visintin, A., Mazzoni, A., Spitzer, J. H., Wyllie, D. H., Dower, S. K., & Segal, D. M. (2001). Regulation of Toll-like receptors in human monocytes and dendritic cells. *Journal of Immunology, 166*(1), 249–255.

Walter, S., Letiembre, M., Liu, Y., Heine, H., Penke, B., Hao, W., Bode, B., Manietta, N., Walter, J., Schulz-Schuffer, W., & Fassbender, K. (2007). Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease. *Cellular Physiology and Biochemistry, 20*(6), 947–956.

Westerman, M. A., Cooper-Blacketer, D., Mariash, A., Kotilinek, L., Kawarabayashi, T., Younkin, L. H., Carlson, G. A., Younkin, S. G., & Ashe, K. H. (2002). The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease. *Journal of Neuroscience, 22*(5), 1858–1867.

Wyss-Coray, T., Yan, F., Lin, A. H., Lambris, J. D., Alexander, J. J., Quigg, R. J., & Masliah, E. (2002). Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *Proceedings of the National Academy of Sciences of the United States of America, 99*(16), 10837–10842.

Yang, L., Lindholm, K., Konishi, Y., Li, R., & Shen, Y. (2002). Target depletion of distinct tumor necrosis factor receptor subtypes reveals hippocampal neuron death and survival through different signal transduction pathways. *Journal of Neuroscience, 22*(8), 3025–3032.

Yong, V. W., & Rivest, S. (2009). Taking advantage of the systemic immune system to cure brain diseases. *Neuron, 64*(1), 55–60.

6-Hydroxydopamine Lesioning of Dopamine Neurons in Neonatal and Adult Rats Induces Age-Dependent Consequences

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Abstract

Over 45 years ago, it was discovered that the compound 6-hydroxydopamine (6-OHDA) destroys catecholamine (CA)-containing neurons when injected directly into the brain. Refinements in the use of 6-OHDA led to the development of dopamine (DA)-denervated rats to model Parkinson's disease (PD) and Lesch-Nyhan syndrome (LNS), in which loss of DA-containing neurons is a prominent feature. Differing functional characteristics were eventually discovered in DA-denervated rats depending on the age at which the DA was reduced. With 6-OHDA lesioning of CA neural systems in the brain, key discoveries were made regarding how these systems control specific physiological functions and behavioral responses. Important neurobiological concepts emerged as well, including receptor supersensitivity, D_1/D_2-DA receptor interaction, and D_1-DA priming of supersensitivity, including neural mechanisms likely relevant to clinical symptoms modeled by the 6-OHDA treatment. In this chapter, behavioral, neurochemical, structural, and pharmacological studies carried out in adult and neonatal 6-OHDA-treated rats are reviewed, and findings are compared and contrasted. To date, age-dependent lesioning of DA neurons in rats with 6-OHDA continues to play an important role in the development and validation of new therapies for clinical disorders with brain DA reduced.

Keywords

Adult 6-OHDA • DA-receptor interactions • Lesch-Nyhan disease • Neonate-6OHDA • Parkinson's disease

1 Introduction

The neurotoxicant 6-OHDA was the first agent to produce long-term depletion and destruction of norepinephrine (NE) in peripheral sympathetic neurons without affecting central CA content (Porter et al. 1963; Laverty et al. 1965; Thoenen and Tranzer 1968). Subsequently, the toxin was found to produce a selective destruction of CA-containing neurons when administered directly into the brain (Ungerstedt 1968; Bloom et al. 1969; Breese and Traylor 1970; Uretsky and Iversen 1970).

Over the last 4 decades, administration of 6-OHDA into the brain has been used effectively in both adult (Ungerstedt 1968; Breese and Traylor 1970; Breese et al. 1974) and neonatal (Breese and Traylor 1972; Smith et al. 1973) animals to investigate age-dependent behavioral, physiological, and biochemical effects of midbrain CAergic neuronal loss in disease symptoms and for identification of drug action. From these efforts, DA and NE neurons were found to be essential for the action of psychostimulant drugs, control of movement, and behavioral processes such as emotion, attention, reward, body temperature, and stress responses. Additionally, the basic information collected with 6-OHDA on brain function allowed for defining the role of these CAs in the pathogenesis and/or symptoms of PD, Lesch-Nyhan syndrome (LNS), and a number of other neuropsychiatric disorders, including attention deficit hyperactivity disorder (ADHD), schizophrenia, and drug addiction.

In the present chapter, an extensive overview of 6-OHDA lesioning in rats is provided, highlighting the distinctions between adult and neonatal CA-depleted animals. First, the history, scientific basis, and application of 6-OHDA lesioning techniques to selectively destroy nigrostriatal DA and/or NE neurons of the adult and neonatal rats are discussed. Next, important contributions from previous investigations regarding the behavioral and compensatory neural responses to age-dependent 6-OHDA lesioning of these neurons are reviewed. Focus is placed on characteristics unique to rats lesioned with 6-OHDA to destroy DA neurons during the neonatal period, particularly with regard to the differential sensitivity of these rats to various agonists and antagonists of DA receptors and to changes in other transmitter receptors in comparison to adult DA-depleted animals. Finally, potential mechanisms associated with the neural and behavioral plasticity in 6-OHDA-lesioned rats are presented, and the usefulness of these animals to model symptoms and features of specific central disorders is emphasized.

2 The 6-OHDA-Lesioned Rat Model

2.1 Mechanism of Action of 6-OHDA

6-OHDA does not pass the blood-brain barrier, so it must be directly injected into the brain to destroy central CA-containing neurons (Schober 2004; Bove et al. 2005). As an analog of DA and NE neurons, the toxin is readily taken up by CA neurons through the high-affinity DA and NE transporters, DAT and NET, respectively (Jonsson and Sachs 1970; Ljungdahl et al. 1971; Saner and Thoenen 1971). Once inside the neuron, the 6-OHDA initiates degeneration of CA neurons likely through a combination of oxidative stress and mitochondrial respiratory dysfunction (Schober 2004; Bove et al. 2005). The 6-OHDA-induced destruction of DA or NE nerve endings, and possibly cell bodies (depending on dose), results in the denervation of DA- and/or NE-containing brain neurons, which ultimately induces development of DA and/or NE receptor supersensitivity and other adaptations to compensate for this neural loss.

2.2 Microinjections of 6-OHDA into the Brain to Lesion Nigrostriatal DA Neurons in Adult Rats

In 1968, Ungerstedt showed that injection of 6-OHDA into the pars compacta region of the SN (SNpc) of adult rats produced anterograde degeneration of the nigrostriatal DA system, thereby generating the first animal model of PD (Ungerstedt 1968). In subsequent studies to disrupt DA function, 6-OHDA was injected either into the medial forebrain bundle (MFB), through which the nigrostriatal pathway ascends, or into the striatum, where axon terminals of SNpc neurons are located (Deumens et al. 2002). With high doses of 6-OHDA (>6 μg) injected bilaterally into either the SNpc or MFB of adult rats, nigral DAergic cell death begins within 12 h of injection, followed by a near-complete lesioning of nigrostriatal DA neurons (>90 %) within 2–3 days (Ungerstedt 1968; Jeon et al. 1995; Zuch et al. 2000) – a loss of neurons which mirrors the pattern of DA loss seen in postmortem brains of PD patients. After injection into the SNpc, neighboring DA neurons in the ventral tegmental area (VTA), which give rise to the mesolimbic and mesocortical pathways, are somewhat compromised by 30–40 % as well (Perese et al. 1989; Carman et al. 1991).

Bilateral injections of 6-OHDA into the SNpc or MFB are usually avoided due to a profound acute aphagia and adipsia that follows – an approach which can result in mortality if rats are not tube-fed to maintain nutrition (Ungerstedt 1968, 1971a). Rather than bilateral site injections of 6-OHDA, administration is typically performed unilaterally, with the opposite, or contralateral, side serving as the control. This approach is particularly suitable for evaluating turning behavior to a drug challenge (Ungerstedt 1971d). However, following unilateral lesioning of the nigrostriatal DA pathway, it should be noted that the rat initially exhibits a severe body circling posture to one side for a few days, after which time this deficit nearly subsides – a recovery that obviously depends upon a neural adaptation to allow this functional correction. Consequently, for defining the basis of this adaptation in these unilateral 6-OHDA-lesioned rats, the inclusion of an unlesioned rat would be wise. Robinson and colleagues have provided extensive data documenting the need for appropriate controls to understand the basis of the initial adaptation to unilateral 6-OHDA lesioning in adult rats (Robinson 1991).

Another approach to investigate the consequences of lesioning DA neurons was to inject 6-OHDA (12–25 μg) into the striatum to produce a more selective and slowly developing partial lesion of nigrostriatal DA neurons (50–70 %) in this site to mimic earlier stages of PD. Though damage to the striatal DAergic terminals is acute (within 24 h of injection) with this method, degeneration of the parent nigral cell bodies begins at 5–7 days postinjection and progresses for at least 4 months after the insult (Sauer and Oertel 1994; Przedborski et al. 1995; Blandini et al. 2007; Berger et al. 1991). This protracted time course of degeneration has provided an in vivo experimental platform for studying neuroprotective strategies aimed at slowing or halting the degeneration of DA neurons in PD (Deumens et al. 2002). Since central administration of 6-OHDA can also reduce brain NE, direct injections of 6-OHDA into the locus coeruleus, the region of the

brainstem where NE neurons originate (Ungerstedt 1971c), have also been performed (Ungerstedt 1971e). This strategy of lesioning NE neurons has allowed for the determination of what functional role these particular neurons play in brain function.

2.3 Intracisternal Injection of 6-OHDA to Lesion CA-Containing Neurons in Adult Rats

Subsequent to the initial work by Ungerstedt (1968), Breese and Traylor administered 6-OHDA into the cisterna magna of adult rats to produce lesioning of CA-containing neurons (Breese and Traylor 1970). This initial use of intracisternal (i.c.) administration of 6-OHDA to lesion CAergic neurons in the brain was then followed by others (Bruno et al. 1984, 1987; Lytle et al. 1972; Smith et al. 1973). Because initial unpublished efforts demonstrated that 6-OHDA i.c. utilization did not produce the profound reduction of DA observed with injection of 6-OHDA into the SNpc (Ungerstedt 1968), Breese and Traylor pretreated rats with the monoamine oxidase (MAO) inhibitor, pargyline, to produce a comparable reduction of brain DA as seen when 6-OHDA was microinjected into the MFB (Breese and Traylor 1970). Like pargyline, several MAO inhibitors were subsequently demonstrated to also enhance the destruction of DA-containing neurons (Breese 1975). Additionally, the degree of destruction of DA neurons was found to be further enhanced if an additional dose of 6-OHDA was administered 7–10 days after the initial pargyline/6-OHDA exposure (Breese and Traylor 1971; Breese et al. 1973, 1974). Because the 6-OHDA also shows high affinity for the NE transporter, NET (Luthman et al. 1989b), systemic injection of the NET inhibitor, desipramine, administered prior to 6-OHDA ensures increased selectivity of the toxin for DA neurons (Evetts and Iversen 1970; Breese and Traylor 1971). Owing to the high affinity of 6-OHDA for NET (Porter et al. 1963), Breese and Traylor found that small doses of 6-OHDA administered i.c. to adult rats reduced brain NE with little effect on DA content in the brain (Breese and Traylor 1971). These approaches allowed evaluating the distinct functions related to either DA or NE in the brain (Breese et al. 1974; Ungerstedt et al. 1974), a strategy which provided a critical understanding of how the loss of CA neurons differentially affected brain functions.

2.4 Intracisternal Injection of 6-OHDA to Lesion CA-Containing Neurons in Neonatal Rats

In 1972, Breese and Traylor administered 6-OHDA into the cisterna magna of developing rats to determine whether the 6-OHDA would destroy CA-containing neurons during this period (Breese and Traylor 1972). They found that this procedure produces a marked reduction in brain DA, NE, and tyrosine hydroxylase activity (TH; the rate limiting enzyme required for DA synthesis) (Breese and Traylor 1972). In subsequent studies to reduce only brain

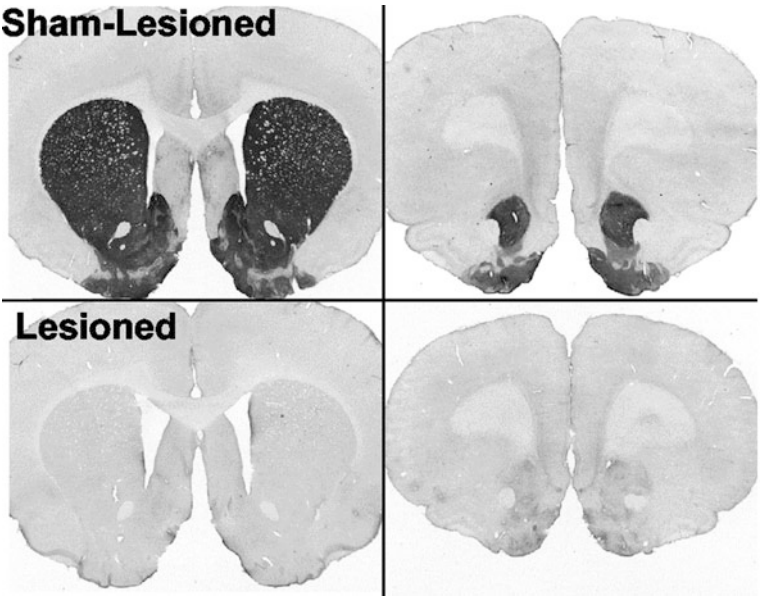

Fig. 1 Tyrosine hydroxylase immunohistochemistry after neonatal 6-OHDA lesioning in rats. Immunohistochemical detection of tyrosine hydroxylase in coronal sections representing the striatum, nucleus accumbens, and medial prefrontal cortex of adult rats after neonatal sham (*top*) and 6-OHDA (*bottom*) lesioning

DA, selective destruction of DA neurons was achieved by pretreating rats with desipramine prior to injection of 6-OHDA either into the cisterna magna or into the cerebral ventricles (Smith et al. 1973). At 100–150 μg doses of 6-OHDA, injection into either of these ventricular sites was found to produce a major destruction of DA- and NE-containing neurons (>95 %) within 72 h (Breese et al. 1984a, 1985a, b, 1987a; Berger et al. 1985; Snyder et al. 1986). In fact, as early as 4 days postinjection into the ventricles, DAergic cell bodies and dendrites were reported to disappear entirely from the SN (Fernandes Xavier et al. 1994) and cause a considerable reduction in number in the VTA (Fernandes Xavier et al. 1994; Breese et al. 1984a, 1985a, b, 1987a; Berger et al. 1985; Snyder et al. 1986). Consequently, the DAergic terminal fibers in the striatum are drastically reduced by 6-OHDA (Snyder et al. 1986; Descarries et al. 1992), as are neurons in certain target regions of the VTA, including the septum, nucleus accumbens, and frontal cortex (Luthman et al. 1990a). This extensive DAergic denervation explains the profound loss of brain DA content measured in these animals during adulthood (Breese and Traylor 1972; Breese et al. 1984a; Stachowiak et al. 1984; Bruno et al. 1987; Jackson et al. 1988; Dewar et al. 1990). In Fig. 1, the extent of DA depletion in the striatum and frontal cortex of adult rats lesioned with 6-OHDA as neonates is revealed through the immunohistochemical detection of TH. This perinatal loss of DA in neonatal

rats has been the focus of investigations aimed at understanding how this loss relates to the pathophysiology of the developmental brain disorder LNS in which DA is reduced during development (Breese et al. 2005).

3 Behavioral Responses After 6-OHDA Lesions

3.1 Behavioral Impairment and Recovery of Function in Rats Lesioned as Adults

As noted earlier, extensive bilateral lesions of nigrostriatal DA neurons in adult rats produce a syndrome that is analogous to PD in humans (Marshall et al. 1974; Ungerstedt 1971a; Zigmond and Stricker 1984). The rats exhibit symptoms such as bradykinesia/akinesia, rigidity, sensorimotor neglect, excessive bracing and clinging reactions, festination, aphagia, adipsia, and cognitive dysfunction (Marshall 1979; Stricker and Zigmond 1984; Ungerstedt 1971a; Zigmond and Stricker 1972; Zis et al. 1974). The occurrence and severity of these behavioral deficits appears to be strongly correlated with the degree of DA depletion in the striatum (Lees et al. 1985). For example, adult rats with essentially total bilateral depletions of striatal DA (>95 %) can perish as a consequence of these behavioral impairments if they are not tube-fed intragastrically with nutrients. In spite of this tube-feeding effort over an extended period, the rats rarely regain normal ingestive behavior and continue to show sensorimotor neglect and profound motor and cognitive impairments (Marshall 1979; Zigmond and Stricker 1973). These 6-OHDA-lesioned adult rats continue to suffer from residual regulatory deficits, particularly in their ability to respond to stress and acute homeostatic imbalances (Snyder et al. 1985; Stricker and Zigmond 1974; Marshall and Teitelbaum 1974). In fact, when challenged with an exposure to stressor, the severe ingestive and motor impairments can be reinstated in these animals to the level of severity seen acutely after the lesion (Robinson et al. 1990). Adult rats with extensive, yet less severe, bilateral 6-OHDA lesions of striatal DA (80–90 %) initially display behavioral deficits (Baez et al. 1977; Fibiger et al. 1973; Spirduso et al. 1985) but show significant recovery over time (Altar et al. 1987; Creese et al. 1977; Dravid et al. 1984; Neve et al. 1982; Nisenbaum et al. 1986). Similarly, patients with PD manifest clinical symptoms only after a loss of 80 % of the DAergic input into the striatum (Bernheimer et al. 1973; Fearnley and Lees 1991). Therefore, while nigrostriatal DAergic activity is required for normal behavioral function, only 10–20 % of nigrostriatal DA input is actually needed to maintain fairly normal function in both rats and humans.

3.2 Sparing of Behavioral Impairment and Recovery of Function in Rats Lesioned as Neonates

The behavior of rats lesioned with 6-OHDA as neonates is very different from that of rats lesioned with 6-OHDA during adulthood. Rats lesioned with 6-OHDA as

neonates do not display any outward symptoms of aphagia or adipsia associated with adult bilateral lesions (Breese and Traylor 1972; Smith et al. 1973). In fact, while these neonatal lesioned rats are smaller in stature (Breese and Traylor 1972), they show few overt behavioral deficits that distinguish them from non-lesioned rats during development or in subsequent adulthood, even when their DA levels are depleted by 99 % (Breese et al. 1984a; Bruno et al. 1984; Erinoff et al. 1979; Smith et al. 1973). Near-total depletion of striatal DA incurred prior to postnatal day 27 (P27) produces no obvious deficits in sensorimotor function either soon after the lesion or after the rats reach adulthood (Weihmuller and Bruno 1989a), nor is there any evidence of profoundly impaired ingestive or motor function (Potter and Bruno 1989; Weihmuller and Bruno 1989b). In early studies, Breese and Traylor showed that neonatal rats with reductions in both DA and NE remained virtually indistinguishable from non-lesioned controls except for a slight decrease in body weight and a lack of self-grooming (Breese and Traylor 1972). Smith and colleagues later showed that the reduced body weight induced by the 6-OHDA reduction in food consumption was related to reduced DA and not to a loss of NE (Smith et al. 1973). Although neonatal rats with DA or with both CA-containing neurons destroyed exhibit reduced body weight (Breese and Traylor 1972; Smith et al. 1973), they are still able to suckle and grow and can be weaned readily onto solid food and water as early as 27 days of age (Bruno et al. 1984, 1986; Weihmuller and Bruno 1989b; Smith et al. 1973).

The apparent sparing of behavioral function in neonatal lesioned rats, however, is not a complete one. Deficiencies in rats with DA selectively reduced during development were eventually uncovered with rigorous behavioral assessments of challenges to functional measures (Smith et al. 1973). For example, Smith and colleagues found that neonatal lesioned rats with selective reduction of brain DA do not ingest sucrose even though intake of water was normal (Smith et al. 1973), whereas rats with only brain NE reduced exhibit an increase in the sucrose drinking over that of controls. Others found that the neonatal lesioned rats with DA reduced do not eat in response to acute glucoprivation and do not drink water or saline in response to hypovolemia (Bruno et al. 1986). These deficits in response to acute homeostatic imbalances are similar to those seen in rats with adult lesions (Bruno et al. 1986). Further, studies showed that neonatal lesioned rats with only DA reduced exhibit a total deficiency in acquiring the shuttle-box avoidance response in adulthood, while rats with only NE reduced exhibit an elevated acquisition in this task (Smith et al. 1973). As adults, the neonatal lesioned rats show significant deficits in classical and aversive conditioning (Raskin et al. 1983; Whishaw et al. 1987), escape and avoidance learning (Shaywitz et al. 1976b; Pappas et al. 1980), spatial navigation (Whishaw et al. 1987; Pappas et al. 1992), and independent limb/tongue use and limb posture (Whishaw et al. 1987), suggestive that the neonatal DA depletion produces lasting impairments in learning ability and skilled motor control. Rats depleted of DA as neonates also show deficits in motoric capacity for high-rate operant responding (lever-pressing) during intracranial self-stimulation to the lateral hypothalamus (Stellar et al. 1988) and VTA (Takeichi et al. 1986), as well as acquisition deficits in operant responding for water and other reinforcing

stimuli (Heffner and Seiden 1983; Moy 1995), just as do adult lesioned rats (Cooper and Breese 1975). Adult rats with DA destroyed as neonates with 6-OHDA also exhibit reduced locomotion, whereas those that had NE selectively reduced exhibit a nearly doubling of locomotion (Smith et al. 1973). Other reports have shown that animals lesioned with 6-OHDA during development exhibit hyperactivity in adolescence similar to patients with ADHD (Shaywitz et al. 1976b; Miller et al. 1981; Luthman et al. 1989a). Thus, while rats with neonatal DA-specific lesions cope quite well in the absence of challenges, exposure of these animals to such confrontations clearly reveals that severe functional deficiencies persist.

4 Compensatory Neural Responses After 6-OHDA Lesions

4.1 Adaptations in the DA System of Adult Lesioned Rats

In both neonatal and adult 6-OHDA-lesioned rats, a number of compensatory responses have been identified in the striatal DA system that could be involved in counterbalancing the effects of the lesion. While adaptive changes related to 6-OHDA lesioning likely occur when the toxin destroys NE-containing neurons, this following section focuses only on adaptations that occur after the selective lesioning of DA neurons with 6-OHDA. The adaptive changes in the DA system that occur in response to 6-OHDA lesioning are largely neurochemical, involving presynaptic changes in the remaining DA terminals as well as postsynaptic changes in target cells that are dependent upon lesion size. Such changes at synapses likely enable the spared DA neurons to upregulate their activity in order to regain functional control of their targets (Zigmond 1997). Initially, the adaptations observed in adult lesioned rats will be reviewed followed by those changes observed after neonatal lesioning of DA neurons.

Presynaptic DA Changes. In adult rats receiving 6-OHDA lesions of the nigrostriatal DA pathway, presynaptic changes occur that are largely related to an enhanced metabolism of surviving DA neurons. Well established is that the remaining DA neurons greatly increase their rate of DA synthesis at striatal terminals (Agid et al. 1973; Hefti et al. 1980, 1985; Wolf et al. 1989), a finding attributed to the ability of these cells to upregulate their intrinsic TH activity presumably to meet the increased demand for releasable DA (Zigmond et al. 1984). In fact, a direct correlation exists between the degree of DA depletion by 6-OHDA and the extent of upregulation of TH activity in the spared terminals, an increase which in most cases can be greater than sixfold (Zigmond et al. 1984). Using intracerebral microdialysis techniques in adult rats with unilateral DA lesions, Robinson and Whishaw showed that the extracellular concentrations of DA in the denervated striatal hemisphere are much higher than predicted given the extent of DA depletion measured in the postmortem tissue (Robinson and Whishaw 1988). Later microdialysis studies in adult rats with bilateral 6-OHDA lesions showed that the extracellular DA concentrations remain close to normal even when the DA destruction in both striatal hemispheres was nearly complete

(Castaneda et al. 1990b). Additionally, an increase in the rate of firing and burst firing activity of the remaining striatal DA neurons occurs (Hollerman and Grace 1990), which together can contribute to the increased levels of DA release as well as to the amount of DA release per action potential in the lesioned striatum (Stachowiak et al. 1987). At the same time, a decrease in DA reuptake, produced by the destruction of DAT on DA terminals by 6-OHDA, contributes to a slower rate of DA clearance from the extracellular space which, in combination with the disappearance of presynaptic DA autoreceptors, results in an increased diffusibility of DA throughout the lesioned striatum (Zigmond et al. 1984; Snyder et al. 1990).

An additional contributing factor to the functional sparing or recovery related to presynaptic function seen after extensive 6-OHDA lesions in adult rats appears to relate to sprouting of remaining DA neuronal terminals into denervated striatal regions. This growth occurs from an undamaged axon, possibly a neighboring axon in the same tract or an axon from a different tract, to fill a vacated adjacent space. Although the nigrostriatal DA projection is predominately ipsilateral, there is also a minor crossed projection that arises from the contralateral SN (Fallon and Loughlin 1982; Gerfen et al. 1982; Morgan et al. 1986). While DA fibers of the ipsilateral projection have a clear capacity to undergo this regenerative sprouting (Bohn et al. 1987; Hansen et al. 1995; Onn et al. 1986), Pritzel and colleagues used a retrograde labeling technique to show an increased number of contralaterally labeled nigral neurons that innervated the striatum 7 days after an extensive unilateral lesion of the ipsilateral nigrostriatal bundle in adult rats (Pritzel et al. 1983). This sprouting of DA neurons to reinnervate the DA-denervated striatum could be a possible basis for the eventual recovery of unilaterally lesioned adult rats from their initial turning posture after exposure to the 6-OHDA. Ultrastructural tracer studies have also revealed the appearance of hypertrophic DAergic fibers and growth-cone-like structures and the expansion of a terminal arbor network over a period of 1–4 months after the adult unilateral 6-OHDA lesioning (Blanchard et al. 1996; Liberatore et al. 1999; Finkelstein et al. 2000). Evidence suggests that this process is regulated by both supersensitive postsynaptic receptors and glial cells activated by the lesion (Batchelor et al. 1999; Parish et al. 2002; Tripanichkul et al. 2003). It has been hypothesized that the enlargement of synaptic varicosities and terminal arborizations observed after adult lesions could help compensate for the loss of DA by delivering larger boluses of DA to the denervated striatum, especially after presynaptic DA reuptake mechanisms and autoreceptors have been disrupted by the 6-OHDA lesion (Castaneda et al. 1990b; van Horne et al. 1992). Studies with DA-rich fetal SN or adrenal medulla transplants showed that replacement of only a very small fraction of the normal complement of DA is sufficient to restore some functional control of the striatum (Carder et al. 1987, 1989; Snyder-Keller et al. 1989).

Collectively, these studies suggested that the presynaptic adaptations in the remaining DA terminals likely exist to normalize the extracellular DA concentrations in the denervated striatum – changes which contribute to the recovery of function in the 6-OHDA-lesioned rats (Robinson and Whishaw 1988; Robinson et al. 1990, 1994; Abercrombie et al. 1990; Castaneda et al. 1990b; Parsons et al. 1991; Touchet and Bennett 1989). However, when striatal DA depletion exceeds 95 %, a marked

drop in extracellular levels of DA occurs, presumably reducing drastically the degree of behavioral function because of the extensive loss of presynaptic DA adaptation (Abercrombie et al. 1990; Castaneda et al. 1990b; Robinson et al. 1990). Therefore, at least 5 % of the DAergic cells must be present to produce an adequate supply of extracellular DA to maintain control over striatal targets and ultimately to sustain some function in the adult lesioned animals (Robinson et al. 1990).

Postsynaptic DA Changes. In adult 6-OHDA-lesioned rats, postsynaptic changes are manifested when the degree of striatal DA depletion by 6-OHDA becomes more extreme – an additional adaptation that is likely to contribute to the recovery process induced by the lesioning (Zigmond 1997; Zigmond and Stricker 1980; Staunton et al. 1981). This recovery process can enable the lesioned animals to amplify a weak DA signal (Zigmond 1997). A number of quantitative autoradiography and small animal positron tomography (PET) studies have consistently reported an increase in the density of postsynaptic D_2-DA receptors in the striatum of rats with extensive 6-OHDA lesioning of DA during adulthood (Creese et al. 1977; Savasta et al. 1987; Staunton et al. 1981; Murrin et al. 1979; Neve et al. 1982; Graham et al. 1990; Przedborski et al. 1995; MacKenzie et al. 1989; Joyce 1991a; Nikolaus et al. 2003; Hume et al. 1995; Nguyen et al. 2000). While the majority of these studies have been conducted in rats with unilateral DA depletions, a similar increase has been found in rats with bilateral loss of 90 % or more of total DA content in both striatal hemispheres (MacKenzie et al. 1989; Joyce 1991a). Autoradiography studies using [^{3}H] spiroperidol to localize the changes in D_2 receptors revealed increases in the lateral parts of the striatum after short survival times (Savasta et al. 1987), with a more diffuse upregulation into medial parts by 7 months after such lesions (Chritin et al. 1992). Other studies showed that the increase in D_2 receptor density is accompanied by an upregulation in D_2 receptor mRNA levels (Gerfen et al. 1990; Angulo et al. 1991; Neve et al. 1991; Cadet et al. 1992; Lisovoski et al. 1992; Chritin et al. 1993). Brene and colleagues found a D_2 receptor mRNA increase on medium-sized γ-aminobutyric acid (GABA)-containing neurons in the lateral striatum 3 weeks after a unilateral 6-OHDA lesion (Brene et al. 1990). Reports have suggested that the increase in D_2 receptors is due to a propensity of mRNA for the D_2-long spliced variant (Martres et al. 1992). Interestingly, this isoform of the D_2 receptor is preferentially involved in postsynaptic responses to DA under normal conditions (Usiello et al. 2000; Wang et al. 2000). Consistent with the changes in D_2 receptor synthesis, the recovery rate of D_2 receptor binding following irreversible blockade with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was found to be enhanced in lesioned striatum 4–5 weeks after unilateral 6-OHDA treatment in adult rats (Neve et al. 1985). This increase in recovery was attributed to an increase in the rate of reappearance of D_2 receptors into synaptic membranes. Irrespective of these findings, the physiological consequences of D_2 receptor upregulation on striatal function remain unknown, as increased D_2 receptor density was found to have no effect on the inhibitory responsiveness of spontaneously firing striatal neurons after exposure to iontophoresed DA or to the D_2 receptor agonist 2-(*N*-phenyl-ethyl-*N*-propyl)amino-5-hydroxytetralin in adult lesioned animals (Radja et al. 1993b). Nonetheless, this

adaptive increase in D_2 receptors could provide another means by which to amplify a weak DA signal induced by the DA lesioning (Zigmond 1997).

In adult lesioned rats, the effects of 6-OHDA-induced destruction of DA neurons on the regulation of other DA receptor subtypes, in particular the D_1 receptor, remain a contentious issue (Reader and Dewar 1999). Studies have reported that the density of D_1 receptors is either increased (Buonamici et al. 1986; Fornaretto et al. 1993; Narang and Wamsley 1995), unaltered (Altar and Marien 1987; Savasta et al. 1988; Lawler et al. 1995), or decreased in the striatum of these animals (Marshall et al. 1989; Stromberg et al. 1995). Further studies have reported either no change (Brene et al. 1990; Fornaretto et al. 1993; Narang and Wamsley 1995) or a slight decrease in D_1 mRNA levels after such lesions (Stromberg et al. 1995). Such variability might be due to (1) the amount of damage induced by the adult 6-OHDA lesion (Iwata et al. 1996; Reader and Dewar 1999), (2) the period of survival following the lesion (Fornaretto et al. 1993; Narang and Wamsley 1995), and/or (3) the site and conditions selected for the 6-OHDA treatment (Narang and Wamsley 1995; Reader and Dewar 1999). Therefore, parameters such as time course and spatial distribution of the lesion must be considered when comparing and interpreting the various changes reported for D_1 receptor function after 6-OHDA lesions in adult rats.

4.2 Adaptations in the DA System of Neonatal Lesioned Rats

Presynaptic DA Changes. While adult 6-OHDA-lesioned rats remain exclusively dependent upon the amount of DA that remains after 6-OHDA treatment, rats lesioned as neonates survive quite well and continue to exhibit sparing of function even when only 1–2 % of the nigrostriatal DA neurons remain. In this respect, an adaptive change which appears to be unique to neonatal 6-OHDA-lesioned rats includes an increased ability of post-lesion residual neurons to release DA, even when 99 % of the DA content in both striatal hemispheres is lost (Carder et al. 1989; Castaneda et al. 1990b). The few surviving DA neurons after neonatal lesions undergo presynaptic metabolic changes that persist well into adulthood and are similar to those seen after adult lesions (Molina-Holgado et al. 1994). These changes include an increase in the synthesis of DA, suggestive of an enhanced release of DA from spared DA terminals (Molina-Holgado et al. 1994). As with adult lesions, it has been suggested that these presynaptic adaptations in the neonatal lesioned rats account for the maintenance of a relatively high extracellular DA concentration in the denervated striatum during the resting state, as measured by intracerebral microdialysis (Castaneda et al. 1990a). These higher levels of extraneuronal DA are quite surprising given the meager 1–2 % survival rate of striatal terminals after such lesions. It has been hypothesized that the storage capacity of the remaining terminals may be increased to protect DA from intraneuronal oxidative deamination (Reader and Dewar 1999), particularly since studies have shown that the actual size of the terminals becomes larger (Pickel et al. 1992). An alternative hypothesis is that the membrane properties of the

DA-containing vesicles may become altered to facilitate the increased transmitter release (Reader and Dewar 1999). Regardless, the normalization of extracellular DA levels in the denervated striatum is likely a crucial component in the sparing of behavioral function seen in rats after destruction of neonatal DA neurons.

When neonatal lesioned rats are administered an additional 6-OHDA treatment in adulthood to further reduce presynaptic function, these animals show symptoms of aphagia, adipsia, and akinesia similar to adult lesioned rats, suggesting that the residual DA activity is involved in the sparing of these particular functions subsequent to the neonatal lesion (Rogers and Dunnett 1989b). Likewise, neonatal lesioned rats are hypersensitive to the disruption of residual DA function with the catecholamine synthesis inhibitor α-methyl-para-tyrosine (α-MT) that results in deficits in feeding as well as other behaviors (Rogers and Dunnett 1989a), just as observed in adult 6-OHDA-lesioned rats (Cooper et al. 1972, 1973b). Collectively, such outcomes provide convincing evidence that the residual DA neurons are necessary for functional sparing in these neonate lesioned animals.

Postsynaptic DA Receptor Changes. In rats lesioned as neonates, early studies reported no changes in the binding of [^{3}H]-spiperone to D_2 receptors after i.c. administration of 6-OHDA (Breese et al. 1987a; Duncan et al. 1987; Kostrzewa and Brus 1991; Kostrzewa and Hamdi 1991). However, subsequent studies in lesioned rats using [^{3}H]-raclopride demonstrated significant increases of 30–40 % in D_2 receptor density over that in controls (Dewar et al. 1990). This change occurred in the rostral portion of the striatum between 1 and 3 months after the 6-OHDA lesion (Dewar et al. 1990), but increased throughout the entirety of the striatum at a later time after the lesion (Radja et al. 1993b). Because studies showed that the recovery of D_2 binding after in vivo receptor alkylation with EEDQ was unaltered, this D_2 receptor proliferation after the neonatal lesioning was proposed to be the result of posttranscriptional modifications (Dewar et al. 1997).

Unlike adult 6-OHDA-lesioned animals, the increase in density of the D_2 receptors in the bilaterally lesioned neonates is not accompanied by a sustained increase in mRNA levels (Chen and Weiss 1991; Radja et al. 1993b). In contrast to this finding, unilateral lesions of the nigrostriatal DA pathway in neonatal rats were found to produce a 20–25 % increase in the density of D_2 receptors (radiolabeled with the D_2 antagonist quinpirole, LY 171555) which was accompanied by an upregulation of D_2 mRNA levels in the rostral striatum 9 months after the lesion (Mennicken et al. 1995). This latter finding is consistent with the increase in the density of D_2 receptors being related to an increase in the synthesis of the D_2 receptor. Further, since binding in a control was the same as the contralateral side to the lesion, receptor changes were not likely related to adaptation on the unlesioned side of the brain (Mennicken et al. 1995). The increase in D_2 receptor binding and mRNA levels was not observed in similarly lesioned rats that received DA-rich grafts intrastriatally 5 days after the 6-OHDA lesion (Mennicken et al. 1995), a finding supportive of the view that the adaptive increase in D_2 receptors was related to the destruction of the striatal DA neurons.

Similar to the D_1 receptor binding studies in adult 6-OHDA-lesioned rats, there are conflicting reports of slight increases (Broaddus and Bennett 1990), slight

decreases (Dewar et al. 1990; Dewar et al. 1997; Molina-Holgado et al. 1995), large decreases (Gelbard et al. 1990), or no change at all (Breese et al. 1987a; Luthman et al. 1990b) in the density of striatal D_1 receptors in neonatal 6-OHDA-lesioned rats. Also, there is no change in the percentage of low-affinity (R_L) and high-affinity (R_H) binding sites for striatal D_1 receptors in adult rats that were lesioned as neonates with 6-OHDA (Gong, Kostrzewa et al. 1994). So far, studies attempting to explore changes in mRNA encoding for the D_1 receptor in these neonatal lesioned rats have failed to find any significant changes in these rats compared to controls (Duncan et al. 1993). In contrast to the latter findings, Gong et al. found that D_1 receptor mRNA is reduced by 25–50 % in neonatally 6-OHDA-lesioned rats at 6 weeks post-birth and that 4 weekly treatments with the D_1 receptor agonist SKF 38393 restore D_1 receptor mRNA to control levels (Gong et al. 1994). It is also important to note here that there are at least six different forms of the DA receptor, and the terms D_1 and D_2 refer more to subfamilies of receptor type and not to a single DA receptor isoform (Strange 1993). The D_1 class of DA receptors, which consists of the D_1 and D_5 subtypes, stimulates adenylate cyclase (AC) activity, while the D_2 class, comprised of the D_2 short, D_2 long, D_3, and D_4 subtypes, generally inhibits it. It is possible that some of the compensation seen after 6-OHDA lesioning in rats is related to changes in DA receptor types other than D_1 and D_2 receptors, although such changes could also facilitate some of the behavioral disturbances seen in neonatal lesioned animals. Specifically, Zhang and colleagues found an increase in the density of D_4 receptors in the striatum and a decrease in the nucleus accumbens at P25, but not at P37 or P60 after neonatal 6-OHDA lesions (Zhang et al. 2002a, b), with only minor changes in D_1 and D_2 receptor binding in various forebrain regions. The change in D_4 receptors, but not in D_1 and D_2 receptors, was positively correlated with the time course of motor hyperactivity exhibited by the neonatal lesioned animals, suggesting that D_4 receptors may play a pivotal role in this response (Zhang et al. 2002a, b). In subsequent studies, Zhang and colleagues also showed that the administration of D_4 antagonists L-745,870 and U-101,958 dose-dependently inhibits the motor hyperactivity in neonatal lesioned animals (Zhang et al. 2002a).

4.3 Adaptations in the 5-HT System by 6-OHDA Lesions

5-HT Hyperinnervation. An alternative view to the direct role of residual DA and receptor changes in the sparing or recovery of function in rats after 6-OHDA lesions is that compensatory mechanisms within the 5-HT system might provide some replacement for the loss of DA function. In this respect, following lesioning of DA neurons in neonatal animals, an extensive hyperinnervation of 5-HT of axon terminals or varicosities arising from the dorsal raphe nucleus is found in the striatum that is accompanied by an increase in 5-HT levels (Breese et al. 1984a; Berger et al. 1985; Luthman et al. 1987; Snyder et al. 1986; Stachowiak et al. 1984; Towle et al. 1989; Descarries et al. 1992; Molina-Holgado et al. 1994). So long as 80 % of the striatal DA is lost, both unilateral and bilateral DA depletions can

produce this 5-HT hyperinnervation in neonatal lesioned rats (Towle et al. 1989; Gong et al. 1993b; Penit-Soria et al. 1997). In rats lesioned at 3 days of age, this extent of DA loss can produce a severalfold increase in 5-HT content in the rostral striatum 2–5 months post-lesioning, along with an upregulation in high-affinity 5-HT uptake (Breese et al. 1984a; Stachowiak et al. 1984) and 5-HT transporter density (Molina-Holgado et al. 1994). While initially reported that this adaptive 5-HT hyperinnervation was not seen in adult rats after a comparable bilateral DA lesion of the striatum (Breese et al. 1984a), striatal 5-HT hyperinnervation was later reported to occur after unilateral 6-OHDA lesions in adult rats if striatal DA content was severely reduced by over 95 % (Zhou et al. 1991; Guerra et al. 1997; Maeda et al. 2003).

Apparently, the trigger for this proliferation of 5-HT-containing fibers is the extensive loss of striatal DA neurons (Kostrzewa et al. 1993; Towle et al. 1989) even though the loss of DA occurs several days before the development of 5-HT innervation (Lidov and Molliver 1982). Further evidence in accord with this view is that the 5-HT terminals do not occupy synaptic sites vacated by the loss of DA neurons and do not interfere with DA outgrowth arising from transplanted DA-rich cells derived from the ventral mesencephalon (Snyder-Keller et al. 1989; Abrous et al. 1993a). Additionally, replacement of lost DA innervation by transplanted DA-rich cells shortly after the lesion prevents the 5-HT hyperinnervation (Snyder-Keller et al. 1989; Abrous et al. 1993a). The sprouted axons that accumulated significant amounts of 5-HT (Molina-Holgado et al. 1994) tripled their extracellular 5-HT levels under stimulatory release conditions (Jackson and Abercrombie 1992). However, intracerebral microdialysis studies under resting conditions demonstrated that the striatal 5-HT hyperinnervation is not associated with enhanced levels of basal extracellular 5-HT, probably due to an increase in high-affinity 5-HT uptake (Jackson and Abercrombie 1992). Further, Descarries et al. revealed that the hyperinnervation is not accompanied by an increase in the number of synaptic contacts made by the 5-HT varicosities (Descarries et al. 1992). On the other hand, Jackson and colleagues found that that endogenously released 5-HT could affect acetylcholine (Ach) release in neonatally denervated striatum (Jackson et al. 1988). Additionally, 5-HT release could exert effects on striatal DA terminals through indirect diffusion processes (Descarries and Mechawar 2000; Umbriaco et al. 1995). For example, the release of 5-HT from hyperinnervated fibers could contribute to enhancing DA release from residual DA terminals by entry into the presynaptic DA terminals, which could in turn facilitate DA release from these remaining neurons (Navailles et al. 2010).

While initially hypothesized that the increased striatal 5-HT might be responsible for the sparing phenomenon in neonatal DA-lesioned animals (Towle et al. 1989), a combination of 6-OHDA with the 5-HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) (Breese et al. 1978) given to 3-day-old rat pups to lesion both 5-HT and DA neurons produced no greater behavioral dysfunction than 6-OHDA lesions alone (Bruno et al. 1987). Consequently, if indeed 5-HT was serving as a substitute for the lost DA, then it would be expected that rats with co-lesions of the two transmitter systems would exhibit severe impairments,

perhaps similar to rats lesioned in adulthood. Further, behavioral sparing was still observed in rats treated with 6-OHDA at P15 and P27 (Bruno et al. 1987), even though the neurochemical effects of 6-OHDA lesions given at this point in development do not include the elevated 5-HT levels typical of early lesioning of neonatal DA neurons (Bruno et al. 1987). Given these various findings, the 5-HT hyperinnervation does not appear to be a critical component in the functional sparing seen in neonatal 6-OHDA-lesioned rats.

5-HT Receptor Binding. In adult rats, the effect of 6-OHDA lesions on various 5-HT receptors and their mRNA levels in the brain is controversial, as a number of receptor autoradiographic and in situ hybridization studies have provided conflicting results. Early studies suggested that 6-OHDA-induced DA depletion in adult rats does not alter either 5-HT receptor densities or the inhibitory responsiveness of striatal neurons to iontophoretic application of 5-HT or its agonists (el Mansari et al. 1994). Despite this reported lack of effect of DA destruction on 5-HT receptor function, later studies found an increase in 5-HT_{2A} receptor mRNA levels in the striatum after unilateral 6-OHDA lesions in adult rats (Numan et al. 1995; Zhang et al. 2007) in which 5-HT is elevated (Zhou et al. 1991; Guerra et al. 1997; Maeda et al. 2003). This striatal 5-HT receptor increase was abolished by L-dopa treatment (Zhang et al. 2007), suggestive that the regulation of striatal 5-HT_{2A} receptors is primarily dependent upon DA acting on DA receptors. At odds with these results, a more recent study performed in adult 6-OHDA-lesioned animals using [^{3}H] ketanserin-binding autoradiography showed reduced 5-HT_{2A} receptor levels in the striatum, as well as in the cingulate, insular, prefrontal, and primary somatosensory cortices (Li et al. 2010). In contrast to this increase, studies in adult 6-OHDA-lesioned rats have also shown a decrease in 5-HT_{2C} receptor mRNA levels in the striatum (Zhang et al. 2007). L-dopa treatment had no effect on 5-HT_{2C} mRNA levels in this region (Zhang et al. 2007), suggesting that the 5-HT_{2C} and 5-HT_{2A} receptors are differentially regulated by the nigrostriatal DA function. Also in adult 6-OHDA-lesioned animals, one study found no change in striatal 5-HT_4 receptor levels upon binding with the selective radioligand [^{125}I] SB 207710 (Patel et al. 1995), while another found increases in the caudal, but not rostral, striatum and globus pallidus upon binding with the 5-HT_4 receptor antagonist [^{3}H] GR113803 (Compan et al. 1996). Such discrepancies could be due to methodological factors, such as the type of ligand used, the state of receptor affinity measured, the actual placement of the 6-OHDA lesion, and/or the part of the brain region analyzed.

In striatum of neonatal 6-OHDA-lesioned rats in which 5-HT was elevated, quantitative ligand binding utilizing autoradiography has revealed changes in several 5-HT receptor subtypes at adulthood (Radja et al. 1993a; Reader and Dewar 1999; Kostrzewa et al. 1998; Laprade et al. 1996). Binding of [^{3}H] 5-HT revealed an increase throughout the striatum and SN after neonatal lesioning, but not in the globus pallidus (Radja et al. 1993a), suggestive that this upregulation occurs primarily on striatonigral as opposed to striatopallidal projection neurons. Labeling of 5-HT_{1B} receptors with the nonselective radioligand [^{125}I] cyanopindolol revealed an increase in the binding density of these receptors

throughout the striatum (i.e., both rostral and caudal halves), globus pallidus, and SN 3 months after the neonatal lesions (Radja et al. 1993a). Since previous studies showed that 5-HT_{1B} mRNA was present in the striatum but not in SN or globus pallidus of rats (Bruinvels et al. 1994), the increase in 5-HT_{1B} binding seen in the striatum, globus pallidus, and SN was suggested to result from an upregulation and increased transport of these receptors on striatal GABAergic projection neurons (Reader and Dewar 1999). Moreover, labeling of 5-HT_{2A} receptors with [^{125}I] iodimethoxyphenylamino-propane, or [^{125}I] DOI, revealed a marked increase in this receptor subtype in the rostral striatum of adult rats lesioned as neonates (Radja et al. 1993a). The anatomical distribution of increased 5-HT_{2A} receptors strongly correlated with that of 5-HT hyperinnervation in the rostral striatum, initially suggestive of a relationship between the two phenomena. However, studies established that the majority of 5-HT_{2A} receptors are postsynaptic to 5-HT neurons (Mengod et al. 1990) and that the increase in 5-HT_{2A} receptors occurs independent of the 5-HT hyperinnervation (Laprade et al. 1996; Basura and Walker 1999; Fox and Brotchie 2000; Kostrzewa et al. 1998). The increased 5-HT_{2A} binding density in the rostral striatum was also associated with a considerable increase in mRNA encoding for this receptor within lateral parts of the striatum of the neonatal lesioned rats (Laprade et al. 1996). In these neonatal lesioned animals, both changes were abolished after chronic treatment with apomorphine or the D_1 receptor agonist SKF-38393, suggesting that D_1 receptor activation participates in the degree of 5-HT_{2A} receptor expression (Laprade et al. 1996). Examination of the 5-HT_{1A} receptor subtype upon specific labeling with [^{3}H] 8-hydroxy-dipropyl-aminotetralin or [^{3}H] 8-OH-DPAT revealed no changes in the binding density of these receptors in adult striatum after neonatal 6-OHDA lesions (Radja et al. 1993a), nor was there any changes in 5-HT_{1A} mRNA levels (Numan et al. 1995).

4.4 Adaptations in GABA and Other Amino Acid Transmitter Systems by 6-OHDA

Discrete populations of GABAergic medium-sized spiny neurons in the striatum project their axons to the globus pallidus, entopeduncular nucleus (internal pallidum), and SN (Beckstead and Cruz 1986; Bolam and Smith 1990; Loopuijt and van der Kooy 1985). The inhibitory activity of these GABAergic neurons (van der Heyden et al. 1980) appears to be modulated by the ascending nigrostriatal DA fibers arising from the SNpc (van der Heyden et al. 1980; Samuel et al. 1988; Vernier et al. 1988; Reid et al. 1990). This efferent GABAergic population from the striatum is predominately subdivided into two subclasses, defined by the nature of their co-transmitters (Penny et al. 1986; Reiner and Anderson 1990) and the differential expression of D_1 and D_2 receptor subtypes on their surface. The striatal GABAergic neurons that project to the SN and entopeduncular nucleus mainly express D_1 receptors and their axons contain the neuropeptides substance P, neurokinin A, and dynorphin, while those that project to the globus pallidus predominately express D_2 receptors and contain the neuropeptide enkephalin

(Gerfen and Young 1988; Gerfen et al. 1990, 1991; Gerfen 1992). These striatonigral and striatopallidal pathways work in concert to regulate thalamic output to motor areas of the cortex and thus play an integral role in the execution of movement. More recently, a third, smaller population of striatal GABAergic neurons has been identified that co-expresses both D_1 and D_2 receptors as well as the neuropeptides substance P and enkephalin (Perreault et al. 2011). Unlike the striatal GABAergic neurons expressing only D_1 or D_2 receptors, these D_1/D_2 receptor striatal projection neurons terminate in regions within both the striatonigral and striatopallidal pathways, as well as regions containing DAergic cell bodies such as the VTA (Deng et al. 2006; Wang et al. 2006, 2007). While little is known about the functional relevance of these latter neurons, they are thought to contribute to the regulation of thalamic neurotransmission, perhaps with the purpose of maintaining homeostatic balance between the striatonigral and striatopallidal pathways (Perreault et al. 2011). An imbalance between the striatonigral and striatopallidal pathways, such as seen with DAergic degeneration in humans and in animals (Albin et al. 1989; DeLong 1990; Hikosaka et al. 2000; Bezard et al. 2001; Obeso et al. 2004), can affect the final outflow to the thalamus and contribute to motor disturbances (Gerfen et al. 1990; Gerfen 2000; Mallet et al. 2006).

Increased GABA Levels. In adult rats, unilateral DA depletion with 6-OHDA has been reported to increase endogenous GABA levels (Tanaka et al. 1986; Lindefors et al. 1989), to increase potassium (K^+)-stimulated in vivo release of GABA (Lindefors et al. 1989; Tossman et al. 1986), and to increase the activity of glutamic acid decarboxylase (GAD), the enzyme responsible for converting glutamic acid to GABA (Nagy et al. 1978; Segovia et al. 1990; Samuel et al. 1988), and GAD mRNA levels (Vernier et al. 1988; Lindefors et al. 1990; Segovia et al. 1990; O'Connor et al. 1991). In neonatal rats, bilateral DA depletions have also resulted in increased tissue GABA levels in the striatum as well as in target regions of the striatopallidal and striatonigral projections, namely, the globus pallidus and SN 2–3 months after the lesion (Molina-Holgado et al. 1993a, b). This increase in GABA levels in the neonatally lesioned striatum was found to correspond to increased GAD mRNA expression (Soghomonian 1993; Laprade and Soghomonian 1999). Because nigrostriatal DA terminals that synapse on GABAergic medium-sized neurons exert an inhibitory effect over striatal GABA neurons (van der Heyden et al. 1980; Reid et al. 1990), attenuation of this inhibition after lesioning DA neurons is likely responsible for the increase in striatal GABA levels. In this respect, studies have shown that enhanced GABAergic activity after neonatal 6-OHDA lesions is influenced substantially by DA receptor status, since irreversible blockade of DA receptors by N-ethoxycarbonyl-2ethoxy-1,2-dihydroquinoline (EEDQ) decreased both D_1 and D_2 sites (73–84 %) in control and neonatal lesioned striatum, but increased GABA levels by 25 % only in rats that received 6-OHDA after birth (Molina-Holgado et al. 1995). Further, following acute inhibition of DA synthesis or catabolism, GABA levels remain unchanged. While not found in control rats, a positive correlation occurred between increased GABA levels and the density of D_2 receptors, but not D_1 receptors, in the striatum of neonatal

6-OHDA-lesioned rats, a finding suggestive of a direct role for D_2 receptors in this regulation (Molina-Holgado et al. 1995). The increase in D_2 receptors in the striatum, presumably on GABAergic neurons that project to the globus pallidus (striatopallidal pathway) may play a key role in maintaining this permanent increase in GABAergic transmission in rats with neonatal 6-OHDA lesions.

Altered GABA Receptors. Accompanying the 6-OHDA increase in GABA-containing neurons is an increase in density of some GABA receptors in the striatum of neonatal lesioned rats (Molina-Holgado et al. 1995) – a change which likely produces plasticity of GABA-mediated signaling in neurons that form the major output systems of the striatum (i.e., the striatonigral and striatopallidal neurons). In rats lesioned unilaterally with 6-OHDA as adults, $GABA_A$ receptors were decreased in the striatum and globus pallidus, but increased in the SNr and entopeduncular nucleus (Pan et al. 1985). In neonatal 6-OHDA-lesioned rats, $GABA_A$ receptors labeled with the radioligand [^{3}H] muscimol were increased in the striatum, but not in the cerebral cortex or thalamus (Molina-Holgado et al. 1993a). It has been postulated that the increased [^{3}H] muscimol labeling in the striatum of neonatal lesioned rats targets $GABA_A$ receptors situated on hyperinnervated 5-HT fibers in the rostral striatum (Molina-Holgado et al. 1993a). Intracerebral microdialysis studies have shown that the striatal 5-HT hyperinnervation is not associated with enhanced basal levels of extracellular 5-HT (Jackson and Abercrombie 1992).

Changes in Other Amino Acid Transmitters. In addition to GABA, studies have demonstrated lasting increases in several other amino acids in the striatum and other regions of the basal ganglia in neonatal 6-OHDA-lesioned rats (Molina-Holgado et al. 1993a, b). In the striatum, aspartic acid and glycine were increased 3 months after the lesion, while in the globus pallidus, taurine, glutamine, aspartic acid, serine, and alanine were elevated (Molina-Holgado et al. 1993a, b). In the SN, only increases in taurine, glutamic acid, and glutamine were documented. When the amino acid profiles were compared to the relative amino acid composition of the tissues, the ratio of glutamic acid to GABA was decreased in the striatum and SN, but remained unchanged in the globus pallidus (Molina-Holgado et al. 1993b). Since the glutamic acid/GABA ratio can serve as an index for precursor product availability for GABA (Molina-Holgado et al. 1993b; Berl 1973), the decreased glutamic acid/GABA ratio implied an enhanced inhibitory influence relative to glutamic acid-mediated excitations in the striatum and SN (Molina-Holgado et al. 1993b). Such persistent and regionally distinct biochemical adjustments in amino acids within these basal ganglia regions demonstrate the extraordinary ability of the developing system to reorganize itself after early neonatal damage to DA neurons.

4.5 Altered Cholinergic Function

Studies using the technique of in vivo microdialysis have demonstrated a decrease in the release of endogenous acetylcholine (ACh) in the striatum in response to D_2 receptor agonists administered either systemically or locally through the dialysis

probe (Damsma et al. 1990; de Boer et al. 1990; De Boer et al. 1992). This finding is consistent with evidence that there is an important functional and anatomical relationship between D_2 receptors and cholinergic interneurons in the striatum. In addition to changes in striatal D_2 receptor density after 6-OHDA lesions to DA neurons, an increase in [^{3}H]hemicholinium-3 binding, a marker for cholinergic terminal density has been reported (Joyce 1991b). Moreover, systemic or local administration of D_2 blockers produces an increase in endogenous ACh release in the striatum (Damsma et al. 1991; De Boer et al. 1992). A study by Guyenet et al. revealed that there is a loss of tonic DA inhibitory control over the release of ACh after 6-OHDA treatment in adult rats (Guyenet et al. 1975). This effect was found to be reversed several weeks later, suggesting that the DA control is eventually reinstated in the lesioned animals (Guyenet et al. 1975; MacKenzie et al. 1989). This reinstatement has been suggested to be dependent upon increases in both the number of D_2 receptors and the activity of the high-affinity choline transport system associated with cholinergic terminals (Joyce 1991b).

4.6 Altered Expression of Neuropeptides

The enkephalin and tachykinin (substance P and neurokinin A) peptides have been shown to be present in high concentrations in the basal ganglia (Graybiel and Ragsdalev 1983). As mentioned earlier, two predominant subpopulations of GABAergic output neurons have been identified in the striatum that have distinct neuropeptide profiles. The striatopallidal pathway expresses enkephalin, whereas the striatonigral pathway expresses tachykinins and dynorphin (Gerfen and Young 1988; Gerfen et al. 1990, 1991; Gerfen 1992). Early ultrastructural studies showed that nigrostriatal DA neurons directly synapse on the enkephalin- and tachykinin-containing GABAergic neurons in rats (Kubota et al. 1986a, b; Kawai et al. 1987). This observation is consistent with the work demonstrating that these neuropeptide systems are regulated by nigrostriatal DA input (Bannon et al. 1987; Basura and Walker 2000; Campbell et al. 2001; Engber et al. 1992; Gerfen et al. 1990; Hanson et al. 1981; Li et al. 1987; Sivam 1989; Sivam et al. 1986, 1987; Sivam and Krause 1990; Walker et al. 1991; Young et al. 1986; Obeso et al. 2000).

6-OHDA Treatments on Neuropeptides. In adult unilateral 6-OHDA-lesioned rats, immunohistochemical studies showed that striatal enkephalin is increased ipsilateral to the lesion (Voorn et al. 1987; Engber et al. 1991; Taylor et al. 1992; Cenci and Bjorklund 1993; Campbell and Bjorklund 1994; Thal et al. 1983), while substance P is decreased in the striatum (Voorn et al. 1987; Engber et al. 1991; Sivam 1989, 1991; Sivam et al. 1987). Electron microscopic analysis revealed that the enkephalin-immunoreactive synaptic bouton profiles in the striatum are increased by the lesion, without affecting the boutons on postsynaptic targets (Ingham et al. 1991). Furthermore, the unilateral 6-OHDA lesions were found to be accompanied by an increase in mRNA enkephalin levels (Gerfen et al. 1991; Campbell et al. 1992; Cenci et al. 1993; Nisenbaum et al. 1994a, b; Voorn et al. 1994; Zeng et al. 1995) and a decrease in preprotachykinin mRNA levels

(Campbell et al. 1992; Campbell and Bjorklund 1994; Nisenbaum et al. 1994a, b; Zeng et al. 1995). Studies also showed that transplants with DA-rich fetal cells are able to reverse the increased striatal enkephalin levels (Abrous et al. 1993b; Bal et al. 1993) consistent with striatal enkephalin levels being controlled by nigrostriatal DA neurons (Bannon et al. 1987; Basura and Walker 2000; Campbell et al. 2001; Engber et al. 1992; Gerfen et al. 1990; Hanson et al. 1981; Li et al. 1987; Sivam 1989; Sivam et al. 1986, 1987; Sivam and Krause 1990; Walker et al. 1991; Young et al. 1986; Obeso et al. 2000).

Although bilateral 6-OHDA lesions in both neonatal and adult rats have consistently increased both enkephalin protein and its mRNA levels (Sivam et al. 1986, 1987, 1991; Sivam and Krause 1990; Kurumaji et al. 1988; Cimino et al. 1991; Soghomonian 1994), only neonatal lesions consistently produce a decrease in the tachykinin peptides substance P and neurokinin A in the striatum (Sivam 1989; Sivam et al. 1987, 1991; Sivam and Krause 1990). Snyder-Keller and colleagues reported that the neonatal DA depletions do not affect the patchy distribution of substance P immunostaining, nor do they affect the distribution of enkephalin in the matrix of the striatum (Snyder-Keller 1991), suggesting that the basic organization of the striatal patch and matrix compartments develop normally in the absence of DA innervation early in development. In addition, while neonatal DA depletion was found to enhance the release of ACh, it has no effect on the action of substance P- or neurokinin A-induced ACh release in rat striatal slices (Perez-Navarro et al. 1993), indicating that striatal cholinergic neurons are modulated by tachykinin peptides independently of the presence of nigrostriatal DA neurons.

The effect of adult and neonatal 6-OHDA lesions on enkephalin, tachykinin, and other peptide levels has also been examined in the SNr, globus pallidus, and nucleus accumbens of rats. Immunohistochemistry and in situ hybridization studies showed that enkephalin and its mRNA levels are enhanced in the SNr after either adult unilateral or neonatal 6-OHDA lesions (Sivam et al. 1987; Vankova et al. 1991; Gerfen et al. 1991). In rats with adult unilateral lesions, neurotensin was reported to be increased and substance P reduced in the SNr (Taylor et al. 1992). In the SNr of rats with neonatal 6-OHDA lesions, no change in neurotensin was reported (Luthman et al. 1990a), although both substance P and neurokinin A were significantly reduced, an effect not observed in adult bilateral-lesioned rats (Sivam et al. 1987). Moreover, enkephalin and its mRNA (Schuller et al. 1999), neurotensin, and substance P were all increased in the globus pallidus of adult rats with unilateral lesions, but the increases were found within distinct neuronal subpopulations (Martorana et al. 2003). The contents of mRNA for enkephalin and peptides for leu- and met-enkephalin were increased, whereas mRNA for dynorphin and substance P were decreased in more rostral parts than caudal parts of the nucleus accumbens after adult unilateral 6-OHDA lesions (Taylor et al. 1992; Voorn et al. 1994). Similarly, neonatal 6-OHDA lesions produced reductions in substance P in the nucleus accumbens (Luthman et al. 1990a). Generally, the changes in peptide levels observed in the SNr, globus pallidus, and nucleus accumbens appear to reflect the peptide and mRNA changes documented in the striatum. A functional relationship between the nigrostriatal DA system and enkephalin or tachykinin

systems in the basal ganglia has been suggested by several investigators (Hong et al. 1978a, b, 1979, 1985; Joyce and Iversen 1979; Bannon et al. 1986; Hanson et al. 1981; Tang et al. 1983).

Evidence has also indicated the participation of corticostriatal afferents and cholinergic function in the regulation of these neuropeptides in the striatum. For example, frontal cortical transections after 6-OHDA treatment in adult animals were found to reverse the lesion-induced increase in preproenkephalin mRNA in the medial striatum, but have no effect on the upregulation found in the lateral striatum (Campbell and Bjorklund 1994). The decrease in preprotachykinin mRNA within the striatum was unaffected by interruption of the corticostriatal afferents (Campbell and Bjorklund 1994). Systemic administration of the muscarinic cholinergic receptor antagonist scopolamine to adult 6-OHDA-lesioned rats prevented the increase in enkephalin mRNA levels in the striatum (Nisenbaum et al. 1994b). Similarly, high doses of systemic scopolamine blocked the lesion-induced decrease in striatal substance P mRNA levels, possibly as a result from an action of scopolamine within other brain areas (Nisenbaum et al. 1994b). In addition to cortical influences, neuropeptide changes induced by adult unilateral 6-OHDA lesions have been shown to be inhibited by lesions of the subthalamic nucleus (Delfs et al. 1995), suggesting that this region can influence peptide function in the striatum through polysynaptic pathways. This latter conclusion is based upon the observation that lesions of the subthalamic nucleus inhibit neuropeptide changes induced by the 6-OHDA in brain regions that do not receive a direct input from the subthalamus.

In addition to the enkephalin and tachykinin peptides, 6-OHDA lesions have also been shown to alter levels of other peptides, including cholecystokinin, neurotensin, and somatostatin in the striatum. In adult rats, nigrostriatal DA depletion induced cholecystokinin and neurotensin mRNA in the striatum (Schiffmann and Vanderhaeghen 1992; Engber et al. 1992). The cholecystokinin mRNA expression was limited to dorsolateral parts of the ipsilateral denervated striatum (Schiffmann and Vanderhaeghen 1992). The increase in cholecystokinin was particularly unexpected, since cholecystokinin co-localizes with TH in the SNpc and VTA, even though not all DA neurons contain this peptide (Savasta et al. 1989). Furthermore, dynorphin mRNA expression in striatal patch neurons showed a marked reduction, while matrix neurons showed no change after lesioning of DA neurons in adult animals (Gerfen et al. 1991). Finally, a unilateral 6-OHDA lesion performed in neonatal rats had no effect on somatostatin mRNA (Cimino et al. 1991), but was found to increase neurokinin B, a member of the tachykinin family, on the ipsilateral side of the lesion (Burgunder 1991).

Drug Challenges on Neuropeptides After 6-OHDA. Treatment with DA agonists further implicated nigrostriatal DAergic control of neuropeptides located in the striatum. It has been shown that chronic treatment with either a D_1 or D_2 receptor agonist in adult unilateral 6-OHDA-lesioned rats affects several neuropeptides, but in distinct ways. Systemic administration of the D_2 receptor agonist quinpirole inhibited the increase in enkephalin mRNA levels in these lesioned animals (Nisenbaum et al. 1994b; Engber et al. 1992). Moreover, systemic administration and direct infusion of quinpirole for 7 days to lesioned animals elevated

somatostatin and neuropeptide Y levels in the striatum, but decreased the lesion-induced increase in neurotensin and enkephalin (Nisenbaum et al. 1994b; Engber et al. 1992). Administration of the D_1 receptor agonist SKF-38393 had no effect on enkephalin, but reduced somatostatin and neuropeptide Y levels, and increased neurotensin and dynorphin levels in the striatum of unilateral 6-OHDA-lesioned animals (Engber et al. 1992). These findings suggested that a reciprocal relationship exists between the function of these neuropeptides and the DA receptor subtypes in rats with a unilateral lesioning of DA neurons. In neonatal lesioned animals, administration of SKF-38393 beginning 24 h after 6-OHDA treatment reversed the lesion-induced increase in enkephalin and decrease in substance P in the striatum, while quinpirole (LY-17155) had no effect (Sivam and Cox 2006), suggesting an important role for D_1 receptors in the postnatal development of enkephalin and substance P systems in this region. Kaakkola and colleagues found that circling behavior induced by an enkephalin analog, FK 33-824, microinjected into the SNr is antagonized by 6-OHDA lesions, suggesting that the response is mediated by the nigrostriatal DA pathway (Kaakkola 1980). In contrast, increased locomotor and rearing behavior in rats induced by microinjection of the peptidase-resistant enkephalin analog D-ala-met-enkephalamide (DALA) into the nucleus accumbens was found to be unaffected by the destruction of the mesolimbic DA system with 6-OHDA, indicating that this action occurred independent of an intact mesolimbic DA system (Kalivas et al. 1983).

5 Behavioral Supersensitivity After 6-OHDA Lesions

5.1 Hyperlocomotor and Stereotyped Responses to DA Agonists

Since the early 1970s, it has been established that all treatments resulting in the inhibition or loss of DA transmission in the adult striatum induce a behavioral supersensitivity to agonist administration. This phenomenon is most commonly examined by testing rats for their rotational response to mixed D_1/D_2 receptor agonists (e.g., apomorphine) following adult unilateral lesions of the nigrostriatal DA pathway (Schwarting and Huston 1996). The behavioral supersensitivity after adult unilateral 6-OHDA lesions has been associated with the increase in D_2 receptor number found in the striatum, suggesting that the change in D_2 receptor density is one mechanism by which the DA denervation-induced supersensitivity occurs (Creese et al. 1977; Marshall and Ungerstedt 1977). Although rats unilaterally lesioned with 6-OHDA provide a useful model to examine drug actions in the CNS, one major problem is that the behavioral response to DA agonists cannot be examined in non-lesioned animals. An additional problem is that the intact control hemisphere may undergo a rapid reorganization (morphological and/or neurochemical) subsequent to the unilateral DA depletion which would allow for compensatory function on the contralateral side of the lesion (Tillerson et al. 2001; Woodlee et al. 2008), necessitating the assessment of biochemical changes in non-lesioned striatum as well in order to avoid a possible misinterpretation of data.

Bilateral destruction of DA neurons in adult rats has also been used to examine supersensitive responses to DA agonists. While systemic administration of low doses of apomorphine and L-dopa in controls produces a minor increase in locomotor responsiveness that is accompanied by sniffing, these D_1/D_2 receptor agonists induce a major increase in locomotion in the bilaterally lesioned rats. The elevated locomotor response to apomorphine in the adult rats was found to be blocked by haloperidol, *cis*-flupentixol, and SCH-23390 (Breese et al. 1985a). Likewise, these adult bilaterally lesioned rats exhibited a major increase in activity when administered a D_2 agonist, with only a variable, but modest limited increase being observed after D_1 agonist administration (Breese et al. 1985a). In this latter case, haloperidol prevented the locomotor response to the D_2 agonist, but SCH-23390 did not (Breese et al. 1985a).

Systemic treatment of intact adult rats with amphetamine or apomorphine also induces a repetitive occurrence of distinct stereotypic behaviors (Costall and Naylor 1974; Costall et al. 1975, 1977; Creese and Iversen 1974). The DA agonist-induced stereotypic behaviors are thought to reflect short-term changes in activity of DA receptors in the striatum of intact rats (Breese et al. 1984a; Costall and Naylor 1981; Silbergeld and Pfeiffer 1977). Increased locomotor activity and concomitant floor sniffing are the first components to occur following low doses of DA agonist (Breese et al. 1984a, 1985a), followed by repetitive limb and head movements such as rearing, paw treading, head bobbing, and floor/wall sniffing (Breese et al. 1984a, 1985a). At higher doses, locomotor components drop out and repetitive head and mouth movements (perioral responses) such as licking, biting, and gnawing predominate. Administration of apomorphine or an additional mixed D_1/D_2 receptor agonist, L-dopa, to rats after adult bilateral 6-OHDA lesions produces a marked increase in locomotor activity over non-lesioned animals (Hollister et al. 1974) that is accompanied by stereotypic behaviors such as intense sniffing, rearing, paw treading, and head bobbing, reflecting a supersensitivity of DA receptors (Creese and Iversen 1974). In contrast, D-amphetamine does not produce any behavioral effects following its administration to these adult animals (Hollister et al. 1974). In unilaterally lesioned adults, d-amphetamine induces contralateral turning, a response opposite to that seen with apomorphine (Ungerstedt 1971b, d). Since amphetamine exerts its behavioral effects through the release of DA and blockade of its uptake into the nerve terminal, its ineffectiveness is the consequence of the 6-OHDA-induced DA loss (Breese et al. 1974).

Breese and colleagues were the first to show that certain behavioral responses to apomorphine and L-dopa in neonatal 6-OHDA-lesioned rats were significantly different from those in adult 6-OHDA-lesioned rats (Breese et al. 1984a, b, 1985b). While increased locomotion, sniffing, paw treading, and head nodding were behaviors characteristically associated with the mixed D_1/D_2 agonist administration to rats bilaterally lesioned with 6-OHDA as adults or as neonates, self-biting and a distinct behavior involving the repeated drawing of the forepaws up to and away from the nose referred to as "taffy pulling" were common unique responses in the neonatal lesioned rats. Both groups showed elevated rearing after either L-dopa or apomorphine treatment, indicative of their supersensitivity to these

challenges (Breese et al. 1984a, b, 1985b). However, a high percentage of rats with neonatal 6-OHDA lesions exhibited self-injurious behavior (SIB) to a D_1/D_2 DA agonist challenge, an effect not found in rats bilaterally lesioned with 6-OHDA as adults (Breese et al. 1984a, b, 1985b). While haloperidol reduced some behaviors including SIB induced by L-dopa in the neonatal lesioned rats, Breese and colleagues (1984a) further showed that *cis*-flupentixol was more potent than haloperidol in antagonizing the self-biting induced by L-dopa. In light of the report by Hyttel that *cis*-flupentixol was more selective for the D_1 receptor site than the D_2 receptor site (i.e., the haloperidol binding site) (Hyttel 1978), inhibition of SIB by *cis*-flupentixol suggested that D_1 receptors might be involved in the behavioral supersensitivity observed in rats depleted of DA during development (Breese et al. 1984a). Later studies showed that pretreatment with SCH-23390, a D_1 receptor antagonist, blocked self-biting and the SIB induced by L-dopa in neonatal 6-OHDA-lesioned animals, while D_2 receptor antagonists had a lesser effect (Breese et al. 1985a, 1989). Surprisingly, Breese et al. also reported that individual treatment with either a D_1 or D_2 receptor antagonist did not block the supersensitive locomotor response to L-dopa in adult or neonatal rats lesioned with 6-OHDA (Breese et al. 1985a). However, when these antagonists were combined, the enhanced locomotor activity induced by the L-dopa was virtually eliminated.

In subsequent studies, responses to specific D_1 and D_2 receptor agonists provided further evidence for distinct differences in responsive to these agonists depending on the age at which the DA was destroyed. When administered the specific D_2 receptor agonist LY-171555 (quinpirole), adult rats with bilateral 6-OHDA lesions exhibited a much greater locomotor responsiveness than did neonatal lesioned animals (Breese et al. 1985a). In contrast, neonatal lesioned rats demonstrated a significantly greater behavioral responsiveness to administration of SKF-38393, a specific D_1 receptor agonist, than did the adult lesioned group (Breese et al. 1985a). These findings suggested that the supersensitivity to DA agonists in the neonatal lesioned rats is primarily mediated by D_1 receptors, whereas D_2 receptors show greater sensitivity in the adult lesioned rats (Breese et al. 1985a). Such data provide further support for the view that the neonate and adult 6-OHDA lesions of the DA system induce adaptations that result in an altered functional relationship between D_1 and D_2 receptor function.

Altered Responses to DA Antagonists. As noted before, both neonatal and adult 6-OHDA-lesioned rats can be profoundly affected by doses of the catecholamine synthesis inhibitor α-MT that have no impact in non-lesioned animals – a further demonstration of the importance of residual DA function in the lesioned animals (Cooper et al. 1973a; Luthman et al. 1995; Moy 1995; Potter and Bruno 1989; Rogers and Dunnett 1989a). Therefore, studies were undertaken to evaluate whether treatment with selective DA receptor antagonists to minimize the action of residual DA function in neonatal and adult lesioned rats would provide further support for the concept that age-dependent differences exist between the two groups. In this respect, adult rats lesioned as neonates show a subsensitivity to the D_2 receptor antagonist haloperidol and the D_1 receptor antagonist SCH-23390 compared to the action in adult lesioned rats (Bruno et al. 1984; Duncan et al. 1987).

In non-lesioned controls and adult 6-OHDA-lesioned rats, Duncan and colleagues demonstrated that acute administration of either haloperidol or SCH-23390 produced a dose-dependent increase in akinesia (measured as movement on an inclined screen) (Duncan et al. 1987). In contrast, no significant alteration in immobility was observed in neonatal 6-OHDA-lesioned rats, even at a dose of SCH-23390 twice that necessary to produce akinesia in controls (Duncan et al. 1987). In another study, Bruno and colleagues reported subsensitivity to the akinesia-producing effect by haloperidol and fluphenazine, another neuroleptic, in neonatal rats lesioned at P3, but not those lesioned at P10, when tested during adulthood (Bruno et al. 1987). Rats with neonatal 6-OHDA lesions have also shown a subsensitivity to the effect of DA antagonist pimozide on lateral hypothalamic self-stimulation (Stellar et al. 1988), a finding which led researchers to suggest that a non-DA process mediates the observed behavioral sparing in these animals.

5.2 Priming of D_1 Agonist-Induced Supersensitivity

In rats lesioned as neonates, maximal supersensitivity of locomotor and stereotyped responses to SKF-38393 is achieved by administering multiple doses of the D_1 agonist at weekly intervals over a period of 4 weeks (Breese et al. 1985a). This effect, referred to as "priming of D_1 receptor supersensitivity," does not occur in rats with adult bilateral 6-OHDA lesions (Breese et al. 1985a). Such a sensitization process was not found after repeated D_2 agonist treatment to neonatal 6-OHDA-lesioned rats, nor did a D_2 antagonist block the priming sensitization caused by SKF-38393 (Criswell et al. 1989). From studies involving intra-accumbens and intrastriatal antagonist-induced suppression of these behaviors, the enhanced locomotor effects could be attributed mainly to activation of D_1 receptors in the nucleus accumbens and stereotypies to activation of D_1 receptors in either the accumbens or the striatum (Breese et al. 1987a). Treatment with a full D_1 receptor agonist such as A-68390 or SKF-82958 can also induce priming in neonatal 6-OHDA-lesioned rats (Johnson et al. 1992; Bishop et al. 2003), although a major disadvantage is that large doses of these D_1 receptor agonists desensitize the D_1 receptors. Still, at a reasonable dose, full D_1 agonists are more potent at inducing the priming phenomenon than SKF-38393 (Johnson et al. 1992; Bishop et al. 2003). The subsequent evaluation of priming of D_1 receptor supersensitivity demonstrated that this effect was long-lasting and possibly permanent (Criswell et al. 1989). Another line of research focusing on increased oral activity in neonatal lesioned rats demonstrated a marked supersensitivity of these animals to SKF-38393 even at 8 months of age (Kostrzewa and Gong 1991). Furthermore, the repeated administration of a D_1 receptor agonist to pre-weanling rat pups was found to cause a partial priming effect in adulthood (Gong et al. 1993a). Additionally, Kostrzewa and Kostrzewa found that quinpirole-induced vertical jumping induced during postnatal ontogeny was enhanced by neonatal 6-OHDA lesioning (Kostrzewa and Kostrzewa 2012).

Although adult animals given bilateral 6-OHDA lesions do not exhibit a priming effect with repeated DA agonist treatments, Morelli and DiChiara demonstrated that priming to SKF-38393 can be observed in adult animals given unilateral 6-OHDA lesions (Morelli and Di Chiara 1987). In this case, prior exposure to either a D_1 or D_2 receptor agonist results in an enhanced behavioral response to SKF-38393. While neonatal 6-OHDA-lesioned rats do not show a sensitization process to quinpirole after repeated treatments, they do, like adult unilateral lesioned animals, show a supersensitized response to SKF-38393 following a previous exposure to the selective D_2 agonist (Criswell et al. 1989). This evidence suggested that both neonatal and adult unilateral lesions may result in comparable adaptations of the DA system, while bilateral lesions in adult animals produce different compensatory changes.

5.3 Functional Uncoupling of D_1 and D_2 Receptors by 6-OHDA

In non-lesioned rats, evidence has accumulated that D_1 and D_2 receptors are functionally "coupled." In this regard, Breese and colleagues reported that the locomotor stimulant effect of apomorphine, which has its primary effects on the D_1/D_2 receptor sites, could be inhibited in non-lesioned animals by SCH-23390 (Breese and Mueller 1985; Breese et al. 1986), receptor antagonist with documented specificity for the D_1 receptor (Billard et al. 1984; Molloy and Waddington 1984). Subsequently, Breese and Mueller demonstrated that this specific D_1 receptor antagonist also prevented the locomotor response to the specific D_2 agonist, quinpirole, in control rats, just as did haloperidol, a D_2 receptor antagonist (Breese and Mueller 1985). In contrast to this finding, SCH-23390 was then found to be without effect on the response to this D_2 agonist in rats with DA neurons lesioned with 6-OHDA. These findings suggested that the coupled functional interaction between D_1 and D_2 receptor subtypes to reach a maximal response appears to be dependent upon having an intact DA system (Breese and Mueller 1985). In additional studies, disruption of CAergic activity via the administration of reserpine and α-MT was found to reduce the ability of SCH-23390 to block the effects of quinpirole in non-lesioned animals (Breese et al. 1986). These data collectively demonstrated clearly that D_1 receptors can modulate D_2 receptors in rats by a mechanism dependent upon intact DA function (Breese and Mueller 1985). Such disruption in the functional coupling of D_1 and D_2 receptors after impaired DAergic transmission has subsequently documented by several other investigators (Walters et al. 1987; Jackson and Hashizume 1987; Longoni et al. 1987).

In additional studies by Breese and colleagues in which the role of D_1 and D_2 receptor involvement of L-dopa induced SIB was explored, D_1 and D_2 agonists were administered alone and in combination to neonatal lesioned rats (Breese et al. 1985a). Repeated administration of the selective D_1 agonist SKF-38393 to rats with neonatal 6-OHDA lesions at doses that produced priming did not lead to SIB, even though L-dopa-induced SIB could be blocked by a selective D_1 receptor

antagonist. Moreover, treatment with the D_2 agonist quinpirole alone likewise did not lead to SIB (Breese et al. 1985a). Importantly when a low dose of quinpirole was combined with a low dose of SKF-38393, SIB was observed in the majority of neonatal 6-OHDA-lesioned rats treated (Breese et al. 1985a). This later finding suggested that while D_1 receptor activation is essential for SIB to develop in rats with neonatal lesions, D_2 receptor activation, which also occurs with L-dopa treatment, is needed for the maximal expression of this SIB response. Johnson and Bruno showed that treatment with either a specific D_1 or D_2 receptor antagonist decreased food intake and induced akinesia in non-lesioned rats at doses that had no effect in neonatal lesioned animals (Johnson and Bruno 1992). Only when both agonists were administered together did the lesioned animals exhibit a response comparable to non-lesioned rats. A functional uncoupling of D_1 and D_2 receptors by minimizing DA function may also explain the subsensitivity of neonatal 6-OHDA-lesioned rats to DA receptor antagonists (Johnson and Bruno 1990, 1992). Collectively, these observations provide further evidence for a functional relationship between the two DA receptor subtypes (D_1/D_2 coupling) being required for maximal effectiveness of DA function (Breese et al. 1985a).

The mechanisms involved in the D_1 and D_2 receptor interaction have been suggested to occur by receptor activation on discrete neurons (Gerfen et al. 1995; LaHoste et al. 2000) or by changes in D_2 receptor-mediated cholinergic interneuron activity with subsequent impact on D_1 receptor-expressing striatonigral neuronal transmission (Di Chiara et al. 1994). These possibilities would seem consistent with the fact that GABAergic striatonigral and striatopallidal pathways have distinct DA receptor subtypes (Gerfen 1992) and that lesioning of DA neurons disrupting D_1/D_2 receptor integration (Breese and Mueller 1985). However, more recent studies suggest that an interaction between D_1 and D_2 receptors occurring within the same cell may also be involved. For example, single medium spiny-to-medium spiny neuron synaptic connections in neonatal striatal cultures have been reported to exhibit modulation by both D_1 and D_2 receptor agonists (Geldwert et al. 2006), and the selective activation of D_1 or D_2 receptors in single striatal neurons has been shown to differentially modulate tetrodotoxin-sensitive sodium channels (Aizman et al. 2000). It is also now known that D_1 and D_2 receptors can form a heteromeric complex via a direct protein-protein interaction on medium spiny neurons that requires activation of both DA receptor subtypes for activation of the G-protein-coupled pathway (Rashid et al. 2007), an observation consistent with the striatal GABAergic neurons that co-expresses both D_1 and D_2 receptors (Perreault et al. 2011). Consequently, possibly neurons with single as well as both DA receptor subtypes could account for the "uncoupling" of DA function described with lesioning. Regardless, the uncoupling of D_1 and D_2 receptor function has been suggested to be linked to a number of psychiatric illnesses related to DA dysfunction, including schizophrenia, drug abuse, and depression (Lee et al. 2009; Pei et al. 2010; Fuxe et al. 2008).

5.4 Hyperlocomotor Responses to NMDA Antagonists

Distinct pharmacological profiles also emerge when neonatal and adult 6-OHDA-lesioned rats are administered MK-801, a noncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor (Criswell et al. 1993; Morelli and Di Chiara 1990a). Rats lesioned as neonates show a latent supersensitivity to the hyperlocomotor stimulant action of MK-801 and CGS-19755, a competitive NMDA receptor antagonist. Rats lesioned as adults, either bilaterally or unilaterally, do not show this enhancement in MK-801-induced locomotor activity (Criswell et al. 1993; Morelli and Di Chiara 1990a; Carlsson and Carlsson 1989; Klockgether and Turski 1990; Morelli et al. 1992). Because pretreatment with α-MT inhibits some, but not all, of the stimulant effects of MK-801 in neonatal lesioned rats (Criswell et al. 1993), a portion of the increased locomotor response can be attributed to some mechanism other than catecholamine or DA release. Additional evidence that the effects of MK-801 are mediated by at least two distinct mechanisms arises from the observation that the coadministration of D_1 and D_2 receptor antagonists attenuates some, but not all, of the actions of the NMDA antagonist in neonatal lesioned animals (Criswell et al. 1993). In contrast, α-MT pretreatment in rats with adult lesions not only prevented the effects of MK-801 but also markedly attenuated any locomotor response (Criswell et al. 1993). The severity of α-MT-induced akinesia seen in the adult lesioned rats further underscores the importance of the residual DAergic activity in these animals.

Studies have also shown that MK-801 and other NMDA antagonists can potentiate the effects of DA agonists. In non-lesioned rats pretreated with reserpine and α-MT, the ability of L-dopa to diminish the resulting motor symptoms can be markedly potentiated by administering a single dose of MK-801 or CPP (a competitive NMDA antagonist), which on their own has no observed effects (Klockgether and Turski 1990). MK-801 also improves the effects of SKF-38393 on contralateral turning in rats given adult unilateral 6-OHDA lesions (Morelli and Di Chiara 1990a; Morelli et al. 1992), although the effects of MK-801 on L-dopa-induced turning remain variable (Morelli et al. 1994; Spooren et al. 2000; Paquette et al. 2010; Dupre et al. 2008). Interestingly, MK-801 can prevent L-dopa-induced SIB in a dose-dependent manner in neonatal 6-OHDA-lesioned rats, while potentiating some stereotyped behaviors, i.e., paw treading, paw licking, and rearing, produced by DA agonists (Criswell et al. 1990). It has been postulated that the differential effects of MK-801 in DA-depleted rats are owed to distinct interactions between MK-801 and the D_1 and D_2 receptor subtypes (Morelli and Di Chiara 1990a, b; Morelli et al. 1992).

In line with these earlier findings with MK-801 and CGS-19755, more recent studies have revealed that neonatal 6-OHDA-lesioned rats also exhibit supersensitivity to the effects of phencyclidine (PCP), another NMDA receptor antagonist (Moy and Breese 2002). The PCP supersensitivity, displayed as an enhanced locomotor activity, was most pronounced after repeated PCP treatment or after a brief priming regimen with a D_1 receptor agonist. This latter observation was

consistent with the process of sensitization of PCP that might be linked to changes in D_1 receptor function as noted in Sect. 5.5 to follow. Nonetheless, non-DAergic mechanisms may play a more important role in the acute attenuation of PCP effects in sensitized animals, since the enhanced locomotor responses in the neonatal lesioned animals could be blocked by the atypical antipsychotic agent olanzapine, a compound with activity at D_1, D_2 and specific 5-HT receptors (Bymaster et al. 1996), but not by haloperidol or SCH-23390 (Moy and Breese 2002). Later studies showed that the selective 5-$HT_{2A/2C}$-receptor antagonist ketanserin could significantly reduce PCP activation in lesioned rats, suggesting that the efficacy of olanzapine against PCP-induced sensitized responses may be mediated by one of these 5-HT_2 receptor subtypes (Moy et al. 2004).

5.5 NMDA Antagonist Inhibition of D_1 Agonist-Induced Supersensitivity

Apart from the stimulant effects on locomotor activity, blockade of the NMDA receptor can suppress some forms of neuronal plasticity, such as long-term potentiation (Morris et al. 1986), and can inhibit excitotoxic cell death induced by ischemia, trauma, and other insults (Hetman and Kharebava 2006). Important to this discussion of DA function, the long-lasting nature of priming of D_1 receptor supersensitivity to repeated doses of SKF-38393 in neonatal 6-OHDA-lesioned rats led to the hypothesis that this form of sensitization involves neural processes involving NMDA receptors similar to other forms of neuronal plasticity in CNS (Criswell et al. 1989). In line with this proposal, Criswell and colleagues showed that coadministration of MK-801 or CGS-19755 along with each priming dose of SKF-38393 blocked the behavioral sensitization (priming) observed by this repeated D_1 agonist administration to neonatal 6-OHDA-lesioned rats (Criswell et al. 1990). Since earlier work had demonstrated that the D_1 antagonist SCH-23390 also blocked the priming effect in neonatal lesioned rats (Criswell et al. 1989), it became clear that the concomitant activation of D_1 and NMDA receptors is likely necessary for priming of D_1 receptor supersensitivity in neonatal lesioned rats to occur. The indication that NMDA receptor activation is also a necessary prerequisite for other responses that convey long-term consequences suggested that these phenomena share a common biochemical mechanism with D_1 receptor-mediated priming (see Sect. 5.2).

5.6 IEG and Signal Transduction Pathway Involvement in D_1 Agonist-Induced Supersensitivity

To date, a number of mechanisms potentially responsible for the development of priming of D_1 receptor sensitivity in neonatal lesioned rats have been explored (Breese et al. 2005). The lack of any substantially significant changes in D_1 receptor mRNA and/or expression in the striatum of rats after neonatal 6-OHDA lesions led

to the speculation that perhaps enhanced intraneuronal second messenger levels or efficiency likewise accompanied D_1 agonist-induced priming in these animals (Gong et al. 1994). However, Gong and colleagues showed that basal as well as DA-, aluminum tetrafluoride-, and forskolin-stimulated adenylyl cyclase activities were not changed following repeated SKF-38393 treatment to neonatal lesioned rats during adulthood (Gong et al. 1994). Simson and colleagues also reported that SKF-38393-stimulated adenylyl cyclase activity was not altered in the striatum of these animals (Simson et al. 1992). These findings suggested that D_1 receptor-linked adenylyl cyclase is not a major determinant of enhanced behavioral responses in neonatal lesioned rats to repeated D_1 agonist administration. Likewise, other studies showed that adenosine 3–5′-monophosphate-regulated phosphoprotein (DARPP-32), a cyclic AMP-regulated phosphoprotein functionally linked to striatal D_1 receptors (Barone et al. 1994), remained unaffected in the adult striatum of neonatal lesioned rats primed with multiple SKF-38393 treatments (Luthman et al. 1990a; Breese et al. 1994).

Because earlier studies had linked the proto-oncogene *c-fos* to behavioral supersensitivity to L-dopa or D_1 agonists in rats either unilaterally or bilaterally lesioned with 6-OHDA as adults (Robertson et al. 1989a, b; Dragunow et al. 1991; Cole et al. 1993; Morelli et al. 1993), *c-fos* expression was examined in neonatal lesioned rats after D_1 agonist treatment. Expression of *c-fos* occurred only after a dose of D_1 agonist that was much higher than the behaviorally active dose and was not increased to a greater extent following repeated D_1 agonist treatments (Johnson et al. 1992). Moreover, while NMDA receptor antagonists could block the D_1 agonist-induced sensitization, it did not block *c-fos* expression in these animals (Johnson et al. 1992). Thus, it was concluded that *c-fos* is not a critical component in the priming phenomenon.

Mitogen-activated protein kinases (MAPKs) are intracellular mediators of signal transduction that are activated in response to a variety of extracellular stimuli (Johnson and Lapadat 2002; Pearson et al. 2001). There are three major classes of MAPKs: extracellular signal-regulated kinases (ERKs) (Sweatt 2001, p. 38; Johnson and Lapadat 2002), and stress-activated protein kinase/c-Jun amino-terminal kinase (SAPK/JNK) (Davis 2000). Among these, the most well studied are the closely related extracellular signal-regulated kinases 1 and 2 (ERK1/2). Biological processes involving ERK1/2 include stimulation of cell proliferation and survival, neoplastic transformation, neuronal differentiation, and plasticity (Seger and Krebs 1995; Paul et al. 1997). Several studies in both non-lesioned and adult 6-OHDA-lesioned animals showed that the stimulation of D_1 receptors can activate ERK1/2 to produce long-lasting adaptive changes in the brain (Vossler et al. 1997; Yao et al. 1998; York et al. 1998; Valjent et al. 2000; Gerfen et al. 2002). Thus, Papadeas and colleagues suggested that ERK1/2 may be involved in the adaptive processes that underlie the behavioral supersensitivity of neonatal lesioned rats to repeated D_1 agonist treatments (Papadeas et al. 2004, 2008). Similar to reports in adult 6-OHDA-lesioned rats (Gerfen et al. 2002), the researchers found that single as well as multiple doses of SKF-38393 to rats with neonatal 6-OHDA lesions produces an acute, transient increase in ERK1/2 activity that peaks at 15 min following

drug treatment (Papadeas et al. 2004). In conjunction with findings in adult lesioned animals, this finding suggested that the D_1 agonist-induced increases in locomotor activity could arise from strengthened ERK1/2-mediated signal transduction mechanisms involving in particular D_1 receptor-expressing striatonigral neurons. The researchers also showed that the priming of D_1 receptor sensitivity to multiple doses of SKF-38393 in rats with neonatal 6-OHDA lesions is coupled to a chronic, NMDA receptor-mediated ERK1/2 kinase activity (>7 days) in the medial prefrontal cortex and other cortical regions (i.e., the ventrolateral orbital, cingulate, motor, somatosensory, and piriform cortices) that promotes the phosphorylation of cAMP response element-binding protein (CREB), a transcription factor integral for neuronal plasticity (Lonze and Ginty 2002), and produces enduring structural changes in prefrontal cortical neurons (Papadeas et al. 2004, 2008). The lasting increase in ERK1/2 activity appears to be dependent upon D_1 receptor function, as SCH-23390 antagonist pretreatment blocked the prolonged ERK1/2 response to repeated administration of SKF-38393 to neonatal lesioned rats (Papadeas et al. 2004). Importantly, the SCH-23390 did not inhibit ERK1/2 activation through its interaction with 5-HT_2 binding sites (Bischoff et al. 1988; McQuade et al. 1988), since systemic injections of the nonselective 5-$HT_{2A/2C}$ antagonist ketanserin prior to SKF-38393 had no effect on the prolonged ERK1/2 response.

In addition, intracerebroventricular or systemic pretreatment with the MAPK kinase (MEK)/ERK1/2 inhibitor SL327, and the structurally dissimilar PD 98059, prior to each of three of four weekly sensitizing doses of SKF-38393 was found to alter the character of the supersensitive behavioral response elicited by D_1 agonism (Papadeas 2006). Priming-induced locomotor behaviors such as running, walking, and trotting were augmented, while stereotyped behaviors such as rearing, paw treading, and taffy pulling were inhibited. It could be hypothesized that the augmentation of locomotor behaviors concomitantly inhibited the expression of rearing and paw-fixation behaviors in these animals (Bernardi et al. 1986; Chinen et al. 2006) or, alternatively, that the opposite effects of MEK inhibitor on locomotor and stereotyped behaviors may have occurred because the behaviors are driven by the activation of different neural systems. The microinjection of PD 98059 directly into the medial prefrontal cortex prior to SKF-38393 treatments inhibited the stereotyped behaviors, but did not affect locomotor behaviors (Papadeas 2006). This documentation suggested that forebrain ERK1/2 activation contributes to some but not all of the sensitized responses seen with D_1 agonist priming of neonatal lesioned rats and further that medial prefrontal cortical ERK1/2 activation may be critical for the sensitization of specific stereotyped behaviors exhibited by these animals.

5.7 Hyperlocomotor and Oral Responses to 5-HT Receptor Agonists

Evidence that neonatal 6-OHDA-lesioned rats show, in addition to the 5-HT hyperinnervation, increased 5-HT receptor density prompted several investigators to explore the role of 5-HT in spontaneous behavior and/or behavioral

responsiveness to DA agonists in these animals. Gong and Kostrzewa showed that systemic administration of the 5-$HT_{1B/2C}$ receptor agonist m-CPP could considerably increase oral activity in neonatal DA-depleted rats compared to control animals (Gong and Kostrzewa 1992). This enhanced oral response was dependent upon a selective supersensitivity of 5-HT_{2C} receptors and was elevated to an even greater extent than that after D_1 agonist treatment in these animals (Gong and Kostrzewa 1992). In addition, the motor hyperactivity characteristic of these rats during adolescence (Shaywitz et al. 1976a; Erinoff et al. 1979; Heffner and Seiden 1982) was found to be reversed by systemic administration of the 5-HT_{2A} receptor antagonists ketanserin and mianserin (Luthman et al. 1991), suggesting that the striatal increase in 5-HT_{2A} receptors facilitates this abnormal behavior. Studies demonstrating the increased responses of striatal neurons to iontophoresed 5-HT and to 5-HT_{1B} (m-CPP) and 5-HT_2 (DOI) agonists after neonatal 6-OHDA lesions further confirmed that increases in the corresponding 5-HT receptors were indeed functional (el Mansari et al. 1994). More recent studies have shown that the stimulation of 5-HT_2 receptors in neonatal or adult unilateral DA-depleted rats with the 5-HT_2 receptor agonist DOI can induce enhanced motor behaviors (Bishop et al. 2004; Bishop and Walker 2003), likely as a consequence of increased 5-HT_{2A} receptor expression in the dorsal striatum (Radja et al. 1993a; Laprade et al. 1996; Basura and Walker 1999) and enhanced 5-HT_2 receptor-mediated activation of the direct striatal pathway (Gresch and Walker 1999; Basura and Walker 2000, 2001). Thus, in neonatal lesioned animals, striatal function may be enhanced significantly due to an elevated number of 5-HT receptors, even in the presence of extracellular 5-HT levels that remain near normal because of increased uptake (Jackson and Abercrombie 1992).

In the intact striatum, studies have consistently demonstrated that intrinsic 5-HT_2 receptors can modify DA function and have postulated divergent roles for 5-HT_{2A} and 5-HT_{2C} receptor subtypes (Lucas and Spampinato 2000; Porras et al. 2002). 5-HT_{2A} antagonists reduce hyperlocomotion induced by cocaine, amphetamine, and 3,4 methylenedioxymethamphetamine (MDMA) (Kehne et al. 1996; O'Neill et al. 1999). Conversely, 5-HT_{2C} receptor antagonists have been shown to enhance or reduce these effects depending upon which compounds and neural sites are studied (Filip and Cunningham 2002; Filip et al. 2004; Fletcher et al. 2002). While less is known about the interaction of these receptors following 6-OHDA-induced DA depletions, DA-related behaviors can be modified by 5-HT_2 receptor antagonists. For example, elevated oral dyskinesia induced by the ventral striatal infusion of SKF-38393 into neonatal 6-OHDA-lesioned rats was diminished by 5-$HT_{2A/2C}$ antagonism (Gong and Kostrzewa 1992; Plech et al. 1995). O'Boyle and colleagues demonstrated that hyperlocomotor activity induced by systemic or intrastriatal administration of SKF-82958 to adult unilateral 6-OHDA-lesioned rats (O'Boyle et al. 1989) can be reduced by pretreatment with the 5$HT_{2A/2C}$ antagonist ritanserin (Bishop et al. 2003). Further studies revealed that the D_1 agonist-induced hyperlocomotor activity could be reduced by antagonism of striatal 5-HT_{2A} receptors with M100907 (volinanserin), but not 5-HT_{2C} receptors with RS102221 (Bishop et al. 2005). Volinanserin also significantly reduced

SKF-82958-induced hyperlocomotor activity in neonatal lesioned animals (Bishop et al. 2005). Taken together, these data suggest that adaptations in 5-HT receptors not only account for various aspects of spontaneous behavior, but can also influence supersensitive D_1 receptors in rats with neonatal or adult unilateral 6-OHDA lesions.

5.8 Increased Stereotyped Responses to GABA Agonists

Differences in the behavioral profiles observed between neonatal and adult 6-OHDA-lesioned rats can also be seen following the administration of the $GABA_A$ receptor agonist, muscimol, into the SNr of these animals (Breese et al. 1987b). As mentioned earlier, the GABAergic neurons are projecting from the striatum into the SNr function as secondary mediators of DAergic activity (Albin et al. 1989; Gerfen et al. 1987). Additionally, DA neurons of the nigrostriatal pathway exert an inhibitory influence over these striatonigral neurons (Reid et al. 1990). Once the neurons of the nigrostriatal DA pathway are destroyed by 6-OHDA, the GABAergic activity in the SNr becomes enhanced. This presumably results in the increase in GABA levels observed in the SNr after neonatal 6-OHDA lesions (Molina-Holgado et al. 1993a). Elevated GABA activity in this region has been shown to play a role in SIB, as injections of muscimol into this region can elicit self-mutilation in normal animals (Baumeister and Frye 1986; Breese et al. 1987b). In both adult and neonatal DA-depleted rats, unilateral injections of muscimol into the SNr produced a supersensitive turning response as compared to non-lesioned animals (Breese et al. 1987b), a finding consistent with the reported increase in [^{3}H]muscimol binding and immunostaining of $GABA_A$ receptors in the SNr after adult unilateral 6-OHDA lesions (Pan et al. 1985; Katz et al. 2005). Only neonatal lesioned rats (and few non-lesioned rats) showed SIB and taffy-pulling behavior in response to bilateral injections of muscimol into the SNr (Breese et al. 1987b). Together, these data support the idea that lesions of DA neurons produce an increased functional responsiveness of $GABA_A$ receptors in the SNr and that the increased susceptibility for SIB in neonatal DA-depleted rats is likely determined by neurons distal to the $GABA_A$ receptor complex in the SNr. Clearly, some other factor in the plasticity of these neurons to neonatal DA loss must account for the SIB that does not occur in the adult DA-depleted animals.

6 Modeling Specific Brain Disorders Using Rats with 6-OHDA Lesions

6.1 Parkinson's Disease

The first of the clinical neurodegenerative disorders known to have reduced levels of DA and its metabolite HVA in the brain and CSF was PD (Hornykiewicz 1975).

PET studies with labeled agents that bind to DA uptake sites or to DA neurons via uptake sites such as fluorodopa confirmed that DA neurons were reduced (Eidelberg 1992; Shinotoh and Calne 1995). The availability of 6-OHDA has enabled the specific lesioning of DA neurons in adult rats to model the DA deficiency seen in PD (Ungerstedt 1968; Albin et al. 1989) and has allowed for the study of pathogenic mechanisms and pathophysiological aspects of PD as well as the testing of various neuroprotective compounds and innovative therapeutic strategies (Duty and Jenner 2011). Recently, adult lesioned rats have been used to investigate abnormal involuntary movements (AIMs) to prolonged use of L-dopa (Cenci et al. 1998; Winkler et al. 1999; Steece-Collier et al. 2003) and have provided invaluable information regarding the potential neurochemical and molecular mechanisms involved in L-dopa-related side effects in PD patients, such as dyskinesia (Cenci et al. 1998; Andersson et al. 1999; Sgambato-Faure et al. 2005; Pavon et al. 2006; Santini et al. 2007; Westin et al. 2007; Cenci and Konradi 2010). Further, it was revealed that striatal 5-HT terminals can cause or aggravate L-dopa-induced dyskinesias by mishandling exogenous L-dopa and by releasing DA from the 5-HT terminals (Carta et al. 2007; Lindgren et al. 2010). In support of these actions related to 5-HT, administration of 5-HT_{1A} and 5-HT_{1B} agonists can have anti-dyskinetic effects (Carta et al. 2007; Lindgren et al. 2010). Currently, clinical investigations are underway to examine the efficacy of such 5-HT agonists in dyskinetic L-dopa-treated PD patients.

Still, the adult 6-OHDA-lesioned rat does not mimic all of the clinical features of PD. The depletion of DA, nigral DA cell loss, and neurobehavioral deficits have been successfully achieved with the model, but this lesioning does not appear to affect changes associated with other brain regions in PD, including olfactory structures, lower brain stem areas, or the locus coeruleus. Also, the adult 6-OHDA lesion does not produce or induce proteinaceous aggregates or Lewy-like inclusions like those seen in PD, although it has been reported that the 6-OHDA interacts with α-synuclein (Blandini et al. 2008). Moreover, the nature of the symptoms in the unilateral adult 6-OHDA-lesioned rat, the most frequently used rodent model of PD, does not reflect that of the human disease. Despite these limitations, the adult 6-OHDA-lesioned rat model has contributed enormously to our understanding of PD and remains at the forefront of preclinical drug discovery for PD.

6.2 Lesch-Nyhan Syndrome

In addition to PD, the developmental disorder LNS (Lesch and Nyhan 1964; Nyhan 1968) is a second clinical disorder in humans with a documented destruction of central DA (Lloyd et al. 1981). The symptoms of LNS include motor dysfunction, mental impairment, and a particularly alarming form of SIB wherein patients bite away their own lips and fingers (Lesch and Nyhan 1964). The SIB is observed in about 85 % of LNS patients (Stout and Caskey 1989). The disease is caused by an almost complete deficiency of the enzyme hypoxanthine-guanine

phosphoribosyltransferase (HPRT) which also is associated with hyperuricemia. The main outcome of this genetically based defect is an increase in purine levels and a buildup of uric acid in the tissues. However, the severe neurological symptoms, including the behavioral abnormalities, are not affected by treatment that attenuates the overproduction of uric acid, indicating that some other consequence of the HPRT deficiency underlies the neurological deficits (Christie et al. 1982; Kopin 1981).

Research involving the examination of postmortem brain tissue revealed that LNS patients have a loss of 65–90 % of DA terminals in the mesolimbic and nigrostriatal pathways (Lloyd et al. 1981). Moreover, the spinal fluid of children with LNS has diminished levels of the DA metabolite HVA (Jankovic et al. 1988; Silverstein et al. 1985). Studies using PET technology to evaluate the integrity of DAergic neurons in LNS showed that a labeled marker for the DA transporter was markedly reduced in the brains of patients with LNS compared to either normal controls or patients with Rett's syndrome (Wong et al. 1996). In addition, labeled fluorodopa accumulation was reduced in the brains of patients with the disease (Ernst et al. 1996). It is likely, then, that these alterations in DA function account for the majority of the neurological symptoms observed in LNS (Jankovic et al. 1988; Lloyd et al. 1981).

Currently, the neonatal 6-OHDA-lesioned rat is the most widely accepted model to emulate the neonatal destruction of DA-containing neurons in LNS (Breese et al. 1984a, b, 1994), as these lesioned rats display characteristics that are similar to neurological changes and symptoms seen in patients with LNS. First, in both LNS patients and the neonatal lesioned rats, reduced DA during development is accompanied by an increase in striatal serotonin levels (Breese et al. 1984b; Lloyd et al. 1981; Stachowiak et al. 1984). Second, patients with LNS suffer from cognitive impairments (Lesch and Nyhan 1964), and considerable evidence suggests that neonatal lesioned rats do not learn behavioral tasks at the rate of non-lesioned animals (Archer et al. 1988; Smith et al. 1973). Third, similar to LNS patients, neonatal lesioned rats show an increased susceptibility for SIB (Breese et al. 1984b). Fourth, patients with LNS also show aggressive and combative behavior under the appropriate conditions (agitation and anxiety) (Lesch and Nyhan 1964), a characteristic feature of neonatal lesioned animals (Breese et al. 1984b). Fifth, patients with LNS are small in stature (Lesch and Nyhan 1964), as are neonatal lesioned rats compared to their non-lesioned counterparts (Smith et al. 1973). Sixth, antipsychotic drugs have little effect on SIB in LNS patients (Jankovic et al. 1988) and in neonatal lesioned rats administered sensitizing doses of L-dopa (Breese et al. 1984b). Finally, similar to neonatal lesioned rats, motor and behavioral symptoms are worsened when LNS patients are administered L-dopa (Jankovic et al. 1988). It is notable that the symptoms in PD patients are alleviated by L-dopa treatment, but neither PD patients nor adult 6-OHDA-lesioned animals exhibit self-biting or SIB. Collectively, the evidence clearly suggests that the early age of onset of loss of DA function is the key factor in the outcome of the DA deficiency in LNS (Breese et al. 1984b; Watts et al. 1982; Baumeister and Frye 1985).

6.3 Other Psychiatric Conditions

There are three additional clinical disease states outlined below in which 6-OHDA lesions may be of potential usefulness in defining the basis of symptoms or sensitivity to drug responses observed after such lesions that are comparable to those observed in schizophrenia, drug addiction, or ADHD.

Schizophrenia. The enhanced susceptibility to NMDA antagonists production of psychosis observed in schizophrenia has led to the suggestion that a state of NMDA receptor hypofunction may be inherent to this psychiatric disorder (Javitt and Zukin 1991; Jentsch and Roth 1999; Olney 1989). Consequently, the exacerbated responsiveness to NMDA antagonists such as MK-801 and PCP in the neonatal 6-OHDA-lesioned rats could serve as a model of this NMDA dysfunction in schizophrenia. Rats with neonatal 6-OHDA lesions have been shown to have impaired habituation and alterations in startle responses and sensory gating (Moy et al. 1994; Schwarzkopf et al. 1992, 1996), as well as a marked susceptibility to the stimulant impact of pharmacological challenges and environmental stressors (Moy et al. 1994; Schallert et al. 1989). These characteristics are also similar to those observed in schizophrenia (Javitt and Zukin 1991; Jentsch and Roth 1999; Malhotra et al. 1997; Olney et al. 1999). Thus, the neonatal 6-OHDA-lesioned rat could be a valuable model to investigate the neural basis of the enhanced negative responsive to NMDA-receptor antagonists observed in schizophrenics.

Drug Addiction. The development of a behavioral sensitization occurs in non-lesioned rats following chronic, intermittent exposure to psychostimulant drugs such as cocaine, amphetamine, and methamphetamine (Segal and Mandell 1974; Nishikawa et al. 1983; Epstein and Altshuler 1978). Furthermore, increasing evidence suggests that this sensitization to psychostimulant drugs involves alterations in the sensitivity of postsynaptic D_1 receptors (McCreary and Marsden 1993; Kuribara 1995a, b; Yoshida et al. 1995; Henry and White 1991). For example, pretreatment with the D_1 receptor antagonist SCH-23390 antagonizes the locomotor-activating effects of repeated cocaine and methamphetamine treatment and prevents the development of sensitization (McCreary and Marsden 1993; Kuribara 1995a, b; Yoshida et al. 1995). Pretreatment with a D_2 receptor agonist, at least in the case of cocaine-induced sensitization, has no such effect on the sensitization (McCreary and Marsden 1993). Pretreatment with NMDA receptor antagonists such as MK-801 also blocks the sensitized response to psychostimulant drugs (Karler et al. 1989; Wolf 1998). These characteristics are similar to those related to priming of the behavioral responsiveness of neonatal 6-OHDA-lesioned rats to repeated doses of D_1 agonist (Criswell et al. 1989). In this respect, both D_1 and NMDA receptor antagonists block the "priming" of neonatal 6-OHDA-lesioned rats to repeated D_1 agonist treatments (Criswell et al. 1990). Finally, both forms of sensitization are accompanied by adaptive responses that converge upon common cellular and molecular signal transduction pathways (e.g., ERK1/2 and CREB) that are associated with D_1 and NMDA receptor activation and long-term plasticity (Fornai et al. 2009; Chao and Nestler 2004; Valjent et al. 2000; Girault et al. 2007; Papadeas et al. 2004). Given these similarities, neonatal lesioned rats primed with

D_1 agonist could provide a model for investigating further the basis of the priming of D_1 receptor sensitivity that occurs in normal rats to multiple doses of psychostimulant drugs. As neural adaptations that underlie the sensitization process in rats are thought to contribute to the development of the compulsive patterns of drug craving that characterizes drug addiction in humans (Robinson and Berridge 2008), investigations of the neural adaptations that develop in response to D_1 priming in neonatal lesioned animals could help uncover D_1 receptor-specific processes involved in human addictive behavior, such as drug craving to stress. One major advantage of this model is the persistence of the sensitized response to D_1 agonists following priming.

ADHD. The neonatal 6-OHDA-lesioned rat has also been proposed to be a useful model for ADHD. Similar to ADHD patients, rat pups lesioned with 6-OHDA exhibit hyperactivity (Shaywitz et al. 1976b) and impaired learning in a spatial discrimination task (Archer et al. 1988) which improves after methylphenidate or amphetamine treatment (Davids et al. 2002, 2003). In addition, as in ADHD, the neonatal lesioned rats show an initial decrease in spontaneous motor behavior when placed in a novel environment, but after repeated testing, increased activity is observed, thus providing useful information regarding the mechanisms that cause hyperactivity (Luthman et al. 1989a). The hyperactivity in neonatal 6-OHDA-lesioned rats is accompanied by decreased striatal DAT and increased D_4 receptor expression, as well as altered 5-HT function (Luthman et al. 1989a; Zhang et al. 2001). While the hyperactivity is not altered by DAT inhibitors, it can be greatly reduced by D_4 antagonists as well as inhibitors of NET and 5-HT transporters (Davids et al. 2002, 2003; Zhang et al. 2001, 2002a). These findings suggested that psychostimulants reduce the hyperactivity of neonatal lesioned rats by inhibiting NET and 5-HT transporters rather than DAT. Consistent with this conclusion, it has been demonstrated that administration of the NET inhibitor atomoxetine strongly antagonizes motor hyperactivity in juvenile rats with neonatal 6-OHDA lesions (Moran-Gates et al. 2005). When neonatally 6-OHDA-lesioned rats are lesioned in adulthood with 5,7-DHT, the level of hyperactivity is increased (Kostrzewa et al. 1994). Also, a 5-HT_2 receptor antagonist attenuates the spontaneous hyperlocomotor activity in neonatal 6-OHDA-lesioned rats (Brus et al. 2004). These findings further add support for involvement of the central 5-HT system in modulating hyperactivity. Furthermore, a possible role for 5-HT in stimulant treatment for ADHD has been proposed based on the demonstration of an inhibitory role of brain 5-HT systems in the actions of methylphenidate and amphetamine (Breese and Cooper 1975; Hollister et al. 1976).

7 Conclusion

The research on monoamine systems has greatly benefited from the discovery of 6-OHDA, the first compound implemented as a selective neurotoxin for CA-containing neurons (Porter et al. 1963; Ungerstedt 1968). Since the 1960s, 6-OHDA has been successfully employed in animals to biochemically dissect the roles of DA and NE neurotransmitter systems in the brain while at the same time

providing interesting models to study compensatory adaptations that occur in the metabolism, uptake mechanisms, sprouting, and plastic properties of the spared neurons and of the alternate neuronal projections that could replace the lesioned terminals. Eventual refinements in the use of 6-OHDA to selectively lesion DA neurons led to the proposal that DA-denervated rats provide a valuable model for human PD, a disease in which the loss of DA neurons is its main pathological hallmark. It soon became evident that while 6-OHDA-lesioned rats display many of the neurochemical characteristics found in human PD, they do not emulate all of the neurological features exhibited by patients. Clearly, the behavioral, neurochemical, morphological, and receptor alterations found in 6-OHDA-lesioned rats are dependent upon the degree of DA denervation, the site and route of 6-OHDA administration, and, most importantly of all, the age at which the lesioning is carried out. Furthermore, behavioral and receptor changes and the activation of associated signaling pathways are found not only in the central DA system as expected, but also in other transmitter systems, as demonstrated by some of the modifications in 5-HT and GABA and their receptors as reviewed above. Nonetheless, studies in both the adult and neonatal 6-OHDA-lesioned rat models still remain of interest, as they enable the examination of such alterations within a reasonable time frame, inevitably leading to the development and a better understanding of innovative therapeutic strategies to treat PD and other DA deficiency states like LNS, ADHD, and schizophrenia.

References

Abercrombie, E. D., Bonatz, A. E., & Zigmond, M. J. (1990). Effects of L-dopa on extracellular dopamine in striatum of normal and 6-hydroxydopamine-treated rats. *Brain Research, 525*(1), 36–44.

Abrous, D. N., Choulli, K., Rouge-Pont, F., Simon, H., Le Moal, M., & Herman, J. P. (1993a). Effects of intracerebral dopaminergic grafts on behavioural deficits induced by neonatal 6-hydroxydopamine lesions of the mesotelencephalic dopaminergic pathway. *Neuroscience, 54*(2), 499–511.

Abrous, D. N., Manier, M., Mennicken, F., Feuerstein, C., Le Moal, M., & Herman, J. P. (1993b). Intrastriatal transplants of embryonic dopaminergic neurons counteract the increase of striatal enkephalin immunostaining but not serotoninergic sprouting elicited by a neonatal lesion of the nigrostriatal dopaminergic pathway. *The European Journal of Neuroscience, 5*(2), 128–136.

Agid, Y., Javoy, F., & Glowinski, J. (1973). Hyperactivity of remaining dopaminergic neurons after partial destruction of the nigro-striatal dopaminergic system in the rat. *Nature: New Biology, 245*(144), 150–151.

Aizman, O., Brismar, H., Uhlen, P., Zettergren, E., Levey, A. I., Forssberg, H., Greengard, P., & Aperia, A. (2000). Anatomical and physiological evidence for D_1 and D_2 dopamine receptor colocalization in neostriatal neurons. *Nature Neuroscience, 3*(3), 226–230.

Albin, R. L., Young, A. B., & Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences, 12*(10), 366–375.

Altar, C. A., & Marien, M. R. (1987). Picomolar affinity of 125I-SCH 23982 for D_1 receptors in brain demonstrated with digital subtraction autoradiography. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 7*(1), 213–222.

Altar, C. A., Marien, M. R., & Marshall, J. F. (1987). Time course of adaptations in dopamine biosynthesis, metabolism, and release following nigrostriatal lesions: Implications for behavioral recovery from brain injury. *Journal of Neurochemistry, 48*(2), 390–399.

Andersson, M., Hilbertson, A., & Cenci, M. A. (1999). Striatal fosB expression is causally linked with l-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. *Neurobiology of Disease, 6*(6), 461–474.

Angulo, J. A., Coirini, H., Ledoux, M., & Schumacher, M. (1991). Regulation by dopaminergic neurotransmission of dopamine D_2 mRNA and receptor levels in the striatum and nucleus accumbens of the rat. *Brain Research. Molecular Brain Research, 11*(2), 161–166.

Archer, T., Danysz, W., Fredriksson, A., Jonsson, G., Luthman, J., Sundstrom, E., & Teiling, A. (1988). Neonatal 6-hydroxydopamine-induced dopamine depletions: Motor activity and performance in maze learning. *Pharmacology, Biochemistry, and Behavior, 31*(2), 357–364.

Baez, L. A., Ahlskog, J. E., & Randall, P. K. (1977). Body weight and regulatory deficits following unilateral nigrostriatal lesions. *Brain Research, 132*(3), 467–476.

Bal, A., Savasta, M., Chritin, M., Mennicken, F., Abrous, D. N., Le Moal, M., Feuerstein, C., & Herman, J. P. (1993). Transplantation of fetal nigral cells reverses the increase of preproenkephalin mRNA levels in the rat striatum caused by 6-OHDA lesion of the dopaminergic nigrostriatal pathway: A quantitative in situ hybridization study. *Brain Research. Molecular Brain Research, 18*(3), 221–227.

Bannon, M. J., Lee, J. M., Giraud, P., Young, A., Affolter, H. U., & Bonner, T. I. (1986). Dopamine antagonist haloperidol decreases substance P, substance K, and preprotachykinin mRNAs in rat striatonigral neurons. *The Journal of Biological Chemistry, 261*(15), 6640–6642.

Bannon, M. J., Elliott, P. J., & Bunney, E. B. (1987). Striatal tachykinin biosynthesis: Regulation of mRNA and peptide levels by dopamine agonists and antagonists. *Brain Research, 427*(1), 31–37.

Barone, P., Morelli, M., Popoli, M., Cicarelli, G., Campanella, G., & Di Chiara, G. (1994). Behavioural sensitization in 6-hydroxydopamine lesioned rats involves the dopamine signal transduction: Changes in DARPP-32 phosphorylation. *Neuroscience, 61*(4), 867–873.

Basura, G. J., & Walker, P. D. (1999). Serotonin 2A receptor mRNA levels in the neonatal dopamine-depleted rat striatum remain upregulated following suppression of serotonin hyperinnervation. *Brain Research. Developmental Brain Research, 116*(1), 111–117.

Basura, G. J., & Walker, P. D. (2000). Stimulated serotonin release from hyperinnervated terminals subsequent to neonatal dopamine depletion regulates striatal tachykinin, but not enkephalin gene expression. *Brain Research. Molecular Brain Research, 81*(1–2), 80–91.

Basura, G. J., & Walker, P. D. (2001). Serotonin 2A receptor regulation of striatal neuropeptide gene expression is selective for tachykinin, but not enkephalin neurons following dopamine depletion. *Brain Research. Molecular Brain Research, 92*(1–2), 66–77.

Batchelor, P. E., Liberatore, G. T., Wong, J. Y., Porritt, M. J., Frerichs, F., Donnan, G. A., & Howells, D. W. (1999). Activated macrophages and microglia induce dopaminergic sprouting in the injured striatum and express brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 19*(5), 1708–1716.

Baumeister, A. A., & Frye, G. D. (1985). The biochemical basis of the behavioral disorder in the Lesch-Nyhan syndrome. *Neuroscience and Biobehavioral Reviews, 9*(2), 169–178.

Baumeister, A. A., & Frye, G. D. (1986). Involvement of the midbrain reticular formation in self-injurious behavior, stereotyped behavior, and analgesia induced by intranigral microinjection of muscimol. *Brain Research, 369*(1–2), 231–242.

Beckstead, R. M., & Cruz, C. J. (1986). Striatal axons to the globus pallidus, entopeduncular nucleus and substantia nigra come mainly from separate cell populations in cat. *Neuroscience, 19*(1), 147–158.

Berger, T. W., Kaul, S., Stricker, E. M., & Zigmond, M. J. (1985). Hyperinnervation of the striatum by dorsal raphe afferents after dopamine-depleting brain lesions in neonatal rats. *Brain Research, 336*(2), 354–358.

Berger, K., Przedborski, S., & Cadet, J. L. (1991). Retrograde degeneration of nigrostriatal neurons induced by intrastriatal 6-hydroxydopamine injection in rats. *Brain Research Bulletin, 26*(2), 301–307.

Berl, S. (1973). Metabolic compartimentation in developing brain. In W. Himwich (Ed.), *Biochemistry of the developing brain* (Vol. 1, pp. 219–252). New York: Marcel Dekker.

Bernardi, M. M., Scavone, C., & Frussa-Filho, R. (1986). Differential effects of single and long-term amphetamine and apomorphine administrations on locomotor activity of rats. *General Pharmacology, 17*(4), 465–468.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *Journal of the Neurological Sciences, 20*(4), 415–455.

Bezard, E., Dovero, S., Prunier, C., Ravenscroft, P., Chalon, S., Guilloteau, D., Crossman, A. R., Bioulac, B., Brotchie, J. M., & Gross, C. E. (2001). Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 21*(17), 6853–6861.

Billard, W., Ruperto, V., Crosby, G., Iorio, L. C., & Barnett, A. (1984). Characterization of the binding of ^{3}H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. *Life Sciences, 35*(18), 1885–1893.

Bischoff, S., Heinrich, M., Krauss, J., Sills, M. A., Williams, M., & Vassout, A. (1988). Interaction of the D_1 receptor antagonist SCH 23390 with the central 5-HT system: Radioligand binding studies, measurements of biochemical parameters and effects on L-5-HTP syndrome. *Journal of Receptor Research, 8*(1–4), 107–120.

Bishop, C., & Walker, P. D. (2003). Combined intrastriatal dopamine D_1 and serotonin 5-HT_2 receptor stimulation reveals a mechanism for hyperlocomotion in 6-hydroxydopamine-lesioned rats. *Neuroscience, 121*(3), 649–657.

Bishop, C., Kamdar, D. P., & Walker, P. D. (2003). Intrastriatal serotonin 5-HT_2 receptors mediate dopamine D_1-induced hyperlocomotion in 6-hydroxydopamine-lesioned rats. *Synapse, 50*(2), 164–170.

Bishop, C., Tessmer, J. L., Ullrich, T., Rice, K. C., & Walker, P. D. (2004). Serotonin 5-HT_{2A} receptors underlie increased motor behaviors induced in dopamine-depleted rats by intrastriatal 5-$HT_{2A/2C}$ agonism. *The Journal of Pharmacology and Experimental Therapeutics, 310*(2), 687–694

Bishop, C., Daut, G. S., & Walker, P. D. (2005). Serotonin 5-HT_{2A} but not 5-HT_{2C} receptor antagonism reduces hyperlocomotor activity induced in dopamine-depleted rats by striatal administration of the D_1 agonist SKF 82958. *Neuropharmacology, 49*(3), 350–358.

Blanchard, V., Anglade, P., Dziewczapolski, G., Savasta, M., Agid, Y., & Raisman-Vozari, R. (1996). Dopaminergic sprouting in the rat striatum after partial lesion of the substantia nigra. *Brain Research, 709*(2), 319–325.

Blandini, F., Levandis, G., Bazzini, E., Nappi, G., & Armentero, M. T. (2007). Time-course of nigrostriatal damage, basal ganglia metabolic changes and behavioural alterations following intrastriatal injection of 6-hydroxydopamine in the rat: New clues from an old model. *The European Journal of Neuroscience, 25*(2), 397–405.

Blandini, F., Armentero, M. T., & Martignoni, E. (2008). The 6-hydroxydopamine model: News from the past. *Parkinsonism & Related Disorders, 14*(Suppl 2), S124–S129.

Bloom, F. E., Algeri, S., Groppetti, A., Revuelta, A., & Costa, E. (1969). Lesions of central norepinephrine terminals with 6-OH-dopamine: Biochemistry and fine structure. *Science, 166*(3910), 1284–1286.

Bohn, M. C., Cupit, L., Marciano, F., & Gash, D. M. (1987). Adrenal medulla grafts enhance recovery of striatal dopaminergic fibers. *Science, 237*(4817), 913–916.

Bolam, J. P., & Smith, Y. (1990). The GABA and substance P input to dopaminergic neurons in the substantia nigra of the rat. *Brain Research, 529*(1–2), 57–78.

Bove, J., Prou, D., Perier, C., & Przedborski, S. (2005). Toxin-induced models of Parkinson's disease. *NeuroRx : The Journal of the American Society for Experimental NeuroTherapeutics, 2*(3), 484–494.

Breese, G. R. (1975). Chemical and immunochemical lesions by specific neurotoxic substances and antisera. In L. L. Iversen & S. H. Snyder (Eds.), *Handbook of psychopharmacology* (Vol. 1, pp. 137–189). New York: Plenum.

Breese, G. R., & Cooper, B. R. (1975). Behavioral and biochemical interactions of 5,7-dihydroxytryptamine with various drugs when administered intracisternally to adult and developing rats. *Brain Research, 98*(3), 517–527.

Breese, G. R., & Mueller, R. A. (1985). SCH-23390 antagonism of a D-2 dopamine agonist depends upon catecholaminergic neurons. *European Journal of Pharmacology, 113*(1), 109–114.

Breese, G. R., & Traylor, T. D. (1970). Effect of 6-hydroxydopamine on brain norepinephrine and dopamine evidence for selective degeneration of catecholamine neurons. *The Journal of Pharmacology and Experimental Therapeutics, 174*(3), 413–420.

Breese, G. R., & Traylor, T. D. (1971). Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. *British Journal of Pharmacology, 42*(1), 88–99.

Breese, G. R., & Traylor, T. D. (1972). Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: Effects of 6-hydroxydopamine. *British Journal of Pharmacology, 44*(2), 210–222.

Breese, G. R., Smith, R. D., Cooper, B. R., & Grant, L. D. (1973). Alterations in consummatory behavior following intracisternal injection of 6-hydroxydopamine. *Pharmacology, Biochemistry, and Behavior, 1*(3), 319–328.

Breese, G. R., Cooper, B. R., & Smith, R. D. (1974). Biochemical and behavioural alterations following 6-hydroxydopamine administration into brain. *Biochemical Pharmacology, 23* (Suppl 1), 574–579.

Breese, G. R., Vogel, R. A., & Mueller, R. A. (1978). Biochemical and behavioral alterations in developing rats treated with 5,7-dihydroxytryptamine. *The Journal of Pharmacology and Experimental Therapeutics, 205*(3), 587–595.

Breese, G. R., Baumeister, A. A., McCown, T. J., Emerick, S. G., Frye, G. D., Crotty, K., & Mueller, R. A. (1984a). Behavioral differences between neonatal and adult 6-hydroxydopamine-treated rats to dopamine agonists: Relevance to neurological symptoms in clinical syndromes with reduced brain dopamine. *The Journal of Pharmacology and Experimental Therapeutics, 231*(2), 343–354.

Breese, G. R., Baumeister, A. A., McCown, T. J., Emerick, S. G., Frye, G. D., & Mueller, R. A. (1984b). Neonatal-6-hydroxydopamine treatment: Model of susceptibility for self-mutilation in the Lesch-Nyhan syndrome. *Pharmacology, Biochemistry, and Behavior, 21*(3), 459–461.

Breese, G. R., Baumeister, A., Napier, T. C., Frye, G. D., & Mueller, R. A. (1985a). Evidence that D-1 dopamine receptors contribute to the supersensitive behavioral responses induced by L-dihydroxyphenylalanine in rats treated neonatally with 6-hydroxydopamine. *The Journal of Pharmacology and Experimental Therapeutics, 235*(2), 287–295.

Breese, G. R., Napier, T. C., & Mueller, R. A. (1985b). Dopamine agonist-induced locomotor activity in rats treated with 6-hydroxydopamine at differing ages: Functional supersensitivity of D-1 dopamine receptors in neonatally lesioned rats. *The Journal of Pharmacology and Experimental Therapeutics, 234*(2), 447–455.

Breese, G. R., Mueller, R. A., Napier, T. C., & Duncan, G. E. (1986). Neurobiology of D_1 dopamine receptors after neonatal-6-OHDA treatment: Relevance to Lesch-Nyhan disease. *Advances in Experimental Medicine and Biology, 204*, 197–215.

Breese, G. R., Duncan, G. E., Napier, T. C., Bondy, S. C., Iorio, L. C., & Mueller, R. A. (1987a). 6-hydroxydopamine treatments enhance behavioral responses to intracerebral microinjection of D_1- and D_2-dopamine agonists into nucleus accumbens and striatum without changing

dopamine antagonist binding. *The Journal of Pharmacology and Experimental Therapeutics, 240*(1), 167–176.

Breese, G. R., Hulebak, K. L., Napier, T. C., Baumeister, A., Frye, G., & Mueller, R. A. (1987b). Enhanced muscimol-induced behavioral responses after 6-OHDA lesions: Relevance to susceptibility for self-mutilation behavior in neonatally lesioned rats. *Psychopharmacology, 91*(3), 356–362.

Breese, G. R., Criswell, H. E., Duncan, G. E., & Mueller, R. A. (1989). Dopamine deficiency in self-injurious behavior. *Psychopharmacology Bulletin, 25*(3), 353–357.

Breese, G. R., Criswell, H. E., Johnson, K. B., O'Callaghan, J. P., Duncan, G. E., Jensen, K. F., Simson, P. E., & Mueller, R. A. (1994). Neonatal destruction of dopaminergic neurons. *Neurotoxicology, 15*(1), 149–159.

Breese, G. R., Knapp, D. J., Criswell, H. E., Moy, S. S., Papadeas, S. T., & Blake, B. L. (2005). The neonate-6-hydroxydopamine-lesioned rat: A model for clinical neuroscience and neurobiological principles. *Brain Research. Brain Research Reviews, 48*(1), 57–73.

Brene, S., Lindefors, N., Herrera-Marschitz, M., & Persson, H. (1990). Expression of dopamine D_2 receptor and choline acetyltransferase mRNA in the dopamine deafferented rat caudate-putamen. *Experimental Brain Research Experimentelle Hirnforschung Experimentation Cerebrale, 83*(1), 96–104.

Broaddus, W. C., & Bennett, J. P., Jr. (1990). Postnatal development of striatal dopamine function. II. Effects of neonatal 6-hydroxydopamine treatments on D_1 and D_2 receptors, adenylate cyclase activity and presynaptic dopamine function. *Brain Research. Developmental Brain Research, 52*(1–2), 273–277.

Bruinvels, A. T., Landwehrmeyer, B., Gustafson, E. L., Durkin, M. M., Mengod, G., Branchek, T. A., Hoyer, D., & Palacios, J. M. (1994). Localization of 5-HT_{1B}, 5-$HT_{1D\ alpha}$, 5-HT_{1E} and 5-HT_{1F} receptor messenger RNA in rodent and primate brain. *Neuropharmacology, 33*(3–4), 367–386.

Bruno, J. P., Snyder, A. M., & Stricker, E. M. (1984). Effect of dopamine-depleting brain lesions on suckling and weaning in rats. *Behavioral Neuroscience, 98*(1), 156–161.

Bruno, J. P., Zigmond, M. J., & Stricker, E. M. (1986). Rats given dopamine-depleting brain lesions as neonates do not respond to acute homeostatic imbalances as adults. *Behavioral Neuroscience, 100*(1), 125–128.

Bruno, J. P., Jackson, D., Zigmond, M. J., & Stricker, E. M. (1987). Effect of dopamine-depleting brain lesions in rat pups: Role of striatal serotonergic neurons in behavior. *Behavioral Neuroscience, 101*(6), 806–811.

Brus, R., Nowak, P., Szkilnik, R., Mikolajun, U., & Kostrzewa, R. M. (2004). Serotoninergics attenuate hyperlocomotor activity in rats. Potential new therapeutic strategy for hyperactivity. *Neurotoxicity Research, 6*(4), 317–325.

Buonamici, M., Caccia, C., Carpentieri, M., Pegrassi, L., Rossi, A. C., & Di Chiara, G. (1986). D-1 receptor supersensitivity in the rat striatum after unilateral 6-hydroxydopamine lesions. *European Journal of Pharmacology, 126*(3), 347–348.

Burgunder, J. M. (1991). Prenatal ontogeny of growth hormone releasing hormone expression in rat hypothalamus. *Developmental Neuroscience, 13*(6), 397–402.

Bymaster, F. P., Hemrick-Luecke, S. K., Perry, K. W., & Fuller, R. W. (1996). Neurochemical evidence for antagonism by olanzapine of dopamine, serotonin, alpha 1-adrenergic and muscarinic receptors in vivo in rats. *Psychopharmacology, 124*(1–2), 87–94.

Cadet, J. L., Zhu, S. M., & Angulo, J. A. (1992). Quantitative in situ hybridization evidence for differential regulation of proenkephalin and dopamine D_2 receptor mRNA levels in the rat striatum: Effects of unilateral intrastriatal injections of 6-hydroxydopamine. *Brain Research. Molecular Brain Research, 12*(1–3), 59–67.

Campbell, K., & Bjorklund, A. (1994). Prefrontal corticostriatal afferents maintain increased enkephalin gene expression in the dopamine-denervated rat striatum. *The European Journal of Neuroscience, 6*(8), 1371–1383.

Campbell, K., Wictorin, K., & Bjorklund, A. (1992). Differential regulation of neuropeptide mRNA expression in intrastriatal striatal transplants by host dopaminergic afferents. *Proceedings of the National Academy of Sciences of the United States of America, 89*(21), 10489–10493.

Campbell, B. M., Gresch, P. J., & Walker, P. D. (2001). Neonatal dopamine depletion reveals a synergistic mechanism of mRNA regulation that is mediated by dopamine (D_1) and serotonin (2) receptors and is targeted to tachykinin neurons of the dorsomedial striatum. *Neuroscience, 105*(3), 671–680.

Carder, R. K., Snyder-Keller, A. M., & Lund, R. D. (1987). Amphetamine- and stress-induced turning after nigral transplants in neonatally dopamine-depleted rats. *Brain Research, 430*(2), 315–318.

Carder, R. K., Jackson, D., Morris, H. J., Lund, R. D., & Zigmond, M. J. (1989). Dopamine released from mesencephalic transplants restores modulation of striatal acetylcholine release after neonatal 6-hydroxydopamine: An in vitro analysis. *Experimental Neurology, 105*(3), 251–259.

Carlsson, M., & Carlsson, A. (1989). The NMDA antagonist MK-801 causes marked locomotor stimulation in monoamine-depleted mice. *Journal of Neural Transmission, 75*(3), 221–226.

Carman, L. S., Gage, F. H., & Shults, C. W. (1991). Partial lesion of the substantia nigra: Relation between extent of lesion and rotational behavior. *Brain Research, 553*(2), 275–283.

Carta, M., Carlsson, T., Kirik, D., & Bjorklund, A. (2007). Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain: A Journal of Neurology, 130*(Pt 7), 1819–1833.

Castaneda, E., Whishaw, I. Q., Lermer, L., & Robinson, T. E. (1990a). Dopamine depletion in neonatal rats: Effects on behavior and striatal dopamine release assessed by intracerebral microdialysis during adulthood. *Brain Research, 508*(1), 30–39.

Castaneda, E., Whishaw, I. Q., & Robinson, T. E. (1990b). Changes in striatal dopamine neurotransmission assessed with microdialysis following recovery from a bilateral 6-OHDA lesion: Variation as a function of lesion size. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 10*(6), 1847–1854.

Cenci, M. A., & Bjorklund, A. (1993). Transection of corticostriatal afferents reduces amphetamine- and apomorphine-induced striatal Fos expression and turning behaviour in unilaterally 6-hydroxydopamine-lesioned rats. *The European Journal of Neuroscience, 5*(8), 1062–1070.

Cenci, M. A., & Konradi, C. (2010). Maladaptive striatal plasticity in L-DOPA-induced dyskinesia. *Progress in Brain Research, 183*, 209–233.

Cenci, M. A., Campbell, K., & Bjorklund, A. (1993). Neuropeptide messenger RNA expression in the 6-hydroxydopamine-lesioned rat striatum reinnervated by fetal dopaminergic transplants: Differential effects of the grafts on preproenkephalin, preprotachykinin and prodynorphin messenger RNA levels. *Neuroscience, 57*(2), 275–296.

Cenci, M. A., Lee, C. S., & Bjorklund, A. (1998). L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *The European Journal of Neuroscience, 10*(8), 2694–2706.

Chao, J., & Nestler, E. J. (2004). Molecular neurobiology of drug addiction. *Annual Review of Medicine, 55*, 113–132.

Chen, J. F., & Weiss, B. (1991). Ontogenetic expression of D_2 dopamine receptor mRNA in rat corpus striatum. *Brain Research. Developmental Brain Research, 63*(1–2), 95–104.

Chinen, C. C., Faria, R. R., & Frussa-Filho, R. (2006). Characterization of the rapid-onset type of behavioral sensitization to amphetamine in mice: Role of drug-environment conditioning. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 31*(1), 151–159.

Christie, R., Bay, C., Kaufman, I. A., Bakay, B., Borden, M., & Nyhan, W. L. (1982). Lesch-Nyhan disease: Clinical experience with nineteen patients. *Developmental Medicine and Child Neurology, 24*(3), 293–306.

Chritin, M., Savasta, M., Mennicken, F., Bal, A., Abrous, D. N., Le Moal, M., Feuerstein, C., & Herman, J. P. (1992). Intrastriatal dopamine-rich implants reverse the increase of dopamine D_2

receptor mRNA Levels caused by lesion of the nigrostriatal pathway: A quantitative in situ hybridization study. *The European Journal of Neuroscience, 4*(7), 663–672.

Chritin, M., Feuerstein, C., & Savasta, M. (1993). Time-course of changes in striatal levels of DA uptake sites, DA D_2 receptor and preproenkephalin mRNAs after nigrostriatal dopaminergic denervation in the rat. *Brain Research. Molecular Brain Research, 19*(4), 318–322.

Cimino, M., Zoli, M., & Weiss, B. (1991). Differential ontogenetic expression and regulation of proenkephalin and preprosomatostatin mRNAs in rat caudate-putamen as studied by in situ hybridization histochemistry. *Brain Research. Developmental Brain Research, 60*(2), 115–122.

Cole, D. G., Growdon, J. H., & DiFiglia, M. (1993). Levodopa induction of Fos immunoreactivity in rat brain following partial and complete lesions of the substantia nigra. *Experimental Neurology, 120*(2), 223–232.

Compan, V., Daszuta, A., Salin, P., Sebben, M., Bockaert, J., & Dumuis, A. (1996). Lesion study of the distribution of serotonin 5-HT_4 receptors in rat basal ganglia and hippocampus. *The European Journal of Neuroscience, 8*(12), 2591–2598.

Cooper, B. R., & Breese, G. R. (1975). A role for dopamine in the psychopharmacology of electrical self-stimulation of the lateral hypothalamus, substantia nigra, and locus coeruleus. *Psychopharmacology Bulletin, 11*(3), 30–31.

Cooper, B. R., Breese, G. R., Howard, J. L., & Grant, L. D. (1972). Enhanced behavioral depressant effects of reserpine and -methyltyrosine after 6-hydroxydopamine treatment. *Psychopharmacologia, 27*(2), 99–110.

Cooper, B. R., Breese, G. R., Grant, L. D., & Howard, J. L. (1973a). Effects of 6-hydroxydopamine treatments on active avoidance responding: Evidence for involvement of brain dopamine. *The Journal of Pharmacology and Experimental Therapeutics, 185*(2), 358–370.

Cooper, B. R., Grant, L. D., & Breese, G. R. (1973b). Comparison of the behavioral depressant effects of biogenic amine depleting and neuroleptic agents following various 6-hydroxydopamine treatments. *Psychopharmacologia, 31*(2), 95–109.

Costall, B., & Naylor, R. J. (1974). Extrapyramidal and mesolimbic involvement with the stereotypic activity of D- and L-amphetamine. *European Journal of Pharmacology, 25*(2), 121–129.

Costall, B., & Naylor, R. J. (1981). The hypotheses of different dopamine receptor mechanisms. *Life Sciences, 28*(3), 215–229.

Costall, B., Naylor, R. J., & Neumeyer, J. L. (1975). Differences in the nature of the stereotyped behaviour induced by aporphine derivatives in the rat and in their actions in extrapyramidal and mesolimbic brain areas. *European Journal of Pharmacology, 31*(1), 1–16.

Costall, B., Naylor, R. J., Cannon, J. G., & Lee, T. (1977). Differentiation of the dopamine mechanisms mediating stereotyped behaviour and hyperactivity in the nucleus accumbens and caudate-putamen. *The Journal of Pharmacy and Pharmacology, 29*(6), 337–342.

Creese, I., & Iversen, S. D. (1974). The role of forebrain dopamine systems in amphetamine induced stereotyped behavior in the rat. *Psychopharmacologia, 39*(4), 345–357.

Creese, I., Burt, D. R., & Snyder, S. H. (1977). Dopamine receptor binding enhancement accompanies lesion-induced behavioral supersensitivity. *Science, 197*(4303), 596–598.

Criswell, H., Mueller, R. A., & Breese, G. R. (1989). Priming of D_1-dopamine receptor responses: Long-lasting behavioral supersensitivity to a D_1-dopamine agonist following repeated administration to neonatal 6-OHDA-lesioned rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 9*(1), 125–133.

Criswell, H. E., Mueller, R. A., & Breese, G. R. (1990). Long-term D_1-dopamine receptor sensitization in neonatal 6-OHDA-lesioned rats is blocked by an NMDA antagonist. *Brain Research, 512*(2), 284–290.

Criswell, H. E., Johnson, K. B., Mueller, R. A., & Breese, G. R. (1993). Evidence for involvement of brain dopamine and other mechanisms in the behavioral action of the N-methyl-D-aspartic acid antagonist MK-801 in control and 6-hydroxydopamine-lesioned rats. *The Journal of Pharmacology and Experimental Therapeutics, 265*(2), 1001–1010.

Damsma, G., de Boer, P., Westerink, B. H., & Fibiger, H. C. (1990). Dopaminergic regulation of striatal cholinergic interneurons: An in vivo microdialysis study. *Naunyn-Schmiedeberg's Archives of Pharmacology, 342*(5), 523–527.

Damsma, G., Robertson, G. S., Tham, C. S., & Fibiger, H. C. (1991). Dopaminergic regulation of striatal acetylcholine release: Importance of D_1 and N-methyl-D-aspartate receptors. *The Journal of Pharmacology and Experimental Therapeutics, 259*(3), 1064–1072.

Davids, E., Zhang, K., Tarazi, F. I., & Baldessarini, R. J. (2002). Stereoselective effects of methylphenidate on motor hyperactivity in juvenile rats induced by neonatal 6-hydroxydopamine lesioning. *Psychopharmacology, 160*(1), 92–98.

Davids, E., Zhang, K., Tarazi, F. I., & Baldessarini, R. J. (2003). Animal models of attention-deficit hyperactivity disorder. *Brain Research. Brain Research Reviews, 42*(1), 1–21.

Davis, R. J. (2000). Signal transduction by the JNK group of MAP kinases. *Cell, 103*(2), 239–252.

de Boer, P., Damsma, G., Fibiger, H. C., Timmerman, W., de Vries, J. B., & Westerink, B. H. (1990). Dopaminergic-cholinergic interactions in the striatum: The critical significance of calcium concentrations in brain microdialysis. *Naunyn-Schmiedeberg's Archives of Pharmacology, 342*(5), 528–534.

De Boer, P., Damsma, G., Schram, Q., Stoof, J. C., Zaagsma, J., & Westerink, B. H. (1992). The effect of intrastriatal application of directly and indirectly acting dopamine agonists and antagonists on the in vivo release of acetylcholine measured by brain microdialysis. The importance of the post-surgery interval. *Naunyn-Schmiedeberg's Archives of Pharmacology, 345*(2), 144–152.

Delfs, J. M., Ciaramitaro, V. M., Parry, T. J., & Chesselet, M. F. (1995). Subthalamic nucleus lesions: Widespread effects on changes in gene expression induced by nigrostriatal dopamine depletion in rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 15*(10), 6562–6575.

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends in Neurosciences, 13*(7), 281–285.

Deng, Y. P., Lei, W. L., & Reiner, A. (2006). Differential perikaryal localization in rats of D_1 and D_2 dopamine receptors on striatal projection neuron types identified by retrograde labeling. *Journal of Chemical Neuroanatomy, 32*(2–4), 101–116.

Descarries, L., & Mechawar, N. (2000). Ultrastructural evidence for diffuse transmission by monoamine and acetylcholine neurons of the central nervous system. *Progress in Brain Research, 125*, 27–47.

Descarries, L., Soghomonian, J. J., Garcia, S., Doucet, G., & Bruno, J. P. (1992). Ultrastructural analysis of the serotonin hyperinnervation in adult rat neostriatum following neonatal dopamine denervation with 6-hydroxydopamine. *Brain Research, 569*(1), 1–13.

Deumens, R., Blokland, A., & Prickaerts, J. (2002). Modeling Parkinson's disease in rats: An evaluation of 6-OHDA lesions of the nigrostriatal pathway. *Experimental Neurology, 175*(2), 303–317.

Dewar, K. M., Soghomonian, J. J., Bruno, J. P., Descarries, L., & Reader, T. A. (1990). Elevation of dopamine D_2 but not D_1 receptors in adult rat neostriatum after neonatal 6-hydroxydopamine denervation. *Brain Research, 536*(1–2), 287–296.

Dewar, K. M., Paquet, M., & Reader, T. A. (1997). Alterations in the turnover rate of dopamine D_1 but not D_2 receptors in the adult rat neostriatum after a neonatal dopamine denervation. *Neurochemistry International, 30*(6), 613–621.

Di Chiara, G., Morelli, M., & Consolo, S. (1994). Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/NMDA interactions. *Trends in Neurosciences, 17*(6), 228–233.

Dragunow, M., Leah, J. D., & Faull, R. L. (1991). Prolonged and selective induction of Fos-related antigen(s) in striatal neurons after 6-hydroxydopamine lesions of the rat substantia nigra pars compacta. *Brain Research. Molecular Brain Research, 10*(4), 355–358.

Dravid, A., Jaton, A. L., Enz, A., & Frei, P. (1984). Spontaneous recovery from motor asymmetry in adult rats with 6-hydroxydopamine-induced partial lesions of the substantia nigra. *Brain Research, 311*(2), 361–365.

Duncan, G. E., Criswell, H. E., McCown, T. J., Paul, I. A., Mueller, R. A., & Breese, G. R. (1987). Behavioral and neurochemical responses to haloperidol and SCH-23390 in rats treated neonatally or as adults with 6-hydroxydopamine. *The Journal of Pharmacology and Experimental Therapeutics, 243*(3), 1027–1034.

Duncan, G. E., Breese, G. R., Criswell, H. E., Johnson, K. B., Schambra, U. B., Mueller, R. A., Caron, M. G., & Fremeau, R. T., Jr. (1993). D_1 dopamine receptor binding and mRNA levels are not altered after neonatal 6-hydroxydopamine treatment: Evidence against dopamine-mediated induction of D_1 dopamine receptors during postnatal development. *Journal of Neurochemistry, 61*(4), 1255–1262.

Dupre, K. B., Eskow, K. L., Steiniger, A., Klioueva, A., Negron, G. E., Lormand, L., Park, J. Y., & Bishop, C. (2008). Effects of coincident 5-HT_{1A} receptor stimulation and NMDA receptor antagonism on L-DOPA-induced dyskinesia and rotational behaviors in the hemi-parkinsonian rat. *Psychopharmacology, 199*(1), 99–108.

Duty, S., & Jenner, P. (2011). Animal models of Parkinson's disease: A source of novel treatments and clues to the cause of the disease. *British Journal of Pharmacology, 164*(4), 1357–1391.

Eidelberg, D. (1992). Positron emission tomography studies in parkinsonism. *Neurologic Clinics, 10*(2), 421–433.

el Mansari, M., Radja, F., Ferron, A., Reader, T. A., Molina-Holgado, E., & Descarries, L. (1994). Hypersensitivity to serotonin and its agonists in serotonin-hyperinnervated neostriatum after neonatal dopamine denervation. *European Journal of Pharmacology, 261*(1–2), 171–178.

Engber, T. M., Susel, Z., Kuo, S., Gerfen, C. R., & Chase, T. N. (1991). Levodopa replacement therapy alters enzyme activities in striatum and neuropeptide content in striatal output regions of 6-hydroxydopamine lesioned rats. *Brain Research, 552*(1), 113–118.

Engber, T. M., Boldry, R. C., Kuo, S., & Chase, T. N. (1992). Dopaminergic modulation of striatal neuropeptides: Differential effects of D_1 and D_2 receptor stimulation on somatostatin, neuropeptide Y, neurotensin, dynorphin and enkephalin. *Brain Research, 581*(2), 261–268.

Epstein, P. N., & Altshuler, H. L. (1978). Changes in the effects of cocaine during chronic treatment. *Research Communications in Chemical Pathology and Pharmacology, 22*(1), 93–105.

Erinoff, L., MacPhail, R. C., Heller, A., & Seiden, L. S. (1979). Age-dependent effects of 6-hydroxydopamine on locomotor activity in the rat. *Brain Research, 164*, 195–205.

Ernst, M., Zametkin, A. J., Matochik, J. A., Pascualvaca, D., Jons, P. H., Hardy, K., Hankerson, J. G., Doudet, D. J., & Cohen, R. M. (1996). Presynaptic dopaminergic deficits in Lesch-Nyhan disease. *The New England Journal of Medicine, 334*(24), 1568–1572.

Evetts, K. D., & Iversen, L. L. (1970). Effects of protriptyline on the depletion of catecholamines induced by 6-hydroxydopamine in the brain of the rat. *The Journal of Pharmacy and Pharmacology, 22*(7), 540–543.

Fallon, J. H., & Loughlin, S. E. (1982). Monoamine innervation of the forebrain: Collateralization. *Brain Research Bulletin, 9*(1–6), 295–307.

Fearnley, J. M., & Lees, A. J. (1991). Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain: A Journal of Neurology, 114*(Pt 5), 2283–2301.

Fernandes Xavier, F. G., Doucet, G., Geffard, M., & Descarries, L. (1994). Dopamine neoinnervation in the substantia nigra and hyperinnervation in the interpeduncular nucleus of adult rat following neonatal cerebroventricular administration of 6-hydroxydopamine. *Neuroscience, 59*(1), 77–87.

Fibiger, H. C., Zis, A. P., & McGeer, E. G. (1973). Feeding and drinking deficits after 6-hydroxydopamine administration in the rat: Similarities to the lateral hypothalamic syndrome. *Brain Research, 55*(1), 135–148.

Filip, M., & Cunningham, K. A. (2002). Serotonin 5-HT(2C) receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine. *Pharmacology, Biochemistry, and Behavior, 71*(4), 745–756.

Filip, M., Bubar, M. J., & Cunningham, K. A. (2004). Contribution of serotonin (5-hydroxytryptamine; 5-HT) 5-HT_2 receptor subtypes to the hyperlocomotor effects of cocaine: Acute and

chronic pharmacological analyses. *The Journal of Pharmacology and Experimental Therapeutics, 310*(3), 1246–1254.

Finkelstein, D. I., Stanic, D., Parish, C. L., Tomas, D., Dickson, K., & Horne, M. K. (2000). Axonal sprouting following lesions of the rat substantia nigra. *Neuroscience, 97*(1), 99–112.

Fletcher, P. J., Grottick, A. J., & Higgins, G. A. (2002). Differential effects of the 5-HT(2A) receptor antagonist M100907 and the 5-HT(2C) receptor antagonist SB242084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 27*(4), 576–586.

Fornai, F., Biagioni, F., Fulceri, F., Murri, L., Ruggieri, S., & Paparelli, A. (2009). Intermittent Dopaminergic stimulation causes behavioral sensitization in the addicted brain and parkinsonism. *International Review of Neurobiology, 88*, 371–398.

Fornaretto, M. G., Caccia, C., Caron, M. G., & Fariello, R. G. (1993). Dopamine receptors status after unilateral nigral 6-OHDA lesion. Autoradiographic and in situ hybridization study in the rat brain. *Molecular and Chemical Neuropathology/SPonsored by the International Society for Neurochemistry and the World Federation of Neurology and Research Groups on Neurochemistry and Cerebrospinal Fluid, 19*(1–2), 147–162.

Fox, S. H., & Brotchie, J. M. (2000). 5-HT_{2C} receptor binding is increased in the substantia nigra pars reticulata in Parkinson's disease. *Movement Disorders: Official Journal of the Movement Disorder Society, 15*(6), 1064–1069.

Fuxe, K., Marcellino, D., Rivera, A., Diaz-Cabiale, Z., Filip, M., Gago, B., Roberts, D. C., Langel, U., Genedani, S., Ferraro, L., de la Calle, A., Narvaez, J., Tanganelli, S., Woods, A., & Agnati, L. F. (2008). Receptor-receptor interactions within receptor mosaics. Impact on neuropsychopharmacology. *Brain Research Reviews, 58*(2), 415–452.

Gelbard, H. A., Teicher, M. H., Baldessarini, R. J., Gallitano, A., Marsh, E. R., Zorc, J., & Faedda, G. (1990). Dopamine D_1 receptor development depends on endogenous dopamine. *Brain Research. Developmental Brain Research, 56*(1), 137–140.

Geldwert, D., Norris, J. M., Feldman, I. G., Schulman, J. J., Joyce, M. P., & Rayport, S. (2006). Dopamine presynaptically and heterogeneously modulates nucleus accumbens medium-spiny neuron GABA synapses in vitro. *BMC Neuroscience, 7*, 53.

Gerfen, C. R. (1992). The neostriatal mosaic: Multiple levels of compartmental organization in the basal ganglia. *Annual Review of Neuroscience, 15*, 285–320.

Gerfen, C. R. (2000). Molecular effects of dopamine on striatal-projection pathways. *Trends in Neurosciences, 23*(10 Suppl), S64–S70.

Gerfen, C. R., & Young, W. S., 3rd. (1988). Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: An in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Research, 460*(1), 161–167.

Gerfen, C. R., Staines, W. A., Arbuthnott, G. W., & Fibiger, H. C. (1982). Crossed connections of the substantia nigra in the rat. *The Journal of Comparative Neurology, 207*(3), 283–303.

Gerfen, C. R., Herkenham, M., & Thibault, J. (1987). The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 7*(12), 3915–3934.

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., Jr., & Sibley, D. R. (1990). D_1 and D_2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science, 250*(4986), 1429–1432.

Gerfen, C. R., McGinty, J. F., & Young, W. S., 3rd. (1991). Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: In situ hybridization histochemical analysis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 11*(4), 1016–1031.

Gerfen, C. R., Keefe, K. A., & Gauda, E. B. (1995). D_1 and D_2 dopamine receptor function in the striatum: Coactivation of D_1- and D_2-dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D_1-containing neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 15*(12), 8167–8176.

Gerfen, C. R., Miyachi, S., Paletzki, R., & Brown, P. (2002). D_1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 22*(12), 5042–5054.

Girault, J. A., Valjent, E., Caboche, J., & Herve, D. (2007). ERK2: A logical AND gate critical for drug-induced plasticity? *Current Opinion in Pharmacology, 7*(1), 77–85.

Gong, L., & Kostrzewa, R. M. (1992). Supersensitized oral responses to a serotonin agonist in neonatal 6-OHDA-treated rats. *Pharmacology, Biochemistry, and Behavior, 41*(3), 621–623.

Gong, L., Kostrzewa, R. M., Brus, R., Fuller, R. W., & Perry, K. W. (1993a). Ontogenetic SKF 38393 treatments sensitize dopamine D_1 receptors in neonatal 6-OHDA-lesioned rats. *Brain Research. Developmental Brain Research, 76*(1), 59–65.

Gong, L., Kostrzewa, R. M., Perry, K. W., & Fuller, R. W. (1993b). Dose-related effects of a neonatal 6-OHDA lesion on SKF 38393- and m-chlorophenylpiperazine-induced oral activity responses of rats. *Brain Research. Developmental Brain Research, 76*(2), 233–238.

Gong, L., Kostrzewa, R. M., & Li, C. (1994). Neonatal 6-hydroxydopamine and adult SKF 38393 treatments alter dopamine D_1 receptor mRNA levels: Absence of other neurochemical associations with the enhanced behavioral responses of lesioned rats. *Journal of Neurochemistry, 63*(4), 1282–1290.

Graham, W. C., Crossman, A. R., & Woodruff, G. N. (1990). Autoradiographic studies in animal models of hemi-parkinsonism reveal dopamine D_2 but not D_1 receptor supersensitivity. I. 6-OHDA lesions of ascending mesencephalic dopaminergic pathways in the rat. *Brain Research, 514*(1), 93–102.

Graybiel, A. M. R., & Ragsdalev, C. W., Jr. (1983). Biochemical anatomy of the striatum. In P. C. Emson (Ed.), *Chemical neuroanatomy* (pp. 427–504). New York: Raven.

Gresch, P. J., & Walker, P. D. (1999). Serotonin-2 receptor stimulation normalizes striatal preprotachykinin messenger RNA in an animal model of Parkinson's disease. *Neuroscience, 93*(3), 831–841.

Guerra, M. J., Liste, I., & Labandeira-Garcia, J. L. (1997). Effects of lesions of the nigrostriatal pathway and of nigral grafts on striatal serotonergic innervation in adult rats. *Neuroreport, 8*(16), 3485–3488.

Guyenet, P. G., Javory, A. F., Beaujouan, J. C., Rossier, B. J., & Glowinski, J. (1975). Effects of dopaminergic receptor agonists and antagonists on the activity of the neo-striatal cholinergic system. *Brain Research, 84*(2), 227–244.

Hansen, J. T., Sakai, K., Greenamyre, J. T., & Moran, S. (1995). Sprouting of dopaminergic fibers from spared mesencephalic dopamine neurons in the unilateral partial lesioned rat. *Brain Research, 670*(2), 197–204.

Hanson, G. R., Alphs, L., Wolf, W., Levine, R., & Lovenberg, W. (1981). Haloperidol-induced reduction of nigral substance P-like immunoreactivity: A probe for the interactions between dopamine and substance P neuronal systems. *The Journal of Pharmacology and Experimental Therapeutics, 218*(2), 568–574.

Heffner, T. G., & Seiden, L. S. (1982). Possible involvement of serotonergic neurons in the reduction of locomotor hyperactivity caused by amphetamine in neonatal rats depleted of brain dopamine. *Brain Research, 244*(1), 81–90.

Heffner, T. G., & Seiden, L. S. (1983). Impaired acquisition of an operant response in young rats depleted of brain dopamine in neonatal life. *Psychopharmacology, 79*(2–3), 115–119.

Hefti, F., Melamed, E., & Wurtman, R. J. (1980). Partial lesions of the dopaminergic nigrostriatal system in rat brain: Biochemical characterization. *Brain Research, 195*(1), 123–137.

Hefti, F., Enz, A., & Melamed, E. (1985). Partial lesions of the nigrostriatal pathway in the rat. Acceleration of transmitter synthesis and release of surviving dopaminergic neurones by drugs. *Neuropharmacology, 24*(1), 19–23.

Henry, D. J., & White, F. J. (1991). Repeated cocaine administration causes persistent enhancement of D_1 dopamine receptor sensitivity within the rat nucleus accumbens. *The Journal of Pharmacology and Experimental Therapeutics, 258*(3), 882–890.

Hetman, M., & Kharebava, G. (2006). Survival signaling pathways activated by NMDA receptors. *Current Topics in Medicinal Chemistry, 6*(8), 787–799.

Hikosaka, O., Takikawa, Y., & Kawagoe, R. (2000). Role of the basal ganglia in the control of purposive saccadic eye movements. *Physiological Reviews, 80*(3), 953–978.

Hollerman, J. R., & Grace, A. A. (1990). The effects of dopamine-depleting brain lesions on the electrophysiological activity of rat substantia nigra dopamine neurons. *Brain Research, 533*(2), 203–212.

Hollister, A. S., Breese, G. R., & Cooper, B. R. (1974). Comparison of tyrosine hydroxylase and dopamine-beta-hydroxylase inhibition with the effects of various 6-hydroxydopamine treatments on d-amphetamine induced motor activity. *Psychopharmacologia, 36*(1), 1–16.

Hollister, A. S., Breese, G. R., Kuhn, C. M., Cooper, B. R., & Schanberg, S. M. (1976). An inhibitory role for brain serotonin-containing systems in the locomotor effects of d-amphetamine. *The Journal of Pharmacology and Experimental Therapeutics, 198*(1), 12–22.

Hong, J. S., Yang, H. T., & Costa, E. (1978a). Substance P content of substantia nigra after chronic treatment with antischizophrenic drugs. *Neuropharmacology, 17*(1), 83–85.

Hong, J. S., Yang, H. Y., Fratta, W., & Costa, E. (1978b). Rat striatal methionine-enkephalin content after chronic treatment with cataleptogenic and noncataleptogenic antischizophrenic drugs. *The Journal of Pharmacology and Experimental Therapeutics, 205*(1), 141–147.

Hong, J. S., Yang, H. Y., Gillin, J. C., Di Giulio, A. M., Fratta, W., & Costa, E. (1979). Chronic treatment with haloperidol accelerates the biosynthesis of enkephalins in rat striatum. *Brain Research, 160*(1), 192–195.

Hong, J. S., Yoshikawa, K., Kanamatsu, T., McGinty, J. F., Mitchell, C. L., & Sabol, S. L. (1985). Repeated electroconvulsive shocks alter the biosynthesis of enkephalin and concentration of dynorphin in the rat brain. *Neuropeptides, 5*(4–6), 557–560.

Hornykiewicz, O. (1975). Brain monoamines and parkinsonism. *National Institute on Drug Abuse Research Monograph Series, 3*, 13–21.

Hume, S. P., Opacka-Juffry, J., Myers, R., Ahier, R. G., Ashworth, S., Brooks, D. J., & Lammertsma, A. A. (1995). Effect of L-dopa and 6-hydroxydopamine lesioning on [11C] raclopride binding in rat striatum, quantified using PET. *Synapse, 21*(1), 45–53.

Hyttel, J. (1978). Effects of neuroleptics on ^{3}H-haloperidol and ^{3}H-cis(Z)-flupenthixol binding and on adenylate cyclase activity in vitro. *Life Sciences, 23*(6), 551–555.

Ingham, C. A., Hood, S. H., & Arbuthnott, G. W. (1991). A light and electron microscopical study of enkephalin-immunoreactive structures in the rat neostriatum after removal of the nigrostriatal dopaminergic pathway. *Neuroscience, 42*(3), 715–730.

Iwata, S., Shimizu, T., Nomoto, M., & Fukuda, T. (1996). Characteristic upregulation of dopamine D_1-receptor in rat striatum after 6-hydroxydopamine treatment. *Japanese Journal of Pharmacology, 71*(3), 255–258.

Jackson, D., & Abercrombie, E. D. (1992). In vivo neurochemical evaluation of striatal serotonergic hyperinnervation in rats depleted of dopamine at infancy. *Journal of Neurochemistry, 58*(3), 890–897.

Jackson, D. M., & Hashizume, M. (1987). Bromocriptine-induced locomotor stimulation in mice is modulated by dopamine D-1 receptors. *Journal of Neural Transmission, 69*(1–2), 131–145.

Jackson, D., Bruno, J. P., Stachowiak, M. K., & Zigmond, M. J. (1988). Inhibition of striatal acetylcholine release by serotonin and dopamine after the intracerebral administration of 6-hydroxydopamine to neonatal rats. *Brain Research, 457*(2), 267–273.

Jankovic, J., Caskey, T. C., Stout, J. T., & Butler, I. J. (1988). Lesch-Nyhan syndrome: A study of motor behavior and cerebrospinal fluid neurotransmitters. *Annals of Neurology, 23*(5), 466–469.

Javitt, D. C., & Zukin, S. R. (1991). Recent advances in the phencyclidine model of schizophrenia. *The American Journal of Psychiatry, 148*(10), 1301–1308.

Jentsch, J. D., & Roth, R. H. (1999). The neuropsychopharmacology of phencyclidine: From NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia.

Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 20(3), 201–225.

Jeon, B. S., Jackson-Lewis, V., & Burke, R. E. (1995). 6-Hydroxydopamine lesion of the rat substantia nigra: Time course and morphology of cell death. *Neurodegeneration: A Journal for Neurodegenerative Disorders, Neuroprotection, and Neuroregeneration, 4*(2), 131–137.

Johnson, B. J., & Bruno, J. P. (1990). D_1 and D_2 receptor contributions to ingestive and locomotor behavior are altered after dopamine depletions in neonatal rats. *Neuroscience Letters, 118*(1), 120–123.

Johnson, B. J., & Bruno, J. P. (1992). D-1 and D-2 receptor mediation of sensorimotor behavior in rats depleted of dopamine during development. *Behavioural Brain Research, 47*(1), 49–58.

Johnson, G. L., & Lapadat, R. (2002). Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science, 298*(5600), 1911–1912.

Johnson, K. B., Criswell, H. E., Jensen, K. F., Simson, P. E., Mueller, R. A., & Breese, G. R. (1992). Comparison of the D_1-dopamine agonists SKF-38393 and A-68930 in neonatal 6-hydroxydopamine-lesioned rats: Behavioral effects and induction of c-fos-like immunoreactivity. *The Journal of Pharmacology and Experimental Therapeutics, 262*(2), 855–865.

Jonsson, G., & Sachs, C. (1970). Effects of 6-hydroxydopamine on the uptake and storage of noradrenaline in sympathetic adrenergic neurons. *European Journal of Pharmacology, 9*(2), 141–155.

Joyce, J. N. (1991a). Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine. I. Effects of intranigral or intracerebroventricular 6-hydroxydopamine lesions of the mesostriatal dopamine system. *Experimental Neurology, 113*(3), 261–276.

Joyce, J. N. (1991b). Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine. II. Effects of 6-hydroxydopamine or colchicine microinjections into the VTA or reserpine treatment. *Experimental Neurology, 113*(3), 277–290.

Joyce, E. M., & Iversen, S. D. (1979). The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. *Neuroscience Letters, 14*(2–3), 207–212.

Kaakkola, S. (1980). Contralateral circling behaviour induced by intranigral injection of morphine and enkephalin analogue FK 33-824 in rats. *Acta Pharmacologica et Toxicologica, 47*(5), 385–393.

Kalivas, P. W., Widerlov, E., Stanley, D., Breese, G., & Prange, A. J., Jr. (1983). Enkephalin action on the mesolimbic system: A dopamine-dependent and a dopamine-independent increase in locomotor activity. *The Journal of Pharmacology and Experimental Therapeutics, 227*(1), 229–237.

Karler, R., Calder, L. D., Chaudhry, I. A., & Turkanis, S. A. (1989). Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. *Life Sciences, 45*(7), 599–606.

Katz, J., Nielsen, K. M., & Soghomonian, J. J. (2005). Comparative effects of acute or chronic administration of levodopa to 6-hydroxydopamine-lesioned rats on the expression of glutamic acid decarboxylase in the neostriatum and $GABA_A$ receptors subunits in the substantia nigra, pars reticulata. *Neuroscience, 132*(3), 833–842.

Kawai, Y., Takagi, H., Kumoi, Y., Shiosaka, S., & Tohyama, M. (1987). Nigrostriatal dopamine neurons receive substance P-ergic inputs in the substantia nigra: Application of the immunoelectron microscopic mirror technique to fluorescent double-staining for transmitter-specific projections. *Brain Research, 401*(2), 371–376.

Kehne, J. H., Ketteler, H. J., McCloskey, T. C., Sullivan, C. K., Dudley, M. W., & Schmidt, C. J. (1996). Effects of the selective 5-HT_{2A} receptor antagonist MDL 100,907 on MDMA-induced locomotor stimulation in rats. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 15*(2), 116–124.

Klockgether, T., & Turski, L. (1990). NMDA antagonists potentiate antiparkinsonian actions of L-dopa in monoamine-depleted rats. *Annals of Neurology, 28*(4), 539–546.

Kopin, I. J. (1981). Neurotransmitters and the Lesch-Nyhan syndrome. *The New England Journal of Medicine, 305*(19), 1148–1150.

Kostrzewa, R. M., & Brus, R. (1991). Ontogenic homologous supersensitization of quinpirole-induced yawning in rats. *Pharmacology, Biochemistry, and Behavior, 39*(2), 517–519.

Kostrzewa, R. M., & Gong, L. (1991). Supersensitized D_1 receptors mediate enhanced oral activity after neonatal 6-OHDA. *Pharmacology, Biochemistry, and Behavior, 39*(3), 677–682.

Kostrzewa, R. M., & Hamdi, A. (1991). Potentiation of spiperone-induced oral activity in rats after neonatal 6-hydroxydopamine. *Pharmacology, Biochemistry, and Behavior, 38*(1), 215–218.

Kostrzewa, R. M., & Kostrzewa, F. P. (2012). Neonatal 6-hydroxydopamine lesioning enhances quinpirole-induced vertical jumping in rats that were quinpirole primed during postnatal ontogeny. *Neurotoxicity Research, 21*(2), 231–235.

Kostrzewa, R. M., Gong, L., & Brus, R. (1993). Serotonin (5-HT) systems mediate dopamine (DA) receptor supersensitivity. *Acta Neurobiologiae Experimentalis, 53*(1), 31–41.

Kostrzewa, R. M., Brus, R., Kalbfleisch, J. H., Perry, K. W., & Fuller, R. W. (1994). Proposed animal model of attention deficit hyperactivity disorder. *Brain Research Bulletin, 34*(2), 161–167.

Kostrzewa, R. M., Reader, T. A., & Descarries, L. (1998). Serotonin neural adaptations to ontogenetic loss of dopamine neurons in rat brain. *Journal of Neurochemistry, 70*(3), 889–898.

Kubota, Y., Inagaki, S., & Kito, S. (1986a). Innervation of substance P neurons by catecholaminergic terminals in the neostriatum. *Brain Research, 375*(1), 163–167.

Kubota, Y., Inagaki, S., Kito, S., Takagi, H., & Smith, A. D. (1986b). Ultrastructural evidence of dopaminergic input to enkephalinergic neurons in rat neostriatum. *Brain Research, 367*(1–2), 374–378.

Kuribara, H. (1995a). Dopamine D_1 receptor antagonist SCH 23390 retards methamphetamine sensitization in both combined administration and early posttreatment schedules in mice. *Pharmacology, Biochemistry, and Behavior, 52*(4), 759–763.

Kuribara, H. (1995b). Modification of cocaine sensitization by dopamine D_1 and D_2 receptor antagonists in terms of ambulation in mice. *Pharmacology, Biochemistry, and Behavior, 51*(4), 799–805.

Kurumaji, A., Takashima, M., Watanabe, S., & Takahashi, K. (1988). An increase in striatal Met-enkephalin-like immunoreactivity in neonatally dopamine-depleted rats. *Neuroscience Letters, 87*(1–2), 109–113.

LaHoste, G. J., Henry, B. L., & Marshall, J. F. (2000). Dopamine D_1 receptors synergize with D_2, but not D_3 or D_4, receptors in the striatum without the involvement of action potentials. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 20*(17), 6666–6671.

Laprade, N., & Soghomonian, J. J. (1999). Gene expression of the GAD67 and GAD65 isoforms of glutamate decarboxylase is differentially altered in subpopulations of striatal neurons in adult rats lesioned with 6-OHDA as neonates. *Synapse, 33*(1), 36–48.

Laprade, N., Radja, F., Reader, T. A., & Soghomonian, J. J. (1996). Dopamine receptor agonists regulate levels of the serotonin $5\text{-}HT_{2A}$ receptor and its mRNA in a subpopulation of rat striatal neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 16*(11), 3727–3736.

Laverty, R., Sharman, D. F., & Vogt, M. (1965). Action of 2, 4, 5-Trihydroxyphenylethylamine on the Storage and Release of Noradrenaline. *British Journal of Pharmacology and Chemotherapy, 24*, 549–560.

Lawler, C. P., Gilmore, J. H., Watts, V. J., Walker, Q. D., Southerland, S. B., Cook, L. L., Mathis, C. A., & Mailman, R. B. (1995). Interhemispheric modulation of dopamine receptor interactions in unilateral 6-OHDA rodent model. *Synapse, 21*(4), 299–311.

Lee, F. J., Pei, L., & Liu, F. (2009). Disruption of the dopamine transporter-dopamine D_2 receptor interaction in schizophrenia. *Synapse, 63*(8), 710–712.

Lees, G. J., Kydd, R. R., & Wright, J. J. (1985). Relationship between sensorimotor neglect and the specificity, degree and locus of mesotelencephalic dopaminergic cell loss following 6-hydroxydopamine. *Psychopharmacology, 85*(1), 115–122.

Lesch, M., & Nyhan, W. L. (1964). A familial disorder of uric acid metabolism and central nervous system function. *The American Journal of Medicine, 36*, 561–570.

Li, S. J., Sivam, S. P., McGinty, J. F., Huang, Y. S., & Hong, J. S. (1987). Dopaminergic regulation of tachykinin metabolism in the striatonigral pathway. *The Journal of Pharmacology and Experimental Therapeutics, 243*(2), 792–798.

Li, Y., Huang, X. F., Deng, C., Meyer, B., Wu, A., Yu, Y., Ying, W., Yang, G. Y., Yenari, M. A., & Wang, Q. (2010). Alterations in 5-HT_{2A} receptor binding in various brain regions among 6-hydroxydopamine-induced Parkinsonian rats. *Synapse, 64*(3), 224–230.

Liberatore, G. T., Finkelstein, D. I., Wong, J. Y., Horne, M. K., Porritt, M. J., Donnan, G. A., & Howells, D. W. (1999). Sprouting of dopaminergic axons after striatal injury: Confirmation by markers not dependent on dopamine metabolism. *Experimental Neurology, 159*(2), 565–573.

Lidov, H. G., & Molliver, M. E. (1982). An immunohistochemical study of serotonin neuron development in the rat: Ascending pathways and terminal fields. *Brain Research Bulletin, 8*(4), 389–430.

Lindefors, N., Brodin, E., Tossman, U., Segovia, J., & Ungerstedt, U. (1989). Tissue levels and in vivo release of tachykinins and GABA in striatum and substantia nigra of rat brain after unilateral striatal dopamine denervation. *Experimental Brain Research Experimentelle Hirnforschung Experimentation Cerebrale, 74*(3), 527–534.

Lindefors, N., Brene, S., & Persson, H. (1990). Increased expression of glutamic acid decarboxylase mRNA in rat substantia nigra after an ibotenic acid lesion in the caudate-putamen. *Brain Research. Molecular Brain Research, 7*(3), 207–212.

Lindgren, H. S., Andersson, D. R., Lagerkvist, S., Nissbrandt, H., & Cenci, M. A. (2010). L-DOPA-induced dopamine efflux in the striatum and the substantia nigra in a rat model of Parkinson's disease: Temporal and quantitative relationship to the expression of dyskinesia. *Journal of Neurochemistry, 112*(6), 1465–1476.

Lisovoski, F., Haby, C., Borrelli, E., Schleef, C., Revel, M. O., Hindelang, C., & Zwiller, J. (1992). Induction of D_2 dopamine receptor mRNA synthesis in a 6-hydroxydopamine parkinsonian rat model. *Brain Research Bulletin, 28*(5), 697–701.

Ljungdahl, A., Hokfelt, T., Jonsson, G., & Sachs, C. (1971). Autoradiographic demonstration of uptake and accumulation of ^{3}H-6-hydroxydopamine in adrenergic nerves. *Experientia, 27*(3), 297–299.

Lloyd, K. G., Hornykiewicz, O., Davidson, L., Shannak, K., Farley, I., Goldstein, M., Shibuya, M., Kelley, W. N., & Fox, I. H. (1981). Biochemical evidence of dysfunction of brain neurotransmitters in the Lesch-Nyhan syndrome. *The New England Journal of Medicine, 305*(19), 1106–1111.

Longoni, R., Spina, L., & Di Chiara, G. (1987). Permissive role of D-1 receptor stimulation for the expression of D-2 mediated behavioral responses: A quantitative phenomenological study in rats. *Life Sciences, 41*(18), 2135–2145.

Lonze, B. E., & Ginty, D. D. (2002). Function and regulation of CREB family transcription factors in the nervous system. *Neuron, 35*(4), 605–623.

Loopuijt, L. D., & van der Kooy, D. (1985). Organization of the striatum: Collateralization of its efferent axons. *Brain Research, 348*(1), 86–99.

Lucas, G., & Spampinato, U. (2000). Role of striatal serotonin 2A and serotonin 2C receptor subtypes in the control of in vivo dopamine outflow in the rat striatum. *Journal of Neurochemistry, 74*(2), 693–701.

Luthman, J., Bolioli, B., Tsutsumi, T., Verhofstad, A., & Jonsson, G. (1987). Sprouting of striatal serotonin nerve terminals following selective lesions of nigro-striatal dopamine neurons in neonatal rat. *Brain Research Bulletin, 19*(2), 269–274.

Luthman, J., Fredriksson, A., Lewander, T., Jonsson, G., & Archer, T. (1989a). Effects of d-amphetamine and methylphenidate on hyperactivity produced by neonatal 6-hydroxydopamine treatment. *Psychopharmacology, 99*(4), 550–557.

Luthman, J., Fredriksson, A., Sundstrom, E., Jonsson, G., & Archer, T. (1989b). Selective lesion of central dopamine or noradrenaline neuron systems in the neonatal rat: Motor

behavior and monoamine alterations at adult stage. *Behavioural Brain Research, 33*(3), 267–277.

Luthman, J., Brodin, E., Sundstrom, E., & Wiehager, B. (1990a). Studies on brain monoamine and neuropeptide systems after neonatal intracerebroventricular 6-hydroxydopamine treatment. *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience, 8*(5), 549–560.

Luthman, J., Lindqvist, E., Young, D., & Cowburn, R. (1990b). Neonatal dopamine lesion in the rat results in enhanced adenylate cyclase activity without altering dopamine receptor binding or dopamine- and adenosine 3′:5′-monophosphate-regulated phosphoprotein (DARPP-32) immunoreactivity. *Experimental Brain Research Experimentelle Hirnforschung Experimentation Cerebrale, 83*(1), 85–95.

Luthman, J., Fredriksson, A., Plaznik, A., & Archer, T. (1991). Ketanserin and mianserin treatment reverses hyperactivity in neonatally dopamine-lesioned rats. *Journal of Psychopharmacology, 5*(4), 418–425.

Luthman, J., Lindqvist, E., & Ogren, S. O. (1995). Hyperactivity in neonatally dopamine-lesioned rats requires residual activity in mesolimbic dopamine neurons. *Pharmacology, Biochemistry, and Behavior, 51*(1), 159–163.

Lytle, L. D., Shoemaker, W. J., Cottman, K., & Wurtman, R. J. (1972). Long-term effects of postnatal 6-hydroxydopamine treatment on tissue catecholamine levels. *The Journal of Pharmacology and Experimental Therapeutics, 183*(1), 56–64.

MacKenzie, R. G., Stachowiak, M. K., & Zigmond, M. J. (1989). Dopaminergic inhibition of striatal acetylcholine release after 6-hydroxydopamine. *European Journal of Pharmacology, 168*(1), 43–52.

Maeda, T., Kannari, K., Shen, H., Arai, A., Tomiyama, M., Matsunaga, M., & Suda, T. (2003). Rapid induction of serotonergic hyperinnervation in the adult rat striatum with extensive dopaminergic denervation. *Neuroscience Letters, 343*(1), 17–20.

Malhotra, A. K., Pinals, D. A., Adler, C. M., Elman, I., Clifton, A., Pickar, D., & Breier, A. (1997). Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 17*(3), 141–150.

Mallet, N., Ballion, B., Le Moine, C., & Gonon, F. (2006). Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 26*(14), 3875–3884.

Marshall, J. F. (1979). Somatosensory inattention after dopamine-depleting intracerebral 6-OHDA injections: Spontaneous recovery and pharmacological control. *Brain Research, 177*(2), 311–324.

Marshall, J. F., & Teitelbaum, P. (1974). Further analysis of sensory inattention following lateral hypothalamic damage in rats. *Journal of Comparative and Physiological Psychology, 86*(3), 375–395.

Marshall, J. F., & Ungerstedt, U. (1977). Supersensitivity to apomorphine following destruction of the ascending dopamine neurons: Quantification using the rotational model. *European Journal of Pharmacology, 41*(4), 361–367.

Marshall, J. F., Richardson, J. S., & Teitelbaum, P. (1974). Nigrostriatal bundle damage and the lateral hypothalamic syndrome. *Journal of Comparative and Physiological Psychology, 87*(5), 808–830.

Marshall, J. F., Navarrete, R., & Joyce, J. N. (1989). Decreased striatal D_1 binding density following mesotelencephalic 6-hydroxydopamine injections: An autoradiographic analysis. *Brain Research, 493*(2), 247–257.

Martorana, A., Fusco, F. R., D'Angelo, V., Sancesario, G., & Bernardi, G. (2003). Enkephalin, neurotensin, and substance P immunoreactivite neurones of the rat GP following 6-hydroxydopamine lesion of the substantia nigra. *Experimental Neurology, 183*(2), 311–319.

Martres, M. P., Sokoloff, P., Giros, B., & Schwartz, J. C. (1992). Effects of dopaminergic transmission interruption on the D_2 receptor isoforms in various cerebral tissues. *Journal of Neurochemistry, 58*(2), 673–679.

McCreary, A. C., & Marsden, C. A. (1993). Cocaine-induced behaviour: Dopamine D_1 receptor antagonism by SCH 23390 prevents expression of conditioned sensitisation following repeated administration of cocaine. *Neuropharmacology, 32*(4), 387–391.

McQuade, R. D., Ford, D., Duffy, R. A., Chipkin, R. E., Iorio, L. C., & Barnett, A. (1988). Serotonergic component of SCH 23390: In vitro and in vivo binding analyses. *Life Sciences, 43*(23), 1861–1869.

Mengod, G., Nguyen, H., Le, H., Waeber, C., Lubbert, H., & Palacios, J. M. (1990). The distribution and cellular localization of the serotonin 1C receptor mRNA in the rodent brain examined by in situ hybridization histochemistry. Comparison with receptor binding distribution. *Neuroscience, 35*(3), 577–591.

Mennicken, F., Savasta, M., Chritin, M., Feuerstein, C., Le Moal, M., Herman, J. P., & Abrous, D. N. (1995). The neonatal lesion of the meso-telencephalic dopaminergic pathway increases intrastriatal D_2 receptor levels and synthesis and this effect is reversed by neonatal dopaminergic rich-graft. *Brain Research. Molecular Brain Research, 28*(2), 211–221.

Miller, F. E., Heffner, T. G., Kotake, C., & Seiden, L. S. (1981). Magnitude and duration of hyperactivity following neonatal 6-hydroxydopamine is related to the extent of brain dopamine depletion. *Brain Research, 229*(1), 123–132.

Molina-Holgado, E., Dewar, K. M., Grondin, L., van Gelder, N. M., & Reader, T. A. (1993a). Amino acid levels and gamma-aminobutyric acidA receptors in rat neostriatum, cortex, and thalamus after neonatal 6-hydroxydopamine lesion. *Journal of Neurochemistry, 60*(3), 936–945.

Molina-Holgado, E., Dewar, K. M., Grondin, L., van Gelder, N. M., & Reader, T. A. (1993b). Changes of amino acid and monoamine levels after neonatal 6-hydroxydopamine denervation in rat basal ganglia, substantia nigra, and Raphe nuclei. *Journal of Neuroscience Research, 35*(4), 409–418.

Molina-Holgado, E., Dewar, K. M., Descarries, L., & Reader, T. A. (1994). Altered dopamine and serotonin metabolism in the dopamine-denervated and serotonin-hyperinnervated neostriatum of adult rat after neonatal 6-hydroxydopamine. *The Journal of Pharmacology and Experimental Therapeutics, 270*(2), 713–721.

Molina-Holgado, E., Van Gelder, N. M., Dewar, K. M., & Reader, T. A. (1995). Dopamine receptor alterations correlate with increased GABA levels in adult rat neostriatum: Effects of a neonatal 6-hydroxydopamine denervation. *Neurochemistry International, 27*(4–5), 443–451.

Molloy, A. G., & Waddington, J. L. (1984). Dopaminergic behaviour stereospecific promoted by the D_1 agonist R-SK & F 38393 and selectively blocked by the D_1 antagonist SCH 23390. *Psychopharmacology, 82*(4), 409–410.

Moran-Gates, T., Zhang, K., Baldessarini, R. J., & Tarazi, F. I. (2005). Atomoxetine blocks motor hyperactivity in neonatal 6-hydroxydopamine-lesioned rats: Implications for treatment of attention-deficit hyperactivity disorder. *The International Journal of Neuropsychopharmacology, 8*(3), 439–444.

Morelli, M., & Di Chiara, G. (1987). Agonist-induced homologous and heterologous sensitization to D-1- and D-2-dependent contraversive turning. *European Journal of Pharmacology, 141*(1), 101–107.

Morelli, M., & Di Chiara, G. (1990a). MK-801 potentiates dopaminergic D_1 but reduces D_2 responses in the 6-hydroxydopamine model of Parkinson's disease. *European Journal of Pharmacology, 182*(3), 611–612.

Morelli, M., & Di Chiara, G. (1990b). Stereospecific blockade of N-methyl-D-aspartate transmission by MK 801 prevents priming of SKF 38393-induced turning. *Psychopharmacology, 101*(2), 287–288.

Morelli, M., Fenu, S., & Di Chiara, G. (1992). Blockade of N-methyl-D-aspartate receptors potentiates dopaminergic responses in the 6-OHDA model of Parkinson: Differential role of D-1 and D-2 receptors. *Neurochemistry International, 20*(Suppl), 261S–264S.

Morelli, M., Cozzolino, A., Pinna, A., Fenu, S., Carta, A., & Di Chiara, G. (1993). L-dopa stimulates c-fos expression in dopamine denervated striatum by combined activation of D-1 and D-2 receptors. *Brain Research, 623*(2), 334–336.

Morelli, M., Pinna, A., Fenu, S., Carta, A., Cozzolino, A., & Di Chiara, G. (1994). Differential effect of MK 801 and scopolamine on c-fos expression induced by L-dopa in the striatum of 6-hydroxydopamine lesioned rats. *Synapse, 18*(4), 288–293.

Morgan, S., Steiner, H., Rosenkranz, C., & Huston, J. P. (1986). Dissociation of crossed and uncrossed nigrostriatal projections with respect to site of origin in the rat. *Neuroscience, 17*(3), 609–614.

Morris, R. G., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature, 319*(6056), 774–776.

Moy, S. S. (1995). Impaired acquisition and operant responding after neonatal dopamine depletion in rats. *Pharmacology, Biochemistry, and Behavior, 52*(2), 433–441.

Moy, S. S., & Breese, G. R. (2002). Phencyclidine supersensitivity in rats with neonatal dopamine loss. *Psychopharmacology, 161*(3), 255–262.

Moy, S. S., Breese, G. R., & Eckerman, D. A. (1994). Altered activity patterns following neonatal 6-hydroxydopamine lesions to dopaminergic neurons: Effect of SKF-38393. *Brain Research, 645*(1–2), 49–60.

Moy, S. S., Fernandes, A., Qian, Y., Rotella, D. J., Kostrewa, R. M., & Breese, G. R. (2004). Effect of acute and chronic olanzapine treatment on phencyclidine-induced behavioral sensitization in rats with neonatal dopamine loss. *Pharmacology, Biochemistry, and Behavior, 78*(1), 47–56.

Murrin, L. C., Gale, K., & Kuhar, M. J. (1979). Autoradiographic localization of neuroleptic and dopamine receptors in the caudate-putamen and substantia nigra: Effects of lesions. *European Journal of Pharmacology, 60*(2–3), 229–235.

Nagy, J. I., Vincent, S. R., & Fibiger, H. C. (1978). Altered neurotransmitter synthetic enzyme activity in some extrapyramidal nuclei after lesions of the nigro-striatal dopamine projection. *Life Sciences, 22*(20), 1777–1782.

Narang, N., & Wamsley, J. K. (1995). Time dependent changes in DA uptake sites, D_1 and D_2 receptor binding and mRNA after 6-OHDA lesions of the medial forebrain bundle in the rat brain. *Journal of Chemical Neuroanatomy, 9*(1), 41–53.

Navailles, S., Bioulac, B., Gross, C., & De Deurwaerdere, P. (2010). Serotonergic neurons mediate ectopic release of dopamine induced by L-DOPA in a rat model of Parkinson's disease. *Neurobiology of Disease, 38*(1), 136–143.

Neve, K. A., Kozlowski, M. R., & Marshall, J. F. (1982). Plasticity of neostriatal dopamine receptors after nigrostriatal injury: Relationship to recovery of sensorimotor functions and behavioral supersensitivity. *Brain Research, 244*(1), 33–44.

Neve, K. A., Loeschen, S., & Marshall, J. F. (1985). Denervation accelerates the reappearance of neostriatal D-2 receptors after irreversible receptor blockade. *Brain Research, 329*(1–2), 225–231.

Neve, K. A., Neve, R. L., Fidel, S., Janowsky, A., & Higgins, G. A. (1991). Increased abundance of alternatively spliced forms of D_2 dopamine receptor mRNA after denervation. *Proceedings of the National Academy of Sciences of the United States of America, 88*(7), 2802–2806.

Nguyen, T. V., Brownell, A. L., Iris Chen, Y. C., Livni, E., Coyle, J. T., Rosen, B. R., Cavagna, F., & Jenkins, B. G. (2000). Detection of the effects of dopamine receptor supersensitivity using pharmacological MRI and correlations with PET. *Synapse, 36*(1), 57–65.

Nikolaus, S., Larisch, R., Beu, M., Forutan, F., Vosberg, H., & Muller-Gartner, H. W. (2003). Bilateral increase in striatal dopamine D_2 receptor density in the 6-hydroxydopamine-lesioned rat: A serial in vivo investigation with small animal PET. *European Journal of Nuclear Medicine and Molecular Imaging, 30*(3), 390–395.

Nisenbaum, E. S., Stricker, E. M., Zigmond, M. J., & Berger, T. W. (1986). Long-term effects of dopamine-depleting brain lesions on spontaneous activity of type II striatal neurons: Relation to behavioral recovery. *Brain Research, 398*(2), 221–230.

Nisenbaum, L. K., Kitai, S. T., Crowley, W. R., & Gerfen, C. R. (1994a). Temporal dissociation between changes in striatal enkephalin and substance P messenger RNAs following striatal dopamine depletion. *Neuroscience, 60*(4), 927–937.

Nisenbaum, L. K., Kitai, S. T., & Gerfen, C. R. (1994b). Dopaminergic and muscarinic regulation of striatal enkephalin and substance P messenger RNAs following striatal dopamine denervation: Effects of systemic and central administration of quinpirole and scopolamine. *Neuroscience, 63*(2), 435–449.

Nishikawa, T., Mataga, N., Takashima, M., & Toru, M. (1983). Behavioral sensitization and relative hyperresponsiveness of striatal and limbic dopaminergic neurons after repeated methamphetamine treatment. *European Journal of Pharmacology, 88*(2–3), 195–203.

Numan, S., Lundgren, K. H., Wright, D. E., Herman, J. P., & Seroogy, K. B. (1995). Increased expression of $5HT_2$ receptor mRNA in rat striatum following 6-OHDA lesions of the adult nigrostriatal pathway. *Brain Research. Molecular Brain Research, 29*(2), 391–396.

Nyhan, W. L. (1968). Clinical features of the Lesch-Nyhan syndrome. Introduction–clinical and genetic features. *Federation Proceedings, 27*(4), 1027–1033.

O'Boyle, K. M., Gaitanopoulos, D. E., Brenner, M., & Waddington, J. L. (1989). Agonist and antagonist properties of benzazepine and thienopyridine derivatives at the D_1 dopamine receptor. *Neuropharmacology, 28*(4), 401–405.

O'Connor, W. T., Lindefors, N., Brene, S., Herrera-Marschitz, M., Persson, H., & Ungerstedt, U. (1991). Short-term dopaminergic regulation of GABA release in dopamine deafferented caudate-putamen is not directly associated with glutamic acid decarboxylase gene expression. *Neuroscience Letters, 128*(1), 66–70.

O'Neill, M. F., Heron-Maxwell, C. L., & Shaw, G. (1999). $5\text{-}HT_2$ receptor antagonism reduces hyperactivity induced by amphetamine, cocaine, and MK-801 but not D_1 agonist C-APB. *Pharmacology, Biochemistry, and Behavior, 63*(2), 237–243.

Obeso, J. A., Rodriguez-Oroz, M. C., Rodriguez, M., Lanciego, J. L., Artieda, J., Gonzalo, N., & Olanow, C. W. (2000). Pathophysiology of the basal ganglia in Parkinson's disease. *Trends in Neurosciences, 23*(10 Suppl), S8–S19.

Obeso, J. A., Rodriguez-Oroz, M., Marin, C., Alonso, F., Zamarbide, I., Lanciego, J. L., & Rodriguez-Diaz, M. (2004). The origin of motor fluctuations in Parkinson's disease: Importance of dopaminergic innervation and basal ganglia circuits. *Neurology, 62*(1 Suppl 1), S17–S30.

Olney, J. W. (1989). Excitatory amino acids and neuropsychiatric disorders. *Biological Psychiatry, 26*(5), 505–525.

Olney, J. W., Newcomer, J. W., & Farber, N. B. (1999). NMDA receptor hypofunction model of schizophrenia. *Journal of Psychiatric Research, 33*(6), 523–533.

Onn, S. P., Berger, T. W., Stricker, E. M., & Zigmond, M. J. (1986). Effects of intraventricular 6-hydroxydopamine on the dopaminergic innervation of striatum: Histochemical and neurochemical analysis. *Brain Research, 376*(1), 8–19.

Pan, H. S., Penney, J. B., & Young, A. B. (1985). Gamma-aminobutyric acid and benzodiazepine receptor changes induced by unilateral 6-hydroxydopamine lesions of the medial forebrain bundle. *Journal of Neurochemistry, 45*(5), 1396–1404.

Papadeas, S. T. (2006). *Relationship of ERK1/2 phosphorylation to D_1 dopamine receptor activation, behavioral sensitization, and structural plasticity in the neonate 6-hydroxydopamine-lesioned rat*. University of North Carolina at Chapel Hill, ProQuest/UMI.

Papadeas, S. T., Blake, B. L., Knapp, D. J., & Breese, G. R. (2004). Sustained extracellular signal-regulated kinase 1/2 phosphorylation in neonate 6-hydroxydopamine-lesioned rats after repeated D_1-dopamine receptor agonist administration: Implications for NMDA receptor involvement. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 24*(26), 5863–5876.

Papadeas, S. T., Halloran, C., McCown, T. J., Breese, G. R., & Blake, B. L. (2008). Changes in apical dendritic structure correlate with sustained ERK1/2 phosphorylation in medial

prefrontal cortex of a rat model of dopamine D_1 receptor agonist sensitization. *The Journal of Comparative Neurology, 511*(2), 271–285.

Pappas, B. A., Gallivan, J. V., Dugas, T., Saari, M., & Ings, R. (1980). Intraventricular 6-hydroxydopamine in the newborn rat and locomotor responses to drugs in infancy: No support for the dopamine depletion model of minimal brain dysfunction. *Psychopharmacology, 70*(1), 41–46.

Pappas, B. A., Murtha, S. J., Park, G. A., Condon, K. T., Szirtes, R. M., Laventure, S. I., & Ally, A. (1992). Neonatal brain dopamine depletion and the cortical and behavioral consequences of enriched postweaning environment. *Pharmacology, Biochemistry, and Behavior, 42*(4), 741–748.

Paquette, M. A., Anderson, A. M., Lewis, J. R., Meshul, C. K., Johnson, S. W., & Paul Berger, S. (2010). MK-801 inhibits L-DOPA-induced abnormal involuntary movements only at doses that worsen parkinsonism. *Neuropharmacology, 58*(7), 1002–1008.

Parish, C. L., Stanic, D., Drago, J., Borrelli, E., Finkelstein, D. I., & Horne, M. K. (2002). Effects of long-term treatment with dopamine receptor agonists and antagonists on terminal arbor size. *The European Journal of Neuroscience, 16*(5), 787–794.

Parsons, L. H., Smith, A. D., & Justice, J. B., Jr. (1991). The in vivo microdialysis recovery of dopamine is altered independently of basal level by 6-hydroxydopamine lesions to the nucleus accumbens. *Journal of Neuroscience Methods, 40*(2–3), 139–147.

Patel, S., Roberts, J., Moorman, J., & Reavill, C. (1995). Localization of serotonin-4 receptors in the striatonigral pathway in rat brain. *Neuroscience, 69*(4), 1159–1167.

Paul, A., Wilson, S., Belham, C. M., Robinson, C. J., Scott, P. H., Gould, G. W., & Plevin, R. (1997). Stress-activated protein kinases: Activation, regulation and function. *Cellular Signalling, 9*(6), 403–410.

Pavon, N., Martin, A. B., Mendialdua, A., & Moratalla, R. (2006). ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. *Biological Psychiatry, 59*(1), 64–74.

Pearson, G., Robinson, F., Beers Gibson, T., Xu, B. E., Karandikar, M., Berman, K., & Cobb, M. H. (2001). Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions. *Endocrine Reviews, 22*(2), 153–183.

Pei, L., Li, S., Wang, M., Diwan, M., Anisman, H., Fletcher, P. J., Nobrega, J. N., & Liu, F. (2010). Uncoupling the dopamine D_1-D_2 receptor complex exerts antidepressant-like effects. *Nature Medicine, 16*(12), 1393–1395.

Penit-Soria, J., Durand, C., Herve, D., & Besson, M. J. (1997). Morphological and biochemical adaptations to unilateral dopamine denervation of the neostriatum in newborn rats. *Neuroscience, 77*(3), 753–766.

Penny, G. R., Afsharpour, S., & Kitai, S. T. (1986). The glutamate decarboxylase-, leucine enkephalin-, methionine enkephalin- and substance P-immunoreactive neurons in the neostriatum of the rat and cat: Evidence for partial population overlap. *Neuroscience, 17*(4), 1011–1045.

Perese, D. A., Ulman, J., Viola, J., Ewing, S. E., & Bankiewicz, K. S. (1989). A 6-hydroxydopamine-induced selective parkinsonian rat model. *Brain Research, 494*(2), 285–293.

Perez-Navarro, E., Alberch, J., & Marsal, J. (1993). Postnatal development of functional dopamine, opioid and tachykinin receptors that regulate acetylcholine release from rat neostriatal slices. Effect of 6-hydroxydopamine lesion. *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience, 11*(6), 701–708.

Perreault, M. L., Hasbi, A., O'Dowd, B. F., & George, S. R. (2011). The dopamine d1-d2 receptor heteromer in striatal medium spiny neurons: Evidence for a third distinct neuronal pathway in basal ganglia. *Frontiers in Neuroanatomy, 5*, 31.

Pickel, V. M., Johnson, E., Carson, M., & Chan, J. (1992). Ultrastructure of spared dopamine terminals in caudate-putamen nuclei of adult rats neonatally treated with intranigral 6-hydroxydopamine. *Brain Research. Developmental Brain Research, 70*(1), 75–86.

Plech, A., Brus, R., Kalbfleisch, J. H., & Kostrzewa, R. M. (1995). Enhanced oral activity responses to intrastriatal SKF 38393 and m-CPP are attenuated by intrastriatal mianserin in neonatal 6-OHDA-lesioned rats. *Psychopharmacology, 119*(4), 466–473.

Porras, G., Di Matteo, V., Fracasso, C., Lucas, G., De Deurwaerdere, P., Caccia, S., Esposito, E., & Spampinato, U. (2002). 5-HT_{2A} and 5-$HT_{2C/2B}$ receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 26*(3), 311–324.

Porter, C. C., Totaro, J. A., & Stone, C. A. (1963). Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. *The Journal of Pharmacology and Experimental Therapeutics, 140*, 308–316.

Potter, B. M., & Bruno, J. P. (1989). Food intake of rats depleted of dopamine as neonates is impaired by inhibition of catecholamine biosynthesis. *Neuroscience Letters, 107*(1–3), 295–300.

Pritzel, M., Huston, J. P., & Sarter, M. (1983). Behavioral and neuronal reorganization after unilateral substantia nigra lesions: Evidence for increased interhemispheric nigrostriatal projections. *Neuroscience, 9*(4), 879–888.

Przedborski, S., Levivier, M., Jiang, H., Ferreira, M., Jackson-Lewis, V., Donaldson, D., & Togasaki, D. M. (1995). Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine. *Neuroscience, 67*(3), 631–647.

Radja, F., Descarries, L., Dewar, K. M., & Reader, T. A. (1993a). Serotonin 5-HT_1 and 5-HT_2 receptors in adult rat brain after neonatal destruction of nigrostriatal dopamine neurons: A quantitative autoradiographic study. *Brain Research, 606*(2), 273–285.

Radja, F., el Mansari, M., Soghomonian, J. J., Dewar, K. M., Ferron, A., Reader, T. A., & Descarries, L. (1993b). Changes of D_1 and D_2 receptors in adult rat neostriatum after neonatal dopamine denervation: Quantitative data from ligand binding, in situ hybridization and iontophoresis. *Neuroscience, 57*(3), 635–648.

Rashid, A. J., So, C. H., Kong, M. M., Furtak, T., El-Ghundi, M., Cheng, R., O'Dowd, B. F., & George, S. R. (2007). D_1-D_2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proceedings of the National Academy of Sciences of the United States of America, 104*(2), 654–659.

Raskin, L. A., Shaywitz, B. A., Anderson, G. M., Cohen, D. J., Teicher, M. H., & Linakis, J. (1983). Differential effects of selective dopamine, norepinephrine or catecholamine depletion on activity and learning in the developing rat. *Pharmacology, Biochemistry, and Behavior, 19*(5), 743–749.

Reader, T. A., & Dewar, K. M. (1999). Effects of denervation and hyperinnervation on dopamine and serotonin systems in the rat neostriatum: Implications for human Parkinson's disease. *Neurochemistry International, 34*(1), 1–21.

Reid, M. S., O'Connor, W. T., Herrera-Marschitz, M., & Ungerstedt, U. (1990). The effects of intranigral GABA and dynorphin A injections on striatal dopamine and GABA release: Evidence that dopamine provides inhibitory regulation of striatal GABA neurons via D_2 receptors. *Brain Research, 519*(1–2), 255–260.

Reiner, A., & Anderson, K. D. (1990). The patterns of neurotransmitter and neuropeptide co-occurrence among striatal projection neurons: Conclusions based on recent findings. *Brain Research. Brain Research Reviews, 15*(3), 251–265.

Robertson, G. S., Herrera, D. G., Dragunow, M., & Robertson, H. A. (1989a). L-dopa activates c-fos in the striatum ipsilateral to a 6-hydroxydopamine lesion of the substantia nigra. *European Journal of Pharmacology, 159*(1), 99–100.

Robertson, H. A., Peterson, M. R., Murphy, K., & Robertson, G. S. (1989b). D_1-dopamine receptor agonists selectively activate striatal c-fos independent of rotational behaviour. *Brain Research, 503*(2), 346–349.

Robinson, T. E. (1991). Controls for lesions of the nigrostriatal dopamine system. *Science, 253*(5017), 332.

Robinson, T. E., & Berridge, K. C. (2008). Review. The incentive sensitization theory of addiction: Some current issues. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 363*(1507), 3137–3146.

Robinson, T. E., & Whishaw, I. Q. (1988). Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: A microdialysis study in freely moving rats. *Brain Research, 450*(1–2), 209–224.

Robinson, T. E., Castaneda, E., & Whishaw, I. Q. (1990). Compensatory changes in striatal dopamine neurons following recovery from injury induced by 6-OHDA or methamphetamine: A review of evidence from microdialysis studies. *Canadian Journal of Psychology, 44*(2), 253–275.

Robinson, T. E., Mocsary, Z., Camp, D. M., & Whishaw, I. Q. (1994). Time course of recovery of extracellular dopamine following partial damage to the nigrostriatal dopamine system. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 14*(5 Pt 1), 2687–2696.

Rogers, D. C., & Dunnett, S. B. (1989a). Hypersensitivity to alpha-methyl-p-tyrosine suggests that behavioural recovery of rats receiving neonatal 6-OHDA lesions is mediated by residual catecholamine neurones. *Neuroscience Letters, 102*(1), 108–113.

Rogers, D. C., & Dunnett, S. B. (1989b). Neonatal dopamine-rich grafts and 6-OHDA lesions independently provide partial protection from the adult nigrostriatal lesion syndrome. *Behavioural Brain Research, 34*(1–2), 131–146.

Samuel, D., Kumar, U., & Nieoullon, A. (1988). Gamma-Aminobutyric acid function in the rat striatum is under the double influence of nigrostriatal dopaminergic and thalamostriatal inputs: Two modes of regulation? *Journal of Neurochemistry, 51*(6), 1704–1710.

Saner, A., & Thoenen, H. (1971). Model experiments on the molecular mechanism of action of 6-hydroxydopamine. *Molecular Pharmacology, 7*(2), 147–154.

Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., Girault, J. A., Herve, D., Greengard, P., & Fisone, G. (2007). Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 27*(26), 6995–7005.

Sauer, H., & Oertel, W. H. (1994). Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: A combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience, 59*(2), 401–415.

Savasta, M., Dubois, A., Feuerstein, C., Manier, M., & Scatton, B. (1987). Denervation supersensitivity of striatal D_2 dopamine receptors is restricted to the ventro- and dorsolateral regions of the striatum. *Neuroscience Letters, 74*(2), 180–186.

Savasta, M., Dubois, A., Benavides, J., & Scatton, B. (1988). Different plasticity changes in D_1 and D_2 receptors in rat striatal subregions following impairment of dopaminergic transmission. *Neuroscience Letters, 85*(1), 119–124.

Savasta, M., Ruberte, E., Palacios, J. M., & Mengod, G. (1989). The colocalization of cholecystokinin and tyrosine hydroxylase mRNAs in mesencephalic dopaminergic neurons in the rat brain examined by in situ hybridization. *Neuroscience, 29*(2), 363–369.

Schallert, T., Petrie, B. F., & Wishaw, I. Q. (1989). Neonatal dopamine depletion: Spared and unspared sensorimotor and attentional disorders and effects of further depletion in adulthood. *Psychobiology, 17*, 386–396.

Schiffmann, S. N., & Vanderhaeghen, J. J. (1992). Lesion of the nigrostriatal pathway induces cholecystokinin messenger RNA expression in the rat striatum. An in situ hybridization histochemistry study. *Neuroscience, 50*(3), 551–557.

Schober, A. (2004). Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. *Cell and Tissue Research, 318*(1), 215–224.

Schuller, J. J., Billings, L. M., & Marshall, J. F. (1999). Dopaminergic modulation of pallidal preproenkephalin mRNA. *Brain Research. Molecular Brain Research, 69*(1), 149–153.

Schwarting, R. K., & Huston, J. P. (1996). Unilateral 6-hydroxydopamine lesions of meso-striatal dopamine neurons and their physiological sequelae. *Progress in Neurobiology, 49*(3), 215–266.

Schwarzkopf, S. B., Mitra, T., & Bruno, J. P. (1992). Sensory gating in rats depleted of dopamine as neonates: Potential relevance to findings in schizophrenic patients. *Biological Psychiatry, 31*(8), 759–773.

Schwarzkopf, S. B., Bruno, J. P., Mitra, T., & Ison, J. R. (1996). Effects of haloperidol and SCH 23390 on acoustic startle in animals depleted of dopamine as neonates: Implications for neuropsychiatric syndromes. *Psychopharmacology, 123*(3), 258–266.

Segal, D. S., & Mandell, A. J. (1974). Long-term administration of d-amphetamine: Progressive augmentation of motor activity and stereotypy. *Pharmacology, Biochemistry, and Behavior, 2*(2), 249–255.

Seger, R., & Krebs, E. G. (1995). The MAPK signaling cascade. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 9*(9), 726–735.

Segovia, J., Tillakaratne, N. J., Whelan, K., Tobin, A. J., & Gale, K. (1990). Parallel increases in striatal glutamic acid decarboxylase activity and mRNA levels in rats with lesions of the nigrostriatal pathway. *Brain Research, 529*(1–2), 345–348.

Sgambato-Faure, V., Buggia, V., Gilbert, F., Levesque, D., Benabid, A. L., & Berger, F. (2005). Coordinated and spatial upregulation of arc in striatonigral neurons correlates with L-dopa-induced behavioral sensitization in dyskinetic rats. *Journal of Neuropathology and Experimental Neurology, 64*(11), 936–947.

Shaywitz, B. A., Klopper, J. H., Yager, R. D., & Gordon, J. W. (1976a). Paradoxical response to amphetamine in developing rats treated with 6-hydroxydopamine. *Nature, 261*(5556), 153–155.

Shaywitz, B. A., Yager, R. D., & Klopper, J. H. (1976b). Selective brain dopamine depletion in developing rats: An experimental model of minimal brain dysfunction. *Science, 191*(4224), 305–308.

Shinotoh, H., & Calne, D. B. (1995). The use of PET in Parkinson's disease. *Brain and Cognition, 28*(3), 297–310.

Silbergeld, E. K., & Pfeiffer, R. F. (1977). Differential effects of three dopamine agonists: Apomorphine, bromocriptine and lergotrile. *Journal of Neurochemistry, 28*(6), 1323–1326.

Silverstein, F. S., Johnston, M. V., Hutchinson, R. J., & Edwards, N. L. (1985). Lesch-Nyhan syndrome: CSF neurotransmitter abnormalities. *Neurology, 35*(6), 907–911.

Simson, P. E., Johnson, K. B., Jurevics, H. A., Criswell, H. E., Napier, T. C., Duncan, G. E., Mueller, R. A., & Breese, G. R. (1992). Augmented sensitivity of D_1-dopamine receptors in lateral but not medial striatum after 6-hydroxydopamine-induced lesions in the neonatal rat. *The Journal of Pharmacology and Experimental Therapeutics, 263*(3), 1454–1463.

Sivam, S. P. (1989). D_1 dopamine receptor-mediated substance P depletion in the striatonigral neurons of rats subjected to neonatal dopaminergic denervation: Implications for self-injurious behavior. *Brain Research, 500*(1–2), 119–130.

Sivam, S. P. (1991). Dopamine dependent decrease in enkephalin and substance P levels in basal ganglia regions of postmortem parkinsonian brains. *Neuropeptides, 18*(4), 201–207.

Sivam, S. P., & Cox, J. (2006). Postnatal administration of D_1 dopamine agonist reverses neonatal dopaminergic lesion-induced changes in striatal enkephalin and substance P systems. *Brain Research, 1073–1074*, 159–163.

Sivam, S. P., & Krause, J. E. (1990). The adaptation of enkephalin, tachykinin and monoamine neurons of the basal ganglia following neonatal dopaminergic denervation is dependent on the extent of dopamine depletion. *Brain Research, 536*(1–2), 169–175.

Sivam, S. P., Breese, G. R., Napier, T. C., Mueller, R. A., & Hong, J. S. (1986). Dopaminergic regulation of proenkephalin-A gene expression in the basal ganglia. *NIDA Research Monograph, 75*, 389–392.

Sivam, S. P., Breese, G. R., Krause, J. E., Napier, T. C., Mueller, R. A., & Hong, J. S. (1987). Neonatal and adult 6-hydroxydopamine-induced lesions differentially alter tachykinin and enkephalin gene expression. *Journal of Neurochemistry, 49*(5), 1623–1633.

Sivam, S. P., Krause, J. E., Breese, G. R., & Hong, J. S. (1991). Dopamine-dependent postnatal development of enkephalin and tachykinin neurons of rat basal ganglia. *Journal of Neurochemistry, 56*(5), 1499–1508.

Smith, R. D., Cooper, B. R., & Breese, G. R. (1973). Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine: Evidence for involvement of brain dopamine. *The Journal of Pharmacology and Experimental Therapeutics, 185*(3), 609–619.

Snyder, A. M., Stricker, E. M., & Zigmond, M. J. (1985). Stress-induced neurological impairments in an animal model of parkinsonism. *Annals of Neurology, 18*(5), 544–551.

Snyder, A. M., Zigmond, M. J., & Lund, R. D. (1986). Sprouting of serotoninergic afferents into striatum after dopamine-depleting lesions in infant rats: A retrograde transport and immunocytochemical study. *The Journal of Comparative Neurology, 245*(2), 274–281.

Snyder, G. L., Keller, R. W., Jr., & Zigmond, M. J. (1990). Dopamine efflux from striatal slices after intracerebral 6-hydroxydopamine: Evidence for compensatory hyperactivity of residual terminals. *The Journal of Pharmacology and Experimental Therapeutics, 253*(2), 867–876.

Snyder-Keller, A. M. (1991). Development of striatal compartmentalization following pre- or postnatal dopamine depletion. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 11*(3), 810–821.

Snyder-Keller, A. M., Carder, R. K., & Lund, R. D. (1989). Development of dopamine innervation and turning behavior in dopamine-depleted infant rats receiving unilateral nigral transplants. *Neuroscience, 30*(3), 779–794.

Soghomonian, J. J. (1993). Effects of neonatal 6-hydroxydopamine injections on glutamate decarboxylase, preproenkephalin and dopamine D_2 receptor mRNAs in the adult rat striatum. *Brain Research, 621*(2), 249–259.

Soghomonian, J. J. (1994). Differential regulation of glutamate decarboxylase and preproenkephalin mRNA levels in the rat striatum. *Brain Research, 640*(1–2), 146–154.

Spirduso, W. W., Gilliam, P. E., Schallert, T., Upchurch, M., Vaughn, D. M., & Wilcox, R. E. (1985). Reactive capacity: A sensitive behavioral marker of movement initiation and nigrostriatal dopamine function. *Brain Research, 335*(1), 45–54.

Spooren, W. P., Gasparini, F., Bergmann, R., & Kuhn, R. (2000). Effects of the prototypical mGlu(5) receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine on rotarod, locomotor activity and rotational responses in unilateral 6-OHDA-lesioned rats. *European Journal of Pharmacology, 406*(3), 403–410.

Stachowiak, M. K., Bruno, J. P., Snyder, A. M., Stricker, E. M., & Zigmond, M. J. (1984). Apparent sprouting of striatal serotonergic terminals after dopamine-depleting brain lesions in neonatal rats. *Brain Research, 291*(1), 164–167.

Stachowiak, M. K., Keller, R. W., Jr., Stricker, E. M., & Zigmond, M. J. (1987). Increased dopamine efflux from striatal slices during development and after nigrostriatal bundle damage. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 7*(6), 1648–1654.

Staunton, D. A., Wolfe, B. B., Groves, P. M., & Molinoff, P. B. (1981). Dopamine receptor changes following destruction of the nigrostriatal pathway: Lack of a relationship to rotational behavior. *Brain Research, 211*(2), 315–327.

Steece-Collier, K., Collier, T. J., Danielson, P. D., Kurlan, R., Yurek, D. M., & Sladek, J. R., Jr. (2003). Embryonic mesencephalic grafts increase levodopa-induced forelimb hyperkinesia in parkinsonian rats. *Movement Disorders: Official Journal of the Movement Disorder Society, 18*(12), 1442–1454.

Stellar, J. R., Waraczynski, M., & Bruno, J. P. (1988). Neonatal dopamine depletions spare lateral hypothalamic stimulation reward in adult rats. *Pharmacology, Biochemistry, and Behavior, 30*(2), 365–370.

Stout, J. T. C., & Caskey, C. T. (1989). Hypoxanthine phosphoribosyltransferase deficiency: The Lesch-Nyhan syndrome and gouty arthritis. In C. R. Scriver, A. L. Beaudet, W. S. Sly, & D. Valle (Eds.), *The metabolic basis of inherited disease* (Vol. 1, pp. 1007–1028). New York: McGraw-Hill.

Strange, P. G. (1993). New insights into dopamine receptors in the central nervous system. *Neurochemistry International, 22*(3), 223–236.

Stricker, E. M., & Zigmond, M. J. (1974). Effects on homeostasis of intraventricular injections of 6-hydroxydopamine in rats. *Journal of Comparative and Physiological Psychology, 86*(6), 973–994.

Stricker, E. M., & Zigmond, M. J. (1984). Brain catecholamines and the central control of food intake. *International Journal of Obesity, 8*(Suppl 1), 39–50.

Stromberg, I., Adams, C., Bygdeman, M., Hoffer, B., Boyson, S., & Humpel, C. (1995). Long-term effects of human-to-rat mesencephalic xenografts on rotational behavior, striatal dopamine receptor binding, and mRNA levels. *Brain Research Bulletin, 38*(3), 221–233.

Sweatt, J. D. (2001). The neuronal MAP kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. *Journal of Neurochemistry, 76*(1), 1–10.

Takeichi, T., Kurumiya, S., Umemoto, M., & Olds, M. E. (1986). Roles of catecholamine terminals and intrinsic neurons of the ventral tegmentum in self-stimulation investigated in neonatally dopamine-depleted rats. *Pharmacology, Biochemistry, and Behavior, 24*(4), 1101–1109.

Tanaka, Y., Niijima, K., Mizuno, Y., & Yoshida, M. (1986). Changes in gamma-aminobutyrate, glutamate, aspartate, glycine, and taurine contents in the striatum after unilateral nigrostriatal lesions in rats. *Experimental Neurology, 91*(2), 259–268.

Tang, F., Costa, E., & Schwartz, J. P. (1983). Increase of proenkephalin mRNA and enkephalin content of rat striatum after daily injection of haloperidol for 2 to 3 weeks. *Proceedings of the National Academy of Sciences of the United States of America, 80*(12), 3841–3844.

Taylor, M. D., De Ceballos, M. L., Rose, S., Jenner, P., & Marsden, C. D. (1992). Effects of a unilateral 6-hydroxydopamine lesion and prolonged L-3,4-dihydroxyphenylalanine treatment on peptidergic systems in rat basal ganglia. *European Journal of Pharmacology, 219*(2), 183–192.

Thal, L. J., Sharpless, N. S., Hirschhorn, I. D., Horowitz, S. G., & Makman, M. H. (1983). Striatal met-enkephalin concentration increases following nigrostriatal denervation. *Biochemical Pharmacology, 32*(22), 3297–3301.

Thoenen, H., & Tranzer, J. P. (1968). Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. *Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie, 261*(3), 271–288.

Tillerson, J. L., Cohen, A. D., Philhower, J., Miller, G. W., Zigmond, M. J., & Schallert, T. (2001). Forced limb-use effects on the behavioral and neurochemical effects of 6-hydroxydopamine. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 21*(12), 4427–4435.

Tossman, U., Segovia, J., & Ungerstedt, U. (1986). Extracellular levels of amino acids in striatum and globus pallidus of 6-hydroxydopamine-lesioned rats measured with microdialysis. *Acta Physiologica Scandinavica, 127*(4), 547–551.

Touchet, N., & Bennett, J. P., Jr. (1989). The metabolism of systemically-administered L-dihydroxyphenylalanine, by intact and dopamine-denervated striata, as revealed by brain microdialysis. *Neuropharmacology, 28*(11), 1217–1222.

Towle, A. C., Criswell, H. E., Maynard, E. H., Lauder, J. M., Joh, T. H., Mueller, R. A., & Breese, G. R. (1989). Serotonergic innervation of the rat caudate following a neonatal 6-hydroxydopamine lesion: An anatomical, biochemical and pharmacological study. *Pharmacology, Biochemistry, and Behavior, 34*(2), 367–374.

Tripanichkul, W., Stanic, D., Drago, J., Finkelstein, D. I., & Horne, M. K. (2003). D_2 Dopamine receptor blockade results in sprouting of DA axons in the intact animal but prevents sprouting following nigral lesions. *The European Journal of Neuroscience, 17*(5), 1033–1045.

Umbriaco, D., Garcia, S., Beaulieu, C., & Descarries, L. (1995). Relational features of acetylcholine, noradrenaline, serotonin and GABA axon terminals in the stratum radiatum of adult rat hippocampus (CA1). *Hippocampus, 5*(6), 605–620.

Ungerstedt, U. (1968). 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *European Journal of Pharmacology, 5*(1), 107–110.

Ungerstedt, U. (1971a). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiologica Scandinavica. Supplementum, 367*, 95–122.

Ungerstedt, U. (1971b). Postsynaptic supersensitivity after 6-hydroxy-dopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiologica Scandinavica. Supplementum, 367*, 69–93.

Ungerstedt, U. (1971c). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiologica Scandinavica. Supplementum, 367*, 1–48.

Ungerstedt, U. (1971d). Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. *Acta Physiologica Scandinavica. Supplementum, 367*, 49–68.

Ungerstedt, U. (1971e). Use of intracerebral injections of 6-hydroxydopamine as a tool for morphological and functional studies on central catecholamine neurons. In T. Malmfors & H. Thonen (Eds.), *6-hydroxydopamine and catecholamine neurons* (pp. 315–332). Amsterdam: North Holland.

Ungerstedt, U., Ljungberg, T., & Steg, G. (1974). Behavioral, physiological, and neurochemical changes after 6-hydroxydopamine-induced degeneration of the nigro-striatal dopamine neurons. *Advances in Neurology, 5*, 421–426.

Uretsky, N. J., & Iversen, L. L. (1970). Effects of 6-hydroxydopamine on catecholamine containing neurones in the rat brain. *Journal of Neurochemistry, 17*(2), 269–278.

Usiello, A., Baik, J. H., Rouge-Pont, F., Picetti, R., Dierich, A., LeMeur, M., Piazza, P. V., & Borrelli, E. (2000). Distinct functions of the two isoforms of dopamine D_2 receptors. *Nature, 408*(6809), 199–203.

Valjent, E., Corvol, J. C., Pages, C., Besson, M. J., Maldonado, R., & Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 20*(23), 8701–8709.

van der Heyden, J. A., Venema, K., & Korf, J. (1980). In vivo release of endogenous GABA from rat striatum: inhibition by dopamine. *Journal of Neurochemistry, 34*(5), 1338–1341.

van Horne, C., Hoffer, B. J., Stromberg, I., & Gerhardt, G. A. (1992). Clearance and diffusion of locally applied dopamine in normal and 6-hydroxydopamine-lesioned rat striatum. *The Journal of Pharmacology and Experimental Therapeutics, 263*(3), 1285–1292.

Vankova, M., Arluison, M., Boyer, P. A., Bourgoin, S., & Quignon, M. (1991). Enkephalin-containing nerve cell bodies in the substantia nigra of the rat: Demonstration by immunohistochemistry and in situ hybridization. *Brain Research Bulletin, 27*(1), 19–27.

Vernier, P., Julien, J. F., Rataboul, P., Fourrier, O., Feuerstein, C., & Mallet, J. (1988). Similar time course changes in striatal levels of glutamic acid decarboxylase and proenkephalin mRNA following dopaminergic deafferentation in the rat. *Journal of Neurochemistry, 51*(5), 1375–1380.

Voorn, P., Roest, G., & Groenewegen, H. J. (1987). Increase of enkephalin and decrease of substance P immunoreactivity in the dorsal and ventral striatum of the rat after midbrain 6-hydroxydopamine lesions. *Brain Research, 412*(2), 391–396.

Voorn, P., Docter, G. J., Jongen-Relo, A. L., & Jonker, A. J. (1994). Rostrocaudal subregional differences in the response of enkephalin, dynorphin and substance P synthesis in rat nucleus accumbens to dopamine depletion. *The European Journal of Neuroscience, 6*(3), 486–496.

Vossler, M. R., Yao, H., York, R. D., Pan, M. G., Rim, C. S., & Stork, P. J. (1997). cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell, 89*(1), 73–82.

Walker, P. D., Riley, L. A., Hart, R. P., & Jonakait, G. M. (1991). Serotonin regulation of neostriatal tachykinins following neonatal 6-hydroxydopamine lesions. *Brain Research, 557*(1–2), 31–36.

Walters, J. R., Bergstrom, D. A., Carlson, J. H., Chase, T. N., & Braun, A. R. (1987). D_1 dopamine receptor activation required for postsynaptic expression of D_2 agonist effects. *Science, 236*(4802), 719–722.

Wang, Y., Xu, R., Sasaoka, T., Tonegawa, S., Kung, M. P., & Sankoorikal, E. B. (2000). Dopamine D_2 long receptor-deficient mice display alterations in striatum-dependent functions. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 20*(22), 8305–8314.

Wang, H. B., Laverghetta, A. V., Foehring, R., Deng, Y. P., Sun, Z., Yamamoto, K., Lei, W. L., Jiao, Y., & Reiner, A. (2006). Single-cell RT-PCR, in situ hybridization histochemical, and immunohistochemical studies of substance P and enkephalin co-occurrence in striatal projection neurons in rats. *Journal of Chemical Neuroanatomy, 31*(3), 178–199.

Wang, H. B., Deng, Y. P., & Reiner, A. (2007). In situ hybridization histochemical and immunohistochemical evidence that striatal projection neurons co-containing substance P and enkephalin are overrepresented in the striosomal compartment of striatum in rats. *Neuroscience Letters, 425*(3), 195–199.

Watts, R. W., Spellacy, E., Gibbs, D. A., Allsop, J., McKeran, R. O., & Slavin, G. E. (1982). Clinical, post-mortem, biochemical and therapeutic observations on the Lesch-Nyhan syndrome with particular reference to the Neurological manifestations. *The Quarterly Journal of Medicine, 51*(201), 43–78.

Weihmuller, F. B., & Bruno, J. P. (1989a). Age-dependent plasticity in the dopaminergic control of sensorimotor development. *Behavioural Brain Research, 35*(2), 95–109.

Weihmuller, F. B., & Bruno, J. P. (1989b). Drinking behavior and motor function in rat pups depleted of brain dopamine during development. *Developmental Psychobiology, 22*(2), 101–113.

Westin, J. E., Vercammen, L., Strome, E. M., Konradi, C., & Cenci, M. A. (2007). Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D_1 receptors. *Biological Psychiatry, 62*(7), 800–810.

Whishaw, I. Q., Funk, D. R., Hawryluk, S. J., & Karbashewski, E. D. (1987). Absence of sparing of spatial navigation, skilled forelimb and tongue use and limb posture in the rat after neonatal dopamine depletion. *Physiology and Behavior, 40*(2), 247–253.

Winkler, C., Bentlage, C., Nikkhah, G., Samii, M., & Bjorklund, A. (1999). Intranigral transplants of GABA-rich striatal tissue induce behavioral recovery in the rat Parkinson model and promote the effects obtained by intrastriatal dopaminergic transplants. *Experimental Neurology, 155*(2), 165–186.

Wolf, M. E. (1998). The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Progress in Neurobiology, 54*(6), 679–720.

Wolf, M. E., Zigmond, M. J., & Kapatos, G. (1989). Tyrosine hydroxylase content of residual striatal dopamine nerve terminals following 6-hydroxydopamine administration: A flow cytometric study. *Journal of Neurochemistry, 53*(3), 879–885.

Wong, D. F., Harris, J. C., Naidu, S., Yokoi, F., Marenco, S., Dannals, R. F., Ravert, H. T., Yaster, M., Evans, A., Rousset, O., Bryan, R. N., Gjedde, A., Kuhar, M. J., & Breese, G. R. (1996). Dopamine transporters are markedly reduced in Lesch-Nyhan disease in vivo. *Proceedings of the National Academy of Sciences of the United States of America, 93*(11), 5539–5543.

Woodlee, M. T., Kane, J. R., Chang, J., Cormack, L. K., & Schallert, T. (2008). Enhanced function in the good forelimb of hemi-parkinson rats: Compensatory adaptation for contralateral postural instability? *Experimental Neurology, 211*(2), 511–517.

Yao, H., York, R. D., Misra-Press, A., Carr, D. W., & Stork, P. J. (1998). The cyclic adenosine monophosphate-dependent protein kinase (PKA) is required for the sustained activation of mitogen-activated kinases and gene expression by nerve growth factor. *The Journal of Biological Chemistry, 273*(14), 8240–8247.

York, R. D., Yao, H., Dillon, T., Ellig, C. L., Eckert, S. P., McCleskey, E. W., & Stork, P. J. (1998). Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. *Nature, 392*(6676), 622–626.

Yoshida, H., Ohno, M., & Watanabe, S. (1995). Roles of dopamine D_1 receptors in striatal fos protein induction associated with methamphetamine behavioral sensitization in rats. *Brain Research Bulletin, 38*(4), 393–397.

Young, W. S., 3rd, Bonner, T. I., & Brann, M. R. (1986). Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. *Proceedings of the National Academy of Sciences of the United States of America, 83*(24), 9827–9831.

Zeng, B. Y., Jolkkonen, J., Jenner, P., & Marsden, C. D. (1995). Chronic L-DOPA treatment differentially regulates gene expression of glutamate decarboxylase, preproenkephalin and preprotachykinin in the striatum of 6-hydroxydopamine-lesioned rat. *Neuroscience, 66*(1), 19–28.

Zhang, K., Tarazi, F. I., & Baldessarini, R. J. (2001). Role of dopamine D(4) receptors in motor hyperactivity induced by neonatal 6-hydroxydopamine lesions in rats. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 25*(5), 624–632.

Zhang, K., Davids, E., Tarazi, F. I., & Baldessarini, R. J. (2002a). Effects of dopamine D_4 receptor-selective antagonists on motor hyperactivity in rats with neonatal 6-hydroxydopamine lesions. *Psychopharmacology, 161*(1), 100–106.

Zhang, K., Tarazi, F. I., Davids, E., & Baldessarini, R. J. (2002b). Plasticity of dopamine D_4 receptors in rat forebrain: Temporal association with motor hyperactivity following neonatal 6-hydroxydopamine lesioning. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 26*(5), 625–633.

Zhang, X., Andren, P. E., & Svenningsson, P. (2007). Changes on 5-HT_2 receptor mRNAs in striatum and subthalamic nucleus in Parkinson's disease model. *Physiology and Behavior, 92*(1–2), 29–33.

Zhou, F. C., Bledsoe, S., & Murphy, J. (1991). Serotonergic sprouting is induced by dopamine-lesion in substantia nigra of adult rat brain. *Brain Research, 556*(1), 108–116.

Zigmond, M. J. (1997). Do compensatory processes underlie the preclinical phase of neurodegenerative disease? Insights from an animal model of parkinsonism. *Neurobiology of Disease, 4*(3–4), 247–253.

Zigmond, M. J., & Stricker, E. M. (1972). Deficits in feeding behavior after intraventricular injection of 6-hydroxydopamine in rats. *Science, 177*(4055), 1211–1214.

Zigmond, M. J., & Stricker, E. M. (1973). Recovery of feeding and drinking by rats after intraventricular 6-hydroxydopamine or lateral hypothalamic lesions. *Science, 182*(4113), 717–720.

Zigmond, M. J., & Stricker, E. M. (1980). Supersensitivity after intraventricular 6-hydroxydopamine: Relation to dopamine depletion. *Experientia, 36*(4), 436–438.

Zigmond, M. J., & Stricker, E. M. (1984). Parkinson's disease: Studies with an animal model. *Life Sciences, 35*(1), 5–18.

Zigmond, M. J., Acheson, A. L., Stachowiak, M. K., & Stricker, E. M. (1984). Neurochemical compensation after nigrostriatal bundle injury in an animal model of preclinical parkinsonism. *Archives of Neurology, 41*(8), 856–861.

Zis, A. P., Fibiger, H. C., & Phillips, A. G. (1974). Reversal by L-dopa of impaired learning due to destruction of the dopaminergic nigro-neostriatal projection. *Science, 185*(4155), 960–962.

Zuch, C. L., Nordstroem, V. K., Briedrick, L. A., Hoernig, G. R., Granholm, A. C., & Bickford, P. C. (2000). Time course of degenerative alterations in nigral dopaminergic neurons following a 6-hydroxydopamine lesion. *The Journal of Comparative Neurology, 427*(3), 440–454.

Dopamine and L-dopa as Selective Endogenous Neurotoxins

Juan Segura-Aguilar, Ulises Ahumada-Castro, and Irmgard Paris

Contents

Abstract

Selective neurotoxins have the ability to exert their neurotoxic effects in some specific neuronal systems. In dopaminergic neurons, the selectivity of exogenous neurotoxins depends on their affinity to the dopamine transporter. However, dopamine and 3,4-L-dihydroxyphenylalanine (L-dopa) are synthesized in dopaminergic neurons and are likewise able to induce neurotoxicity. The possible molecular mechanisms involved in dopamine and L-dopa neurotoxicity in dopaminergic neurons are discussed. Dopamine seems to be neurotoxic in dopaminergic neurons by undergoing oxidation to aminochrome, which is the precursor to neuromelanin. However,

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aminochrome can be neurotoxic when it forms adducts with proteins such as alpha-synuclein, parkin, mitochondrial complexes I and III, actin, tubulin, and the dopamine transporter, or when aminochrome is one-electron reduced by flavoenzymes that use NADH, generating redox cycling with the concomitant depletion of energy and the formation of reactive oxygen species. L-dopa is also neurotoxic in cell cultures after oxidizing to a quinone species, but L-dopa seems to be a transient precursor of dopamine in that it is not able to induce neurotoxicity in vivo due to the efficient decarboxylation to dopamine catalyzed by amino acid decarboxylase. In fact, the only metabolite found in vivo is L-3-*o*-methyldopa, as detected in microdialysis experiments in animals treated with L-dopa. L-dopa is used in Parkinson's disease treatment, and it is still questionable whether L-dopa accelerates the degeneration of remaining dopaminergic neurons. It seems that L-dopa itself does not accelerate dopaminergic neuron degeneration because L-dopa is efficiently converted to dopamine, both in the peripheral and the central nervous systems. However, L-dopa induces dyskinesias in approximately 40 % patients with 4–6 years of treatment, and although the mechanism for L-dopa-induced dyskinesias is very complex, the rapid oscillation of striatal dopamine during L-dopa treatment has been found to be required for the induction of dyskinesias. The remaining dopaminergic neurons convert L-dopa to dopamine and release dopamine to the striatum under regulated conditions, but the majority of dopamine release to the striatum is mediated by serotonergic neurons without regulation, resulting in dyskinesias.

Keywords

Aminochrome • Dopamine • Dyskinesia • L-dopa • Metabolism • Neurotoxicity • Neurotoxins • Orthoquinones • Oxidation • Parkinson's disease

List of Abbreviations

AADC	Aromatic amino acid decarboxylase
COMT	Catechol ortho-methyltransferase
DA	Dopamine
GST M2-2	Glutathione S-transferase M2-2
L-dopa	L-dihydroxyphenylalanine
MAO	Monoamine oxidases
TH	Tyrosine hydroxylase
VMAT-2	Vesicular monoaminergic transporter-2

1 Synthesis of L-dopa and Dopamine

De novo synthesis of dopamine is mediated by two enzymes using the amino acid tyrosine as precursor. The first step is catalyzed by the enzyme tyrosine hydroxylase (TH), in which tyrosine is used to form L-dihydroxyphenylalanine (L-dopa). L-dopa is decarboxylated by the enzyme aromatic amino acid decarboxylase (AADC) to generate dopamine and CO_2. This dopamine is stored in monoaminergic

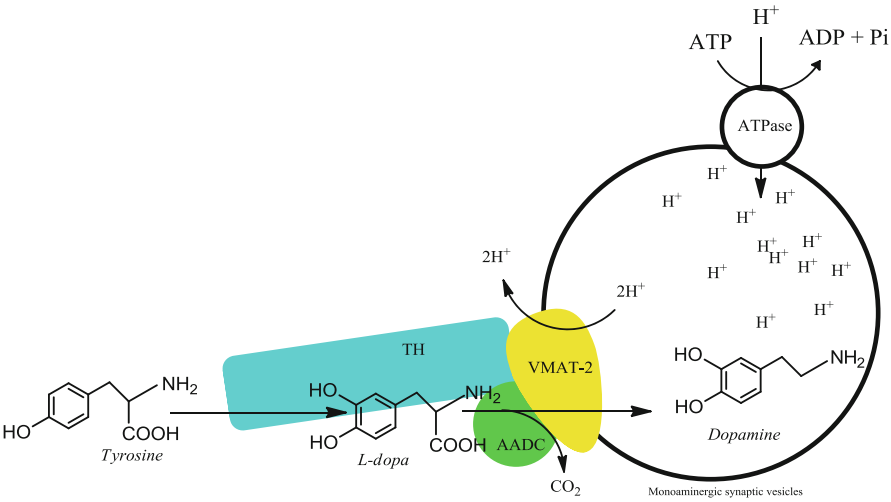
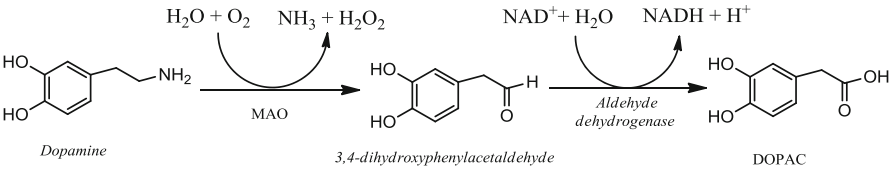


Fig. 1 *Dopamine synthesis*. Dopamine synthesis from tyrosine is catalyzed by tyrosine hydroxylase (TH) and aromatic amino acid decarboxylase (AADC), and uptake into monoaminergic synaptic vesicles is mediated by VMAT-2

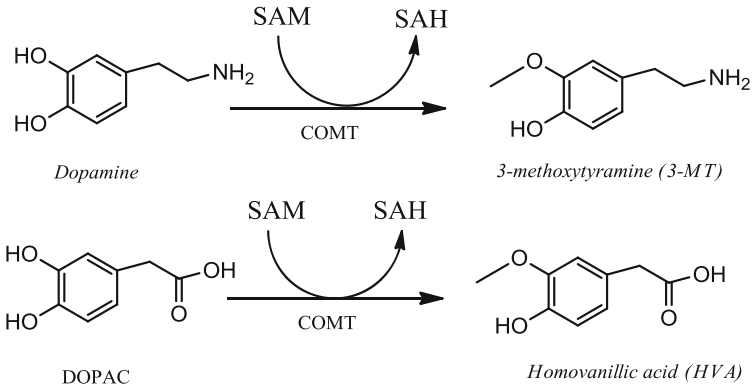
vesicles with a weak acid pH that prevents dopamine oxidation to *o*-quinone species, as dopamine autoxidizes at physiological pH due to the dissociation of the protons of hydroxyl groups. Dopamine oxidation to *o*-quinones after the synthesis of L-dopa and dopamine is prevented by the association of the enzymes TH and AADC with the vesicular monoaminergic transporter-2 (VMAT-2), which is present in the membranes of monoaminergic synaptic vesicles, generating a kind of complex that prevents free cytosolic dopamine (Cartier et al. 2010). Dopamine inside monoaminergic synaptic vesicles is hard protonated, preventing the oxidation of catechol groups to *o*-quinone. Monoaminergic synaptic vesicles express a vesicular ATPase that hydrolyzes ATP to ADP plus inorganic phosphate with one proton (H^+) translocation into the vesicles, creating a proton gradient with a weak acid pH inside the vesicles (Guillot and Miller 2009; Fig. 1).

1.1 Dopamine Degradation

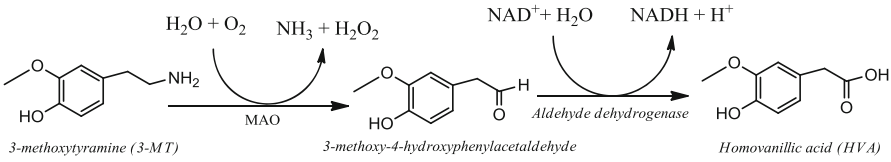
Dopamine accumulates inside monoaminergic synaptic vesicles for neurotransmission, which is an essential process in the control of movements. However, the catechol group of free cytosolic dopamine can be oxidized to neurotoxic *o*-quinones (Arriagada et al. 2004; Paris et al. 2010, 2011). To prevent this effect, dopamine participates in a neurotoxic reaction, and there are two types of enzymes that degrade dopamine. The first is the monoamine oxidases (MAO, E.C. 1.4.3.4), which catalyze the oxidative deamination of the dopamine amino group to 3,4-dihydroxyphenylacetaldehyde with concomitant formation of ammonia and hydrogen peroxide. Aldehyde dehydrogenase then catalyzes the oxidation of 3,4-dihydroxyphenylacetaldehyde to 3,4-dihydroxyphenylacetic acid (DOPAC) with the formation of a molecule of NADH.



The MAO enzymes have multiple isoforms (A and B forms) that are 70 % identical and are localized to the outer membranes of the mitochondria in neurons, glial cells, and other cells (Weyler et al. 1990; Shih et al. 1997). MAO-B is found in histaminergic and serotonergic neurons, as well as in astrocytes, while MAO-A is mainly found in catecholaminergic neurons (Westlund et al. 1988; Saura et al. 1994). MAO-A uses the compounds dopamine, noradrenaline, adrenaline, and serotonin as substrates, while MAO-B uses compounds such as phenylethylamine and tyramine (Strolin-Benedetti et al. 1992). The second enzyme that degrades dopamine is catechol ortho-methyltransferase (COMT; EC 2.1.1.6), which catalyzes the methylation of dopamine by using S-adenosylmethionine (SAM) as a cofactor to form 3-methoxytyramine and S-adenosylhomocysteine (SAH). COMT also plays a role in MAO-catalyzed degradation of dopamine, as DOPAC is methylated to homovanillic acid (HVA).



MAO also participates in dopamine degradation catalyzed by COMT because 3-methoxytyramine is a substrate for MAO, which catalyzes the oxidative deamination of 3-methoxytyramine to 3-methoxy-4-hydroxyphenylacetaldehyde. Aldehyde dehydrogenase catalyzes the oxidation of 3-methoxy-4-hydroxyphenylacetaldehyde to homovanillic acid with the concomitant formation of NADH.



COMT is expressed in pyramidal neurons, striatal spiny neurons, cerebellar Purkinje, and granular cells (Myöhänen et al. 2010), and it has two isoforms – a soluble form (S-COMT) and a membrane-bound form (MB-COMT). Inhibition of COMT by entacapone has been used in the treatment of Parkinson's disease, prolonging the half-life of L-dopa (Marin and Obeso 2010).

2 Dopamine Neurotoxicity

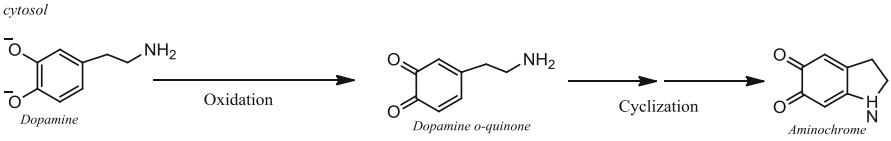
Dopamine exposure at different cell cultures or cell lines resulted in the induction of cell death, suggesting a neurotoxic role for dopamine (Hoyt et al. 1997; Jeon et al. 2010). Dopamine-dependent cell death has been reported to be dependent on an apoptotic cell death mechanism (Ziv et al. 2001). Dopamine exposure of human neuroblastoma NMB cells, which are able to take up dopamine, induces morphological changes, such as cell shrinkage, apoptosis-like atrophy, accumulation of apoptotic particles, DNA fragmentation, and protein synthesis-dependent cell death (Simantov et al. 1996). A study conducted with SH-SY5Y neuroblastoma cells displayed caspase-9 and caspase-3 activation, cleavage of poly(ADP-ribose) polymerase, and nuclear condensation, accompanied by the activation of p38 mitogen-activated protein kinase. The presence of the antioxidant N-acetyl-L-cysteine prevents dopamine-induced p38 kinase activation, caspase-9 and caspase-3 cleavage, and subsequent apoptosis (Junn and Mouradian 2001). Overexpression of Bcl-2, which blocks physiological apoptosis, in PC-12 cells results in a marked resistance to dopamine-induced cell death (Offen et al. 1997). The activation of the JNK pathway precedes dopamine-induced apoptosis and is persistently sustained during the process of apoptosis. Overexpression of a dominant negative mutant SEK1, an upstream kinase of JNK, inhibits both dopamine-induced JNK activation and apoptosis (Luo et al. 1998). Another study reported that dopamine activates SAPK/JNK and p38, but not MEK or ERK/MAPK (Gómez-Santos et al. 2003). It has been reported that dopamine oxidation activates the DNA-binding activity of NF-kappaB and the suppression of NF-kappaB transcriptional activity in PC-12 cells, inducing apoptotic cell death during dopamine oxidation (Lee et al. 2001b). The formation of adducts between alpha-synuclein and the presynaptic human dopamine transporter facilitates the membrane clustering of the dopamine transporter (DAT), thereby accelerating cellular dopamine uptake and dopamine-induced cellular apoptosis (Lee et al. 2001a). Interestingly, parkin prevents dopamine-induced alpha-synuclein-dependent cell death by blocking DAT-mediated dopamine uptake, which is accelerated by alpha-synuclein (Moszczynska et al. 2007). Another study showed that parkin protects against dopamine toxicity by decreasing oxidative stress and the subsequent activation of the JNK/caspase pathway (Jiang et al. 2004). The apoptosis induced by dopamine in PC-12 cells was found to be accompanied by an impairment of mitochondrial bioenergetic functions (Jana et al. 2011). The treatment of human SH-SY5Y cells with dopamine induced an early increase in the expression of hypoxia-inducible factor-1alpha (HIF-1alpha) followed by increases in p53, Puma, and Bnip3, in

which caspase-3, caspase-7, and PARP were activated after 12 h (Giménez-Xavier et al. 2009). The agonist of protein kinase A, forskolin, stimulated dopamine uptake in SK-N-SH cells and blocked dopamine-induced apoptosis (Liu et al. 2001). Dopamine-induced cell death was found to be preceded by a decrease in proteasome activity (Keller et al. 2000). Antioxidants were found to protect against dopamine-induced cell death (Junn and Mouradian 2001; Jana et al. 2011), and the glutathione-depleting compound L-buthionine sulfoximine enhanced dopamine-induced cell death (Stokes et al. 2000).

Why does dopamine induce neurotoxicity when it is an essential neurotransmitter for the control of movement? A possible explanation is that dopamine inside monoaminergic synaptic vesicles is completely inert because the protons of dopamine groups are hardbound to the oxygen as a consequence of the weak acid pH inside the vesicles, which is 2–2.4 pH units lower than the pH in the cytosol (Guillot and Miller 2009). However, the protons of dopamine hydroxyl groups are dissociated at cytosolic pH and can oxidize in the presence of oxygen, even in the absence of metal catalysis (Linert et al. 1996). The dopamine catechol structure oxidizes to orthoquinones, which can induce neurotoxicity.

3 Dopamine Oxidation to Orthoquinones

The protons of dopamine hydroxyl groups are dissociated when dopamine is in the cytosol at physiological pH and spontaneously oxidizes in the presence of oxygen and in the absence of metal traces (Linert et al. 1996). Dopamine oxidizes to dopamine *o*-quinone, which cyclizes to form aminochrome at physiological pH. Dopamine *o*-quinone is not stable at physiological pH because the amino group of dopamine *o*-quinone spontaneously rearranges and undergoes cyclization; thus, dopamine *o*-quinone is only stable at a pH below 2.0 (Segura-Aguilar and Lind 1989).



Dopamine oxidation can also be catalyzed by metals such as manganese(III), copper sulfate(II), iron chloride(III), and sodium periodate (Segura-Aguilar and Lind 1989; Paris et al. 2001, 2005a; Graham et al. 1978) or by enzymes such as prostaglandin H synthase, cytochrome P450, xanthine oxidase, lactoperoxidase, tyrosinase, and dopamine β-monooxygenase (Galzigna et al. 2000; Thompson et al. 2000; Segura-Aguilar 1996; Foppoli et al. 1997; Hastings 1995; Segura-Aguilar et al. 1998; Jimenez et al. 1984). It is important to remember that aminochrome formation is dependent on the presence of free cytosolic dopamine that can oxidize to aminochrome, and VMAT-2 and MAO prevent dopamine oxidation, as VMAT-2 mediates dopamine accumulation in monoaminergic synaptic vesicles and MAO catalyzes the degradation of dopamine.

4 Aminochrome Metabolism

Aminochrome is able to participate in five different reactions, including (i) the polymerization of aminochrome to neuromelanin. Neuromelanin is a pigment present in dopaminergic neurons, which is localized in the substantia nigra and accumulates with age (Zecca et al. 2002) in healthy individuals; it is also dramatically decreased in patients with Parkinson's disease. Therefore, neuromelanin seems to play a protective role in dopaminergic neurons because it chelates metals and binds proteins such as alpha-synuclein (Gerlach et al. 2003; Hong and Simon 2007; Fasano et al. 2006). The localization of neuromelanin in double-membrane vesicles seems to be very important for its protective role, as free neuromelanin has been found to be neurotoxic in cell cultures (Naoi et al. 2008) and extracellular neuromelanin induces microglial activation in the substantia nigra (Zhang et al. 2011; Fig. 2). (ii) Aminochrome also forms adducts with proteins. Aminochrome induces and stabilizes the formation of neurotoxic protofibrils of alpha-synuclein (Conway et al. 2001; Norris et al. 2005). Aminochrome forms adducts with other proteins, such as actin and α- and β-tubulin, and it disrupts the architecture of the cytoskeleton and complexes I and III of the mitochondria (Paris et al. 2010; Van Laar et al. 2009). Dopamine *o*-quinone, the transient precursor of aminochrome at physiological pH, forms adducts with and inactivates parkin, which is a ubiquitin ligase of the proteasomal system (LaVoie et al. 2005); tyrosine hydroxylase; the human dopamine transporter; and tryptophan hydroxylase (Xu et al. 1998; Whitehead et al. 2001; Kuhn and Arthur 1998). (iii) Aminochrome can be one-electron reduced, catalyzed by flavoenzymes that transfer one electron by using NADH or NADPH ions. The leukoaminochrome *o*-semiquinone radical is extremely reactive under aerobic conditions, generating a redox cycle between the leukoaminochrome *o*-semiquinone radical and aminochrome (Baez et al. 1995; Segura-Aguilar et al. 1998). This redox cycling depletes the NADH and O_2 required for ATP production in the mitochondria or the NADPH required for the reduction of oxidized glutathione, which is an important antioxidant. There is significant evidence that supports the neurotoxic role of the one-electron reduction of aminochrome (Paris et al. 2001, 2005a, b, 2009, 2010, 2011; Arriagada et al. 2004; Fuentes et al. 2007; Díaz-Véliz et al. 2008; Muñoz et al. 2012a, b; Fig. 2). (iv) Aminochrome can be two-electron reduced to leukoaminochrome, catalyzed by DT-diaphorase (EC.1.6.99.2), which is a flavoenzyme that uses both NADH and NADPH as electron donors. DT-diaphorase has been proposed to play a protective role in aminochrome metabolism because this enzyme prevents the neurotoxic reactions of aminochrome, including the one-electron reduction of aminochrome and the formation of adducts with proteins (Arriagada et al. 2004; Lozano et al. 2010; Paris et al. 2011; Muñoz et al. 2012a, b; Segura-Aguilar et al. 2006; Cardenas et al. 2008; Paris et al. 2010; Fig. 2). (v) Aminochrome can be glutathione-conjugated by glutathione S-transferase M2-2 (GST M2-2) to 4-*S*-glutathionyl-5,6-dihydroxyindoline, which is a stable molecule that is resistant to biological oxidizing agents (Segura-Aguilar, et al. 1997; Baez et al. 1997). GST M2-2 also conjugates the precursor of aminochrome dopamine *o*-quinone to 5-glutathionyl-dopamine, preventing the

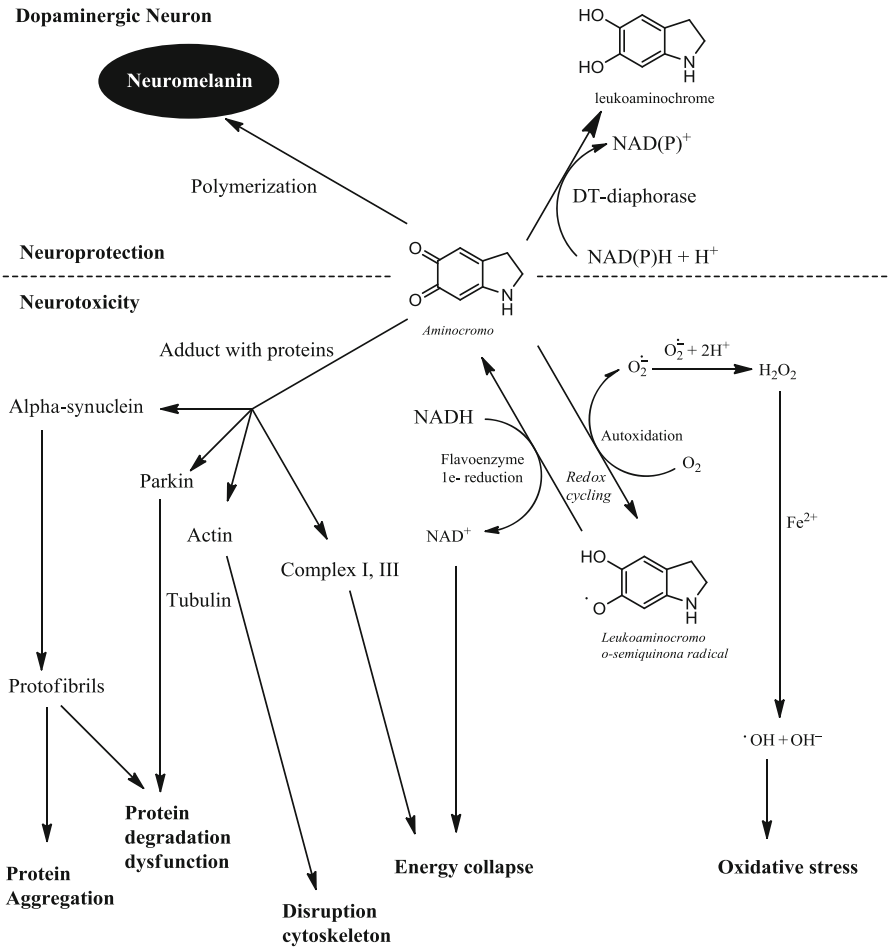


Fig. 2 *Possible metabolism of aminochrome in dopaminergic neurons*. Aminochrome is able to participate in two neurotoxic reactions, such as the formation of aminochrome adducts with alpha-synuclein, parkin, actin, tubulin, and mitochondrial complexes I and III, along with aminochrome one-electron reduction to the leukoaminochrome *o*-semiquinone radical. However, aminochrome can polymerize into neuromelanin, or it can be two-electron reduced to leukoaminochrome, catalyzed by DT-diaphorase, a reaction that prevents aminochrome-induced neurotoxicity

formation of aminochrome (Dagnino-Subiabre et al. 2000). The 5-glutathionyl-dopamine is finally converted to 5-cysteinyl dopamine (Shen et al. 1996), which has been detected in the cerebrospinal fluid of Parkinson's disease patients and in dopamine-rich brain regions such as the caudate nucleus, putamen, globus pallidus, and substantia nigra, as well as in neuromelanin (Cheng et al. 1996; Rosengren et al. 1985; Carstam et al. 1991). Therefore, the conjugation of glutathione has been proposed to be a protective reaction against aminochrome neurotoxicity in astrocytes (Fig. 3).

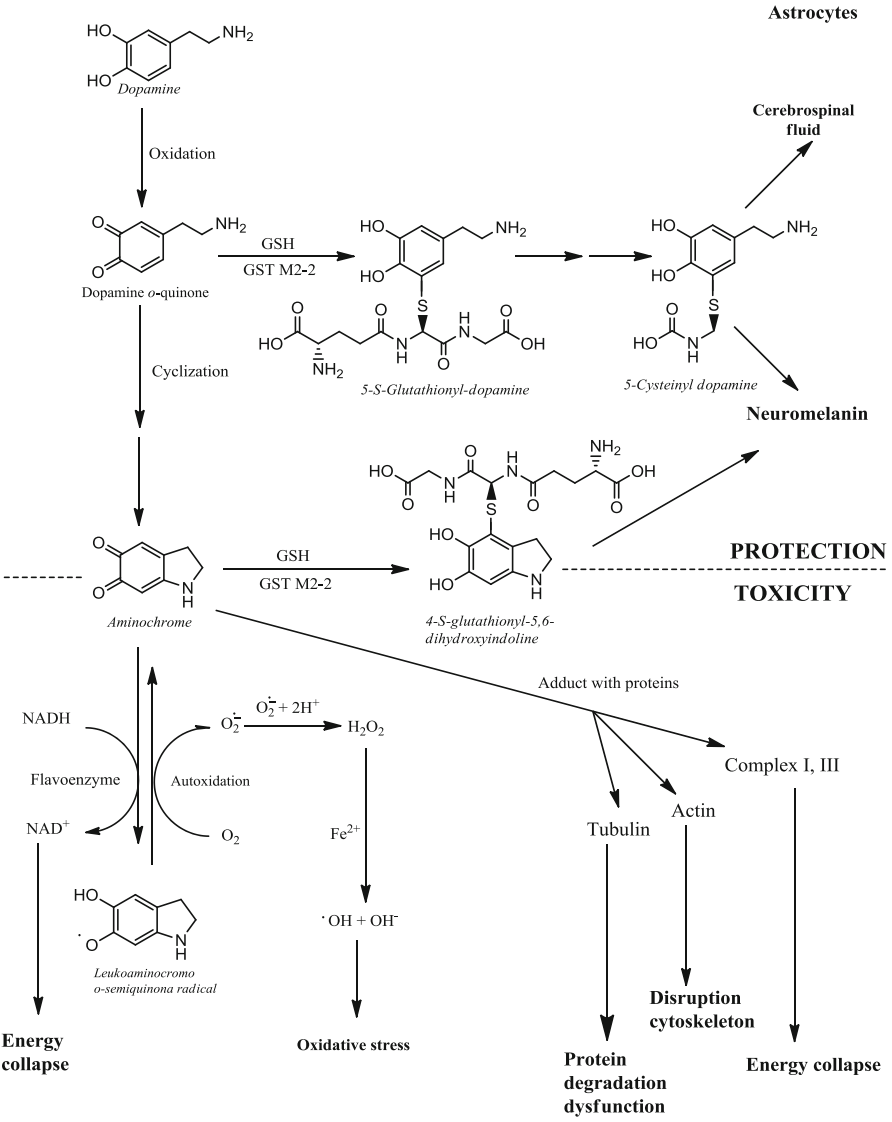


Fig. 3 *Possible metabolism of aminochrome in astrocytes*. Dopamine is taken up into astrocytes and is able to oxidize into dopamine *o*-quinone. GST M2-2 is able to conjugate this compound with glutathione to form 5-glutathionyl-dopamine, which is degraded to 5-S-cysteinyl dopamine, a compound that is found in the cerebrospinal fluid and in neuromelanin. Dopamine o-quinone cyclizes to aminochrome, which can also be conjugated with GSH by GST M2-2 to 4-S-glutathionyl-5,6-dihydroxyindoline, which is resistant to biological oxidizing agents. Both conjugations prevent aminochrome-induced neurotoxicity

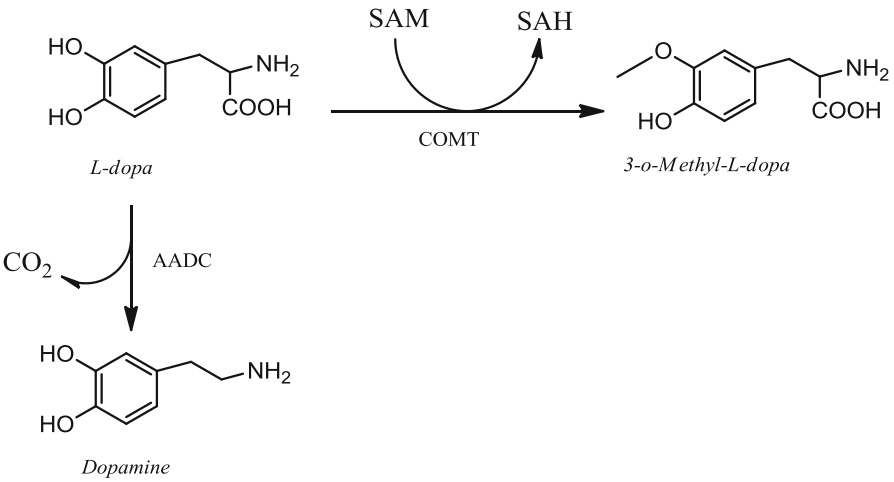
5 Aminochrome and Parkinson's Disease

The progressive loss of nigral dopaminergic neurons containing neuromelanin in the substantia nigra most likely begins long before the symptomatic phases of Parkinson's disease are present (Braak et al. 2004). Intensive research has been conducted over the past few decades to understand the molecular mechanism of the neurodegeneration of neuromelanin-containing dopaminergic neurons, with the discovery of mutations associated with Parkinson's disease. It is generally accepted that the mechanism that results in the loss of dopaminergic neurons in the substantia nigra involves the aggregation of alpha-synuclein to neurotoxic protofibrils, a dysfunction of protein degradation, mitochondrial dysfunction, oxidative stress, and neuroinflammation (Schapira 2011; Conway et al. 2001; McNaught et al. 2004; Cuervo et al. 2010; Schapira and Jenner 2011). There is growing evidence that supports the role of aminochrome in the degeneration of dopaminergic neurons that contain neuromelanin, including the following findings: (i) Aminochrome forms adducts and inactivates complexes I and III and isocitrate dehydrogenase, an enzyme of the citric acid cycle in the mitochondria (Van Laar et al. 2009). One-electron reduction of aminochrome depletes NADH, thereby decreasing ATP production in cell culture (Muñoz et al. 2012b). (ii) Aminochrome was found to induce the formation and stabilization of alpha-synuclein protofibrils (Norris et al. 2005). (iii) Aminochrome inactivates the proteasomal system of degradation of proteins by forming adducts with parkin (Zafar et al. 2006; La Voie et al. 2005). Interestingly, alpha-synuclein protofibrils inhibit chaperone-mediated autophagy (Xilouri et al. 2009) and the 26S proteasomal system (Zhang et al. 2008). Aminochrome forms adducts with tubulin (Van Laar et al. 2009), and one-electron reduction of aminochrome induces the aggregation of α- and β-tubulin, disrupting the cytoskeletal structure (Paris et al. 2010; Muñoz et al. 2012a). Tubulin aggregation prevents the microtubule formation required for the fusion of autophagocytic vacuoles and lysosomes (Monastyrska et al. 2009). (iv) Aminochrome one-electron reduction induces oxidative stress (Arriagada et al. 2004). Interestingly, aminochrome is involved in four of the five mechanisms that are generally accepted to be involved in the degeneration of neuromelanin-containing dopaminergic neurons in Parkinson's disease.

6 L-dopa Metabolism

The amino acid tyrosine is used as a substrate in the production of L-dopa, a reaction catalyzed by tyrosine hydroxylase in dopaminergic neurons. However, L-dopa is immediately converted to dopamine in a reaction catalyzed by AADC. It has been reported that the enzymes TH and AADC are associated with the membrane-bound VMAT-2, forming a kind of complex to prevent L-dopa and dopamine from remaining free in the cytosol (Cartier et al. 2010). It is unknown whether free L-dopa in the cytosol is able to undergo metabolism under

normal conditions because in Parkinson's patients, L-dopa undergoes metabolism, an important part of treatment. L-dopa can be decarboxylated to dopamine even outside the central nervous system, and L-dopa can be converted to L-3-*o*-methyldopa by COMT (Okada et al. 2011).



7 L-dopa Neurotoxicity In Vitro

Experiments in vitro with cultured neurons exposed to L-dopa resulted in apoptosis that included cell shrinkage, membrane blebbing, and nuclear and DNA fragmentation (Melamed et al. 1998; Walkinshaw and Waters 1995). L-dopa was found to be neurotoxic when it increased the expression of cytosolic cytochrome c, cleaved caspase-3, and decreased phosphorylated Akt (Ser473), phosphorylated glycogen synthase kinase-3beta (GSK-3beta) (Ser9), and heat shock transcription factor-1. The activation of PI3K protects the cells against L-dopa neurotoxicity (Park et al. 2009). L-dopa has been reported to induce cell death in SH-SY-5Y cells with the concomitant deactivation of glutaredoxin. This compound was deactivated in a dose-dependent manner, suggesting an irreversible adduction of L-dopachrome to its nucleophilic active-site Cys-22. L-dopa also decreases thioredoxin and thioredoxin reductase activity and protein content. Knockdown of glutaredoxin increases L-dopa-induced apoptosis (Sabens et al. 2010). L-dopa activates apoptosis signaling kinase 1 (ASK1), as indicated by the phosphorylation of its downstream mitogen-activated protein kinases (MAPK), p38 and JNK. The inhibition of either p38 or JNK or the knockdown of ASK1 provides protection against L-dopa-induced apoptotic neuronal cell death in the SH-SY-5Y cell line (Liedhegner et al. 2011). L-dopa has also been found to impair proteasome activity through the D_1 dopamine receptor (Berthet et al. 2012). The ability of L-dopa to be neurotoxic is dependent on its ability to oxidize to L-dopa *o*-quinone, which

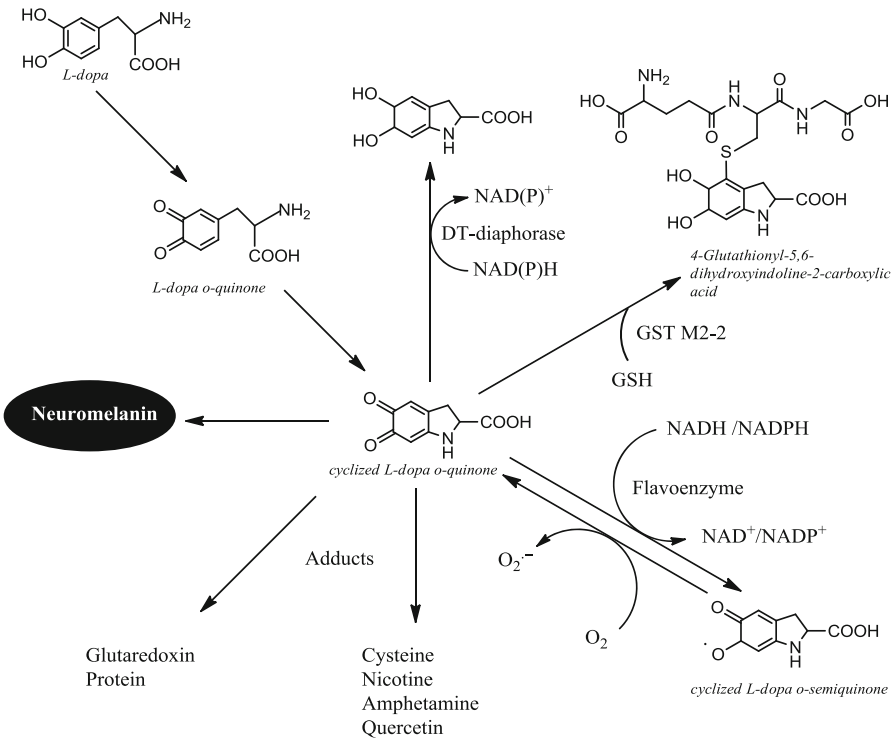


Fig. 4 *Possible metabolism of cyclized L-dopa o-quinone*. L-dopa oxidizes to L-dopa o-quinone, which cyclizes to cyclized L-dopa o-quinone. This compound is able to (i) polymerize to form neuromelanin, (ii) form adducts with proteins, (iii) form adducts with biomolecules, (iv) be one-electron reduced to the cyclized L-dopa o-semiquinone radical, (v) be conjugated by GSH to 4-glutathionyl-5,6-dihydroxyindoline-2-carboxylic acid, or (vi) be two-electron reduced, catalyzed by DT-diaphorase

spontaneously cyclizes to cyclized L-dopa *o*-quinone at physiological pH (Baez et al. 1994; Takeshima et al. 2011; Kostrzewa et al. 2002).

The cyclized L-dopa *o*-quinone is able to participate in several reactions, such as (i) polymerization to melanin (for review, see Prota 1995) and (ii) the formation of adducts with proteins such as glutaredoxin (Sabens et al. 2010). A significant increase in cell death and the formation of quinoproteins were observed when CATH.a cells were incubated with L-dopa (Asanuma et al. 2012). (iii) The formation of adducts with molecules such as cysteine, nicotine, amphetamine, and quercetin has also been reported (Dehn et al. 2001; Claffey and Ruth 2001; Kubo et al. 2007; Caudle et al. 2007; Müller and Muhlack 2012). (iv) L-dopa can be one-electron reduced with flavoenzymes that transfer one electron and use NADH or NADPH. (iv) L-dopa can also be two-electron reduced by DT-diaphorase (Baez et al. 1994), and (v) it can be conjugated by glutathione transferase M2-2 (Baez et al. 1997) (Fig. 4).

8 L-dopa-Induced Dyskinesia

L-dopa induces neurotoxicity in cell lines that are used as a model for in vitro studies of Parkinson's disease or primary cell cultures (Melamed et al. 1998; Walkinshaw and Waters 1995; Park et al. 2009; Sabens et al. 2010; Liedhegner et al. 2011). However, these neurotoxic effects in dopaminergic neurons are not observed in animal models of L-dopa, most likely as a consequence of the efficient conversion of L-dopa to dopamine. In fact, only one L-dopa metabolite is found in the literature (L-3-*o*-methyldopa), supporting the idea that the major route of metabolism of L-dopa is its conversion to dopamine and that under normal conditions, L-dopa is not found, free or accumulated. Although L-dopa does not induce neurotoxicity of dopaminergic neurons, as observed in cell cultures, L-dopa induces dyskinesia in approximately 40 % patients with 4–6 years of treatment (Ahlskog & Muenter 2001). The mechanism for L-dopa-induced dyskinesias is very complex, but a role for serotonergic neurons has been proposed. The rapid oscillation of striatal dopamine during L-dopa treatment has been found to be required for the induction of dyskinesias (Meissner et al. 2006; Pavese et al. 2006). In L-dopa treatment of a Parkinson's disease patient, the dopaminergic neurons that are still functioning convert L-dopa to dopamine, which is stored in monoaminergic synaptic vesicles. Dopamine is released by the axon terminal, which is regulated by D_2 dopamine receptors and the dopamine transporter (DAT). However, serotonergic neurons express both AADC and VMAT-2 and release dopamine, but without the regulation of the D_2 receptor and DAT (for review, see Cheshire and Williams 2012). Studies in animals with experimental Parkinsonism showed that serotonergic neurons are responsible for 80 % of dopamine release during L-dopa treatment (Tanaka et al. 1999)

9 Dopamine and L-dopa as Selective Neurotoxins

Dopamine is an essential neurotransmitter in dopaminergic neurons, accumulating inside monoaminergic synaptic vesicles for use as a neurotransmitter. Dopamine released into the intersynaptic space is recaptured by dopaminergic neurons with DAT, whereas cytosol dopamine can be (i) accumulated into monoaminergic synaptic vesicles mediated by VAMT-2, (ii) degraded by MAO and COMT, or (iii) autoxidized into aminochrome. However, dopamine induces neurotoxicity when undergoing oxidation to aminochrome, which can be neurotoxic by (i) forming adducts with proteins, such as alpha-synuclein, parkin, mitochondrial complexes I and III, isocitrate dehydrogenase, actin, tubulin, or the dopamine transporter or by (ii) being one-electron reduced by flavoenzymes that use NADH or NADPH as an electron donor. It was proposed that aminochrome is the endogenous neurotoxin that is responsible for the neurodegenerative process of neuromelanin-containing dopaminergic neurons (Lozano et al. 2010; Paris et al. 2010, 2011; Muñoz et al. 2012 a, b). Aminochrome is a selective neurotoxin, as it is formed inside a single dopaminergic neuron. Aminochrome can induce

neurotoxicity, resulting in a focalized neurotoxic event, which is in line with the slow neurodegeneration that occurs in neuromelanin-containing dopaminergic neurons, which takes years.

Experiments with cell cultures demonstrate the potential neurotoxic effects of L-dopa, but depending on the cellular model used for these experiments, it is possible that a part or all of the L-dopa added to the cell cultures was decarboxylated to dopamine before it induced neurotoxicity. It seems plausible that L-dopa is only a transient precursor in the synthesis of dopamine because the synthesis of dopamine is performed in a type of complex with TH and AADC, which are associated with VMAT-2, localized in monoaminergic synaptic vesicles. This suggests that L-dopa is not free in the cytosol because free L-dopa is immediately converted to dopamine and transported into the vesicles (Cartier et al. 2010). In Parkinson's therapy, L-dopa also acts as dopamine, as the decarboxylation of L-dopa and the unregulated release of dopamine from serotonergic neurons occur in both the peripheral and central nervous system, a process that seems to be involved in L-dopa-induced dyskinesias (Cheshire and Williams 2012).

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References

Ahlskog, J. E., & Muenter, M. D. (2001). Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Movement Disorders, 16*, 448–458.

Arriagada, A., Paris, I., Matas MJ, S. d. l., Martinez-Alvarado, P., Cardenas, S., Castañeda, P., Graumann, R., Perez-Pastene, C., Olea-Azar, C., Couve, E., Herrero, M. T., Caviedes, P., & Segura-Aguilar, J. (2004). On the neurotoxicity of leukoaminochrome o-semiquinone radical derived of dopamine oxidation: Mitochondria damage, necrosis and hydroxyl radical formation. *Neurobiology of Disease, 16*, 468–477.

Asanuma, M., Miyazaki, I., Kikkawa, Y., Kimoto, N., Takeshima, M., Murakami, S., & Miyoshi, K. (2012). Cyclooxygenase-independent neuroprotective effects of aspirin against dopamine quinone-induced neurotoxicity. *Neurochemical Research, 37*, 1944–1951.

Baez, S., Linderson, Y., & Segura-Aguilar, J. (1994). Superoxide dismutase and catalase prevent the formation of reactive oxygen species during reduction of cyclized dopa ortho-quinone by DT-diaphorase. *Chemico-Biological Interactions, 93*, 103–116.

Baez, S., Linderson, Y., & Segura-Aguilar, J. (1995). Superoxide dismutase and catalase enhance autoxidation during one-electron reduction of aminochrome by NADPH-cytochrome P-450 reductase. *Biochemical and Molecular Medicine, 54*, 12–18.

Baez, S., Segura-Aguilar, J., Widersten, M., Johansson, A. S., & Mannervik, B. (1997). Glutathione transferases catalyse the detoxication of oxidized metabolites (o-quinones) of catecholamines and may serve as an antioxidant system preventing degenerative cellular processes. *The Biochemical Journal, 324*, 25–28.

Berthet, A., Bezard, E., Porras, G., Fasano, S., Barroso-Chinea, P., Dehay, B., Martinez, A., Thiolat, M. L., Nosten-Bertrand, M., Giros, B., Baufreton, J., Li, Q., Bloch, B., & Martin-Negrier, M. L. (2012). L-dopa impairs proteasome activity in parkinsonism through D1 dopamine receptor. *The Journal of Neuroscience, 32*, 681–691.

Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H., & Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell and Tissue Research, 318*, 121–134.

Cardenas, S. P., Perez-Pastene, C., Couve, E., & Segura-Aguilar, J. (2008). The DT-diaphorase prevents the aggregation of α-synuclein induced by aminochrome. *Neurotoxicity Research, 13*, 136.

Carstam, R., Brinck, C., Hindemith-Augustsson, A., Rorsman, H., & Rosengren, E. (1991). The neuromelanin of the human substantia nigra. *Biochimica et Biophysica Acta, 1097*, 152–160.

Cartier, E. A., Parra, L. A., Baust, T. B., Quiroz, M., Salazar, G., Faundez, V., Egaña, L., & Torres, G. E. (2010). A biochemical and functional protein complex involving dopamine synthesis and transport into synaptic vesicles. *The Journal of Biological Chemistry, 151*, 957–666.

Caudle, W. M., Richardson, J. R., Wang, M. Z., Taylor, T. N., Guillot, T. S., McCormack, A. L., Colebrooke, R. E., Di Monte, D. A., Emson, P. C., & Miller, G. W. (2007). Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *The Journal of Neuroscience, 27*, 8138–8148.

Cheng, F. C., Kuo, J. S., Chia, L. G., & Dryhurst, G. (1996). Elevated 5-S-cysteinyldopamine/homovanillic acid ratio and reduced homovanillic acid in cerebrospinal fluid: Possible markers for and potential insights into the pathoetiology of Parkinson's disease. *Journal of Neural Transmission, 103*, 433–446.

Cheshire, P. A., & Williams, D. R. (2012). Serotonergic involvement in levodopa-induced dyskinesias in Parkinson's disease. *Journal of Clinical Neuroscience, 19*, 343–348.

Claffey, D. J., & Ruth, J. A. (2001). Amphetamine adducts of melanin intermediates demonstrated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Chemical Research in Toxicology, 14*, 1339–1344.

Conway, K. A., Rochet, J. C., Bieganski, R. M., & Lansbury, P. T., Jr. (2001). Kinetic stabilization of the a-synuclein protofibril by a dopamine- a-synuclein adduct. *Science, 294*, 1346–1349.

Cuervo, A. M., Wong, E. S., & Martinez-Vicente, M. (2010). Protein degradation, aggregation, and misfolding. *Movement Disorders, 25*(Suppl 1), S49–S54.

Dagnino-Subiabre, A., Cassels, B. K., Baez, S., Johansson, A. S., Mannervik, B., & Segura-Aguilar, J. (2000). Glutathione transferase M2-2 catalyzes conjugation of dopamine and dopa *o*-quinones. *Biochemical and Biophysical Research Communications, 274*, 32–36.

Dehn, D. L., Claffey, D. J., Duncan, M. W., & Ruth, J. A. (2001). Nicotine and cotinine adducts of a melanin intermediate demonstrated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Chemical Research in Toxicology, 14*, 275–279.

Díaz-Véliz, G., Paris, I., Mora, S., Raisman-Vozari, R., & Segura-Aguilar, J. (2008). Copper neurotoxicity in rat substantia nigra and striatum is dependent on DT-diaphorase inhibition. *Chemical Research in Toxicology, 21*, 1180–1185.

Fasano, M., Bergamasco, B., & Lopiano, L. (2006). Is neuromelanin changed in Parkinson's disease? Investigations by magnetic spectroscopies. J. *Neural Transm, 113*, 769–774.

Foppoli, C., Coccia, R., Cini, C., & Rosei, M. A. (1997). Catecholamines oxidation by xanthine oxidase. *Biochimica et Biophysica Acta, 1334*, 200–206.

Fuentes, P., Paris, I., Nassif, M., Caviedes, P., & Segura-Aguilar, J. (2007). Inhibition of VMAT-2 and DT-diaphorase induce cell death in a substantia nigra-derived cell line–an experimental cell model for dopamine toxicity studies. *Chemical Research in Toxicology, 20*, 776–783.

Galzigna, L., De Iuliis, A., & Zanatta, L. (2000). Enzymatic dopamine peroxidation in substantia nigra of human brain. *Clinica Chimica Acta, 300*, 131–138.

Gerlach, M., Double, K. L., Ben-Shachar, D., Zecca, L., Youdim, M. B., & Riederer, P. (2003). Neuromelanin and its interaction with iron as a potential risk factor for dopaminergic neurodegeneration underlying Parkinson's disease. *Neurotoxicity Research, 5*, 35–44.

Giménez-Xavier, P., Francisco, R., Santidrián, A. F., Gil, J., & Ambrosio, S. (2009). Effects of dopamine on LC3-II activation as a marker of autophagy in a neuroblastoma cell model. *Neurotoxicology, 30*, 658–665.

Gómez-Santos, C., Ferrer, I., Santidrián, A. F., Barrachina, M., Gil, J., & Ambrosio, S. (2003). Dopamine induces autophagic cell death and alpha-synuclein increase in human neuroblastoma SH-SY5Y cells. *Journal of Neuroscience Research, 73*, 341–350.

Graham, D. G., Tiffany, S. M., Bell, W. R., Jr., & Gutknecht, W. F. (1978). Autoxidation versus covalent binding of Autoxidation versus covalent binding of quinones as the mechanism of

toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Molecular Pharmacology, 14*, 644–653.

Guillot, T. S., & Miller, G. W. (2009). Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons. *Molecular Neurobiology, 39*, 149–170.

Hastings, T. G. (1995). Enzymatic oxidation of dopamine: The role of prostaglandin H synthase. *Journal of Neurochemistry, 64*, 919–924.

Hong, L., & Simon, J. D. (2007). Current understanding of the binding sites, capacity, affinity, and biological significance of metals in melanin. *The Journal of Physical Chemistry. B, 111*, 7938–4797.

Hoyt, K. R., Reynolds, I. J., & Hastings, T. G. (1997). Mechanisms of dopamine-induced cell death in cultured rat forebrain neurons: Interactions with and differences from glutamate-induced cell death. *Experimental Neurology, 143*, 269–281.

Jana, S., Sinha, M., Chanda, D., Roy, T., Banerjee, K., Munshi, S., Patro, B. S., & Chakrabarti, S. (2011). Mitochondrial dysfunction mediated by quinone oxidation products of dopamine: Implications in dopamine cytotoxicity and pathogenesis of Parkinson's disease. *Biochimica et Biophysica Acta, 1812*, 663–673.

Jeon, S. M., Cheon, S. M., Bae, H. R., Kim, J. W., & Kim, S. U. (2010). Selective susceptibility of human dopaminergic neural stem cells to dopamine-induced apoptosis. *Experimental Neurobiology, 19*, 155–164.

Jiang, H., Ren, Y., Zhao, J., & Feng, J. (2004). Parkin protects human dopaminergic neuroblastoma cells against dopamine-induced apoptosis. *Human Molecular Genetics, 13*, 1745–1754. 15.

Jimenez, M., Garcia-Carmona, F., Garcia-Canovas, F., Iborra, J. L., Lozano, J. A., & Martinez, F. (1984). Chemical intermediates in dopamine oxidation by tyrosinase, and kinetic studies of the process. *Archives of Biochemistry and Biophysics, 235*, 438–448.

Junn, E., & Mouradian, M. M. (2001). Apoptotic signaling in dopamine-induced cell death: The role of oxidative stress, p38 mitogen-activated protein kinase, cytochrome c and caspases. *Journal of Neurochemistry, 78*, 374–383.

Keller, J. N., Huang, F. F., Dimayuga, E. R., & Maragos, W. F. (2000). Dopamine induces proteasome inhibition in neural PC12 cell line. *Free Radical Biology & Medicine, 29*, 1037–1042.

Kostrzewa, R. M., Kostrzewa, J. P., & Brus, R. (2002). Neuroprotective and neurotoxic roles of levodopa (L-dopa) in neurodegenerative disorders relating to Parkinson's disease. *Amino Acids, 23*, 57–63.

Kubo, I., Nitoda, T., & Nihei, K. (2007). Effects of quercetin on mushroom tyrosinase and B16-F10 melanoma cells. *Molecules, 12*, 1045–1056.

Kuhn, D. M., & Arthur, R., Jr. (1998). Dopamine inactivates tryptophan hydroxylase and forms a redox-cycling quinoprotein: Possible endogenous toxin to serotonin neurons. *The Journal of Neuroscience, 18*, 7111–7117.

LaVoie, M. J., Ostaszewski, B. L., Weihofen, A., Schlossmacher, M. G., & Selkoe, D. J. (2005). Dopamine covalently modifies and functionally inactivates parkin. *Nature Medicine, 11*, 1159–1161.

Lee, F. J., Liu, F., Pristupa, Z. B., & Niznik, H. B. (2001a). Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. *The FASEB Journal B, 15*, 916–926.

Lee, H. J., Kim, S. H., Kim, K. W., Um, J. H., Lee, H. W., Chung, B. S., & Kang, C. D. (2001b). Antiapoptotic role of NF-kappaB in the auto-oxidized dopamine-induced apoptosis of PC12 cells. *Journal of Neurochemistry, 76*, 602–609.

Liedhegner, E. A., Steller, K. M., & Mieyal, J. J. (2011). Levodopa activates apoptosis signaling kinase 1 (ASK1) and promotes apoptosis in a neuronal model: Implications for the treatment of Parkinson's disease. *Chemical Research in Toxicology, 24*, 1644–1652.

Linert, W., Herlinger, E., Jameson, R. F., Kienzl, E., Jellinger, K., & Youdim, M. B. (1996). Dopamine, 6-hydroxydopamine, iron, and dioxygen–their mutual interactions and possible

implication in the development of Parkinson's disease. *Biochimica et Biophysica Acta, 1316*, 160–168.

Liu, Z., Zhang, J., Fei, J., & Guo, L. (2001). A novel mechanism of dopamine neurotoxicity involving the peripheral extracellular and the plasma membrane dopamine transporter. *Neuroreport, 12*, 3293–3297.

Lozano, J., Muñoz, P., Nore, B. F., Ledoux, S., & Segura-Aguilar, J. (2010). Stable expression of short interfering RNA for DT-diaphorase induces neurotoxicity. *Chemical Research in Toxicology, 23*, 1492–1496.

Luo, Y., Umegaki, H., Wang, X., Abe, R., & Roth, G. S. (1998). Dopamine induces apoptosis through an oxidation-involved SAPK/JNK activation pathway. *The Journal of Biological Chemistry, 273*, 3756–3764.

Marin, C., & Obeso, J. A. (2010). Catechol-O-methyltransferase inhibitors in preclinical models as adjuncts of L-dopa treatment. *International Review of Neurobiology, 95*, 191–205.

McNaught, K. S., Perl, D. P., Brownell, A. L., & Olanow, C. W. (2004). Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Annals of Neurology, 56*, 149–162.

Meissner, W., Ravenscroft, P., Reese, R., Harnack, D., Morgenstern, R., Kupsch, A., Klitgaard, H., Bioulac, B., Gross, C. E., Bezard, E., & Boraud, T. (2006). Increased slow oscillatory activity in substantia nigra pars reticulata triggers abnormal involuntary movements in the 6-OHDA-lesioned rat in the presence of excessive extracellular striatal dopamine. *Neurobiol, 22*, 586–598. Epub 2006 Mar 10.

Melamed, E., Offen, D., Shirvan, A., Djaldetti, R., Barzilai, A., & Ziv, I. (1998). Levodopa toxicity and apoptosis. *Annals of Neurology, 44*, S149–S154.

Monastyrska, I., Rieter, E., Klionsky, D. J., & Reggiori, F. (2009). Multiple roles of the cytoskeleton in autophagy. *Biological Reviews of the Cambridge Philosophical Society, 84*, 431–448.

Moszczynska, A., Saleh, J., Zhang, H., Vukusic, B., Lee, F. J., & Liu, F. (2007). Parkin disrupts the alpha-synuclein/dopamine transporter interaction: Consequences toward dopamine-induced toxicity. *Journal of Molecular Neuroscience, 32*, 217–227.

Müller, T., & Muhlack, S. (2012). Cysteine decrease following acute Levodopa intake in patients with Parkinson's disease. *Neuroscience Letters, 521*, 37–39.

Muñoz, P., Huenchuguala, S., Paris, I., Cuevas, C., Villa, M., Caviedes, P., Segura-Aguilar, J., & Tizabi, Y. (2012a). Protective effects of nicotine against aminochrome-induced toxicity in substantia nigra derived cells: Implications for Parkinson's disease. *Neurotoxicity Research, 22*, 177–180.

Muñoz, P., Paris, I., Sanders, L. H., Greenamyre, J. T., & Segura-Aguilar, J. (2012b). Overexpression of VMAT-2 and DT-diaphorase protects substantia nigra-derived cells against aminochrome neurotoxicity. *Biochimica et Biophysica Acta, 1822*, 1125–1136.

Myöhänen, T. T., Schendzielorz, N., & Männistö, P. T. (2010). Distribution of catechol-O-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. *Journal of Neurochemistry, 113*, 1632–1643.

Naoi, M., Maruyama, W., Yi, H., Yamaoka, Y., Shamoto-Nagai, M., Akao, Y., Gerlach, M., Tanaka, M., & Riederer, P. (2008). Neuromelanin selectively induces apoptosis in dopaminergic SH-SY5Y cells by deglutathionylation in mitochondria: Involvement of the protein and melanin component. *Journal of Neurochemistry, 105*, 2489–2500.

Norris, E. H., Giasson, B. I., Hodara, R., Xu, S., Trojanowski, J. Q., Ischiropoulos, H., & Lee, V. M. (2005). Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome – mediated conformational alterations. *The Journal of Biological Chemistry, 280*, 21212–21219.

Offen, D., Ziv, I., Panet, H., Wasserman, L., Stein, R., Melamed, E., & Barzilai, A. (1997). Dopamine-induced apoptosis is inhibited in PC12 cells expressing Bcl-2. *Cellular and Molecular Neurobiology, 17*, 289–304.

Okada, M., Nakao, R., Hosoi, R., Zhang, M. R., Fukumura, T., Suzuki, K., & Inoue, O. (2011). Microdialysis with radiometric monitoring of L-[β-^{11}C]DOPA to assess dopaminergic metabolism: Effect of inhibitors of L-amino acid decarboxylase, monoamine oxidase, and

catechol-O-methyltransferase on rat striatal dialysate. *Journal of Cerebral Blood Flow and Metabolism, 31*, 124–131.

Paris, I., Dagnino-Subiabre, A., Marcelain, K., Bennett, L. B., Caviedes, P., Caviedes, R., Olea-Azar, C., & Segura-Aguilar, J. (2001). Copper neurotoxicity is dependent on dopamine-mediated copper uptake and one-electron reduction of aminochrome in a rat substantia nigra neuronal cell line. *Journal of Neurochemistry, 77*, 519–529.

Paris, I., Martinez-Alvarado, P., Cardenas, S., Perez-Pastene, C., Graumann, R., Fuentes, P., Olea-Azar, C., Caviedes, P., & Segura-Aguilar, J. (2005a). Dopamine-dependent iron toxicity in cells derived from rat hypothalamus. *Chemical Research in Toxicology, 18*, 415–419.

Paris, I., Martinez-Alvarado, P., Perez-Pastene, C., Vieira, M. N., Olea-Azar, C., Raisman-Vozari, R., Cardenas, S., Graumann, R., Caviedes, P., & Segura-Aguilar, J. (2005b). Monoamine transporter inhibitors and norepinephrine reduce dopamine-dependent iron dependent iron toxicity in cells derived from the substantia nigra. *Journal of Neurochemistry, 92*, 1021–1032.

Paris, I., Perez-Pastene, C., Couve, E., Caviedes, P., Ledoux, S., & Segura-Aguilar, J. (2009). Copper dopamine complex induces mitochondrial autophagy preceding caspase-independent apoptotic cell death. *The Journal of Biological Chemistry, 284*, 13306–13315.

Paris, I., Perez-Pastene, C., Cardenas, S., Iturriaga-Vasquez, P., Muñoz, P., Couve, E., Caviedes, P., & Segura-Aguilar, J. (2010). Aminochrome induces disruption of actin, alpha-, and beta-tubulin cytoskeleton networks in substantia-nigra-derived cell line. *Neurotoxicity Research, 18*, 82–92.

Paris, I., Muñoz, P., Huenchuguala, S., Couve, E., Sanders, L. H., Greenamyre, J. T., Caviedes, P., & Segura-Aguilar, J. (2011). Autophagy protects against aminochrome-induced cell death in substantia nigra-derived cell line. *Toxicological Sciences, 121*, 376–388.

Park, H. H., Lee, K. Y., Kim, S. H., Lee, Y. J., & Koh, S. H. (2009). L-dopa-induced neurotoxicity is reduced by the activation of the PI3K signaling pathway. *Toxicology, 265*, 80–86. 30.

Pavese, N., Evans, A. H., Tai, Y. F., Hotton, G., Brooks, D. J., Lees, A. J., & Piccini, P. (2006). Clinical correlates of levodopa-induced dopamine release in Parkinson disease: A PET study. *Neurology, 67*, 1612–1617.

Prota, G. (1995). The chemistry of melanins and melanogenesis. *Fortschritte der Chemie Organischer Naturstoffe, 64*, 93–148.

Rosengren, E., Linder-Eliasson, E., & Carlsson, A. (1985). Detection of 5-S-cysteinyldopamine in human brain. *Journal of Neural Transmission, 63*, 247–253.

Sabens, E. A., Distler, A. M., & Mieyal, J. J. (2010). Levodopa deactivates enzymes that regulate thiol-disulfide homeostasis and promotes neuronal cell death: Implications for therapy of Parkinson's disease. *Biochemistry, 49*, 2715–2724.

Saura, J., Luque, J. M., Cesura, A. M., Da Prada, M., Chan-Palay, V., Huber, G., Loffler, J., & Richards, J. G. (1994). Increased monoamine oxidase B activity in plaque-associated astrocytes of alzheimer brains revealed by quantitative enzyme radioautography. *Neuroscience, 62*, 15–30.

Schapira, A. H. (2011). Mitochondrial pathology in Parkinson's disease. *The Mount Sinai Journal of Medicine, 78*, 872–881.

Schapira, A. H., & Jenner, P. (2011). Etiology and pathogenesis of Parkinson's disease. *Movement Disorders, 26*, 1049–1055.

Segura-Aguilar, J. (1996). Peroxidase activity of liver microsomal vitamin D 25-hydroxylase and cytochrome P450 1A2 catalyzes 25-hydroxylation of vitamin D3 and oxidation of dopamine to aminochrome. *Biochemical and Molecular Medicine, 58*, 122–129.

Segura-Aguilar, J., & Lind, C. (1989). On the mechanism of Mn^{3+} induced neurotoxicity of dopamine: Prevention of quinone derived oxygen toxicity by DT-diaphorase and superoxide dismutase. *Chemico-Biological Interactions, 72*, 309–324.

Segura-Aguilar, J., Baez, S., Widersten, M., Welch, C. J., & Mannervik, B. (1997). Human class Mu glutathione transferases, in particular isoenzyme M2-2, catalyze detoxication of the dopamine metabolite aminochrome. *The Journal of Biological Chemistry, 272*, 5727–5731.

Segura-Aguilar, J., Metodiewa, D., & Welch, C. (1998). Metabolic activation of dopamine o-quinones to o-semiquinones by NADPH cytochrome P450 reductase may play an important role in oxidative stress and apoptotic effects. *Biochimica et Biophysica Acta, 1381*, 1–6.

Segura-Aguilar, J., Cardenas, S., Riveros, A., Fuentes-Bravo, P., Lozano, J., Graumann, R., Paris, I., Nassif, M., & Caviedes, P. (2006). DT-diaphorase prevents the formation of alpha-synuclein adducts with aminochrome. *Soc Neurosci Abstr, 824*, 17.

Shen, X. M., Xia, B., Wrona, M. Z., & Dryhurst, G. (1996). Synthesis, redox properties, in vivo formation, and neurobehavioral effects of N-acetylcysteinyl conjugates of dopamine: Possible metabolites of relevance to Parkinson's disease. *Chemical Research in Toxicology, 9*, 1117–1126.

Shih, J. C., Grimsby, J., & Chen, K. (1997). Molecular biology of monoamine oxidase A and B: Their role in the degradation of serotonin. In H. G. Baumgarten & M. Gothert (Eds.), *Handbook of experimental pharmacology, vol 129, Serotoninergic neurons and 5-HT receptors in the CNS* (pp. 655–670). Berlin: Springer.

Simantov, R., Blinder, E., Ratovitski, T., Tauber, M., Gabbay, M., & Porat, S. (1996). Dopamine-induced apoptosis in human neuronal cells: Inhibition by nucleic acids antisense to the dopamine transporter. *Neuroscience, 74*, 39–50.

Stokes, A. H., Lewis, D. Y., Lash, L. H., Jerome, W. G., 3rd, Grant, K. W., Aschner, M., & Vrana, K. E. (2000). Dopamine toxicity in neuroblastoma cells: Role of glutathione depletion by L-BSO and apoptosis. *Brain Research, 858*, 1–8.

Strolin-Benedetti, M., Dostert, P., & Tipton, K. F. (1992). Developmental aspects of the monoamine-degrading enzyme monoamine oxidase. *Developmental Pharmacology and Therapeutics, 18*, 191–200.

Takeshima, M., Murata, M., Urasoe, N., Murakami, S., Miyazaki, I., Asanuma, M., & Kita, T. (2011). Protective effects of baicalein against excess L-dopa-induced dopamine quinone neurotoxicity. *Neurological Research, 33*, 1050–1056.

Tanaka, H., Kannari, K., Maeda, T., Tomiyama, M., Suda, T., & Matsunaga, M. (1999). Role of serotonergic neurons in L-dopa-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *Neuroreport, 10*, 631–634. 25.

Thompson, M., Capdevila, J. H., & Strobel, H. W. (2000). Recombinant cytochrome P450 2D18 metabolism of dopamine and arachidonic acid. *The Journal of Pharmacology and Experimental Therapeutics, 294*, 1120–1130.

Van Laar, V. S., Mishizen, A. J., Cascio, M., & Hastings, T. G. (2009). Proteomic identification of dopamine-conjugated proteins from isolated rat brain mitochondria and SH-SY5Y cells. *Neurobiology of Disease, 34*, 487–500.

Walkinshaw, G., & Waters, C. M. (1995). Induction of apoptosis in catecholaminergic PC12 cells by L-dopa. Implications for the treatment of Parkinson's disease. *The Journal of Clinical Investigation, 95*, 2458–2464.

Westlund, K. N., Denney, R. M., Rose, R. M., & Abell, C. W. (1988). Localization of distinct monoamine oxidase A and monoamine oxidase B cell populations in human brainstem. *Neuroscience, 25*, 439–456.

Weyler, W., Hsu, Y. P., & Breakefield, X. O. (1990). Biochemistry and genetics of monoamine oxidase. *Pharmacology and Therapeutics, 47*, 391–417.

Whitehead, R. E., Ferrer, J. V., Javitch, J. A., & Justice, J. B. (2001). Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter. *Journal of Neurochemistry, 76*, 1242–1251.

Xilouri, M., Vogiatzi, T., Vekrellis, K., Park, D., & Stefanis, L. (2009). Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. *PLoS One, 4*, e5515.

Xu, Y., Stokes, A. H., Roskoski, R., Jr., & Vrana, K. E. (1998). Dopamine, in the presence of tyrosinase, covalently modifies and inactivates tyrosine hydroxylase. *Journal of Neuroscience Research, 54*, 691–697.

Zafar, K. S., Siegel, D., & Ross, D. (2006). A potential role for cyclized quinones derived from dopamine, DOPA, and 3,4-dihydroxyphenylacetic acid in proteasomal inhibition. *Molecular Pharmacology, 70*, 1079–1086.

Zecca, L., Fariello, R., Riederer, P., Sulzer, D., Gatti, A., & Tampellini, D. (2002). The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. *FEBS Letters, 510*, 216–220.

Zhang, N. Y., Tang, Z., & Liu, C. W. (2008). Alpha-Synuclein protofibrils inhibit 26 S proteasome-mediated protein degradation: Understanding the cytotoxicity of protein protofibrils in neurodegenerative disease pathogenesis. *The Journal of Biological Chemistry, 283*, 20288–20298.

Zhang, W., Phillips, K., Wielgus, A. R., Liu, J., Albertini, A., Zucca, F. A., Faust, R., Qian, S. Y., Miller, D. S., Chignell, C. F., Wilson, B., Jackson-Lewis, V., Przedborski, S., Joset, D., Loike, J., Hong, J. S., Sulzer, D., & Zecca, L. (2011). Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: Implications for progression of Parkinson's disease. *Neurotoxicity Research, 19*, 63–72.

Ziv, I., Shirvan, A., Offen, D., Barzilai, A., & Melamed, E. (2001). Molecular biology of dopamine-induced apoptosis: Possible implications for Parkinson's disease. *Methods in Molecular Medicine, 62*, 73–87.

Nature of DSP-4-Induced Neurotoxicity

Aleksandra Bortel

Contents

Abstract

N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) is a noradrenergic neurotoxin which selectively damages noradrenergic projections originating from the locus coeruleus (LC) and transiently alters sympathetic neurons in the periphery. DSP-4 accumulates intraneuronally and produces nerve terminal degeneration via alkylation of diverse neuronal structures. DSP-4 inhibits norepinephrine (NE) reuptake, stimulates NE release, and increases turnover rate of NE. Systemic administration of DSP-4 has a rapid dose-dependent depleting effect on the endogenous NE level. The effect of DSP-4 is largely restricted to

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noradrenergic neurons; however, DSP-4 slightly reduces the level of 5-hydroxytryptamine and dopamine. The rat age at the time of DSP-4 injection is important in determining the nature of the long-term changes in the noradrenergic system. Thus, DSP-4 treatment of adult rats produces morphological changes of NE neurons with a pronounced decrease in NE levels in the cerebral cortex, hippocampus, spinal cord, and cerebellum. Less affected are the hypothalamus and pons-medulla. This process is not long lasting and after several months a regeneration of NE nerve terminals is observed. DSP-4 injected to newborn rats induces an alteration of the postnatal development of noradrenergic system with a permanent NE denervation in brain areas distal to LC cell bodies (cortex and hippocampus) and a hyperinnervation in regions proximal to LC (brainstem, cerebellum, and pons-medulla). This type of hyperinnervation does not exist after DSP-4 treatment of adult animals. The noradrenergic lesion obtained with DSP-4 is highly reproducible; therefore, DSP-4 may represent a suitable tool to discern LC neuron degeneration and recovery and to investigate the projections of non-coerulean NE neurons.

Keywords

Degeneration • Depletion • Dopamine-β-hydroxylase • DSP-4 • Hyperinnervation • Locus coeruleus • Monoamine oxidase • Neurotoxin • Non-coerulean innervation • Noradrenergic neurons • Noradrenergic receptors • Norepinephrine transporter • Norepinephrine • Tyrosine hydroxylase

List of Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	Serotonin
AD	Aldehyde dehydrogenase
AR	Aldehyde reductase
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
DA	Dopamine
DAT	Dopamine transporter
DBH	Dopamine-β-hydroxylase
DHPG	3,4-dihydroxyphenylethylene glycol
DOPAC	3,4-dihydroxyphenylacetic acid
DSP-4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine
GABA	γ-aminobutyric acid
i.p.	Intraperitoneal injection
i.v.	Intravenous injection
LC	Locus coeruleus
MAO	Monoamine oxidase
MHPG	3-methoxy-4-hydroxyphenylethylene glycol
NE	Norepinephrine
NET	Norepinephrine transporter

OCTs	Organic cation transporters
s.c.	Subcutaneous injection
SERT	Serotonin transporter

1 Introduction

N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) was originally described by Ross (1976) who reported its effects on peripheral and central norepinephrine (NE) tissue levels. DSP-4 is a noradrenergic neurotoxin which crosses the blood–brain barrier and selectively damages noradrenergic projections originating from the locus coeruleus (LC). DSP-4 is a 2-chloroethylamine that interacts with the NE transporter (NET), whereby DSP-4 is then accumulated intraneuronally to induce degeneration of noradrenergic terminals (Ransom et al. 1985; Dudley et al. 1990; Howard et al. 1990). DSP-4 administration into rodents permanently inactivates NE reuptake, stimulates NE release, increases NE turnover rate in some brain regions, and produces morphological changes in NE cells (Jaim-Etcheverry 1998; Prieto and Giralt 2001).

2 Molecular Mechanism of Action

DSP-4 undergoes intramolecular cyclization to form an aziridinium ion, an electrophilic intermediate that can subsequently hydrolyze or react with other nucleophiles, including those present in biological tissues (thiols or amines) (Fig. 1). The aziridinium ion is a structural analogue of bretylium that is a substrate for the NET. Thus, the aziridinium ion covalently bonds with electrophilic centers on or near the site of action, disrupts the function of the NET binding site, and thereby inhibits NE reuptake (Ransom et al. 1982;

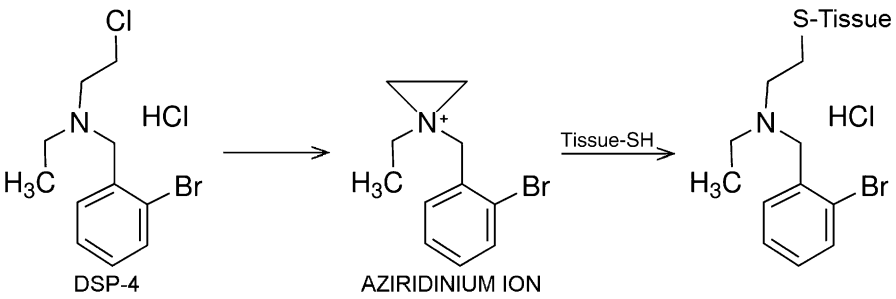

Fig. 1 DSP-4 cyclization and reaction with nucleophiles present in biological tissues (Based on Dudley et al. 1990)

Dudley et al. 1990; Jaim-Etcheverry 1998). Inactivation of the transport system is attributable to DSP-4 alkylation at this site (Lee et al. 1982; Hallman and Jonsson 1984; Dudley et al. 1990) and requires a functional transporter to inhibit NE reuptake (Dudley et al. 1990). DSP-4 in aqueous solutions has a half-life of 7 min at pH 7.4 and 37 °C.

3 DSP-4 Neurotoxic Effects

3.1 DSP-4 Specificity and Monoamine Reuptake

DSP-4 treatment, while having no major influence on NE cell bodies, dendrites, and preterminal axons, exerts neurotoxic effects on the terminal part of NE axons (Jonsson et al. 1981; Fritschy and Grzanna 1989, 1991a). Moreover, the part of NE axons along their pathways positioned between its origin and its entry into a terminal field is not sensitive to DSP-4. Complete loss of NE axons is found in brain regions such as the cerebral cortex, hippocampus, tectum, cerebellum, and spinal cord dorsal horn, and any changes are noted in the basal forebrain, hypothalamus, and some brainstem nuclei (Fritschy and Grzanna 1989, 1991a). Thus, DSP-4 has a preferential, selective neurotoxic effect on nerve terminal projections originating from NE perikarya of LC and does not affect NE axons in brain regions receiving a dense innervation from non-coerulean NE cells (Jonsson et al. 1981; Fritschy and Grzanna 1989, 1991a). Non-coerulean neurons provide the major NE innervation to the regions involved in the regulation of autonomic and motor activities. In contrast, LC neurons innervate brain regions involved in the processing of sensory inputs (Fritschy and Grzanna 1989, 1991a). DSP-4 initially induces degeneration of LC axon terminals that in the long term is responsible for LC cell death. The delayed death of LC neurons is probably a consequence of the loss of terminal axons rather than a result of a direct effect of DSP-4 on LC cell bodies (Fritschy and Grzanna 1991b). Initially, it was suggested that DSP-4 specificity to the noradrenergic projections originating from LC depends on the pharmacologically distinct affinity of DSP-4 for the NET in LC and non-coerulean NE axons (Fritschy and Grzanna 1989, 1991a). Subsequently, Zaczek et al. (1990) proposed that NET binding sites have distinct kinetic characteristics in the cerebral cortex (receiving LC innervation) and hypothalamus (receiving non-coerulean innervation), and therefore, DSP-4 shows a higher affinity for NET in LC versus non-coerulean nerve terminals. There are two components of NE reuptake, and only the high-affinity sites of NET are abolished by DSP-4 treatment. Low-affinity sites remain unaffected by DSP-4 (Lee et al. 1982; Hughes and Stanford 1998a). However, several studies deny NET heterogeneity and demonstrate that NETs are similar in all brain regions (Snyder and Coyle 1969; Ghraf et al. 1983). More plausibly, there may be greater intra-axonal DSP-4 accumulation in LC than non-coerulean NE axons. Interaction of DSP-4 with NET is a prerequisite for NE depletion because the NET is responsible for intracellular accumulation of DSP-4 or its aziridinium ion (Landa et al. 1984). Thus, the cytotoxic DSP-4 effect is not

due to DSP-4 affinity to the transporter but depends on DSP-4 accumulation within the cells by the NET, which then disables DSP-4 outward transport. The outward transport becomes inhibited and DSP-4 remains intracellularly trapped. DSP-4 ultimately leads to ATP depletion and other cytotoxic reactions (Wenge and Bönisch 2009). NE storage is ATP dependent; therefore, DSP-4 inhibition of ATP synthesis results in NE depletion (Dudley et al. 1990).

Neuronally released monoamines are removed from the synaptic cleft by rapid reuptake into presynaptic neurons via Na^+- and Cl^--dependent neuronal monoamine transporters such as NET. Additionally, monoamines are accumulated in extraneuronal tissues by Na^+- and Cl^--independent extraneuronal monoamine transporters and organic cation transporters (OCTs). These transporters are involved in the absorption, distribution, and elimination of endogenous compounds (Wenge and Bönisch 2009). DSP-4 interacts at the level of neuronal membrane and forms covalent bonds with thiol groups of proteins (Jaim-Etcheverry and Zieher 1980; Dudley et al. 1990). DSP-4 and aziridinium ion are competitive inhibitors of NE reuptake, while due to the alkylation of NET, DSP-4 blocks the transporter irreversibly (Zaczek et al. 1990). DSP-4 also inhibits other transporters such as that for dopamine (DAT) and serotonin (SERT). About 80 % and 60 % of the transport activity of DAT and SERT, respectively, is inactivated by the neurotoxin. DSP-4 inhibits, as well, OCTs with a potency 3.2-fold (OCT2), 7.5-fold (OCT1), or 9-fold (OCT3) higher than for the NET. The neurotoxic DSP-4 effect is completely irreversible at NET; only partially irreversible at DAT, SERT, and OCT3; and fully reversible at OCT1 and OCT2 (Wenge and Bönisch 2009).

3.2 Synaptic Norepinephrine Levels and Noradrenergic Receptors

DSP-4-induced imbalance between NE release and reuptake leads to an increase in the extracellular NE concentration and NE efflux in the frontal cortex of freely moving rats (Hughes and Stanford 1998a, b). However, DSP-4 treatment does not alter the basal extracellular NE level in the frontal cortex of anesthetized rats, although NE release evoked by administration of an α_2-adrenergic receptor antagonist results in lower but still significant increase in extracellular NE levels (Kask et al. 1997; Harro et al. 1999). The same, lower but still significant increase in NE release induced by a $GABA_A$ antagonist (bicuculline) was observed by Giorgi et al. (2003) in the piriform cortex. Long-term NE depletion and the resulting decreased presynaptic NE release affect the function of pre- and postsynaptic NE receptors that are exposed to the environment of reduced synaptic NE concentration (Dudley et al. 1990). DSP-4 is a blocker of α-adrenergic receptors; therefore, DSP-4 initially reduces α-adrenergic receptor binding. This blocking effect is not long lasting because already 14 days after the neurotoxin application α- and β-adrenergic receptor binding recovers (Jonsson et al. 1981). DSP-4 exerts a consistent upregulation of β-adrenergic receptors in the somatosensory cortex and hippocampus at 1–3 weeks after treatment (Zahniser et al. 1986). DSP-4 likewise increases

the number of β-adrenergic receptors and does not change the affinity of these receptors. The density of binding sites of β-adrenergic receptors is enhanced at 1 day, 7 days, and up to 1 year after central NE denervation induced by DSP-4 (Theron et al. 1993; Wolfman et al. 1994). The number and the affinity of α_1-adrenergic receptors are unchanged in somatosensory cortex and hippocampus 1–3 weeks after DSP-4 treatment (Zahniser et al. 1986; Szot et al. 2010). As well, the properties of α_2-adrenergic receptors are unaltered (Zahniser et al. 1986). Another study shows that the activity of α_2-adrenergic receptors is reduced in the parietal cortex and hippocampus 3 weeks after DSP-4 treatment, but not in the hypothalamus (Prieto and Giralt 2001). Interestingly, it was reported that the number of α_2-adrenergic receptors is reduced in the cortex (20 %), hippocampus (18 %), cerebellum (24 %), and hypothalamus (39 %) at 3 days after DSP-4 (100 mg/kg; DSP-4 dose is mentioned only when is different from 50 mg/kg) treatment and unchanged or even increased 15 days after treatment. Thus, decreased density of cortical presynaptic α_2-adrenergic receptors is progressively masked by increased sensitivity of postsynaptic α_2-adrenergic receptors (Heal et al. 1993). The density of binding sites of α_1- and α_2-adrenergic receptors is increased in cerebral cortex and cerebellum 7 days after DSP-4 administration, while α_1- and α_2-adrenergic receptor number attains control values 3 months and 1 year later, respectively (Theron et al. 1993; Wolfman et al. 1994).

The enhancement of α-adrenergic receptor density is an adaptative change of postsynaptic receptors as a consequence of the long-term disruption in NE transmission. However, an irreversible increase of β-adrenergic receptors may be linked with the adaptative changes that are independent of the endogenous NE concentration (Dooley et al. 1987).

3.3 Norepinephrine Synthesizing and Metabolizing Enzymes

DSP-4 induces a profound loss of both NE and dopamine-β-hydroxylase (DBH) from noradrenergic axons, with an uncorrelated time course (Fig. 2) (Ross 1976; Jaim-Etcheverry and Zieher 1980; Fritschy et al. 1990). Tissue NE levels decline within hours, and DBH activity is reduced within 4 days after DSP-4 treatment (Fritschy et al. 1990). Therefore, two phases in the response of NE axons to DSP-4 are suggested: (1) an acute phase that is characterized by a marked loss of neurotransmitter and (2) a neurodegenerative phase associated with loss of DBH and structural disintegration of NE axons (Fritschy et al. 1990). LC cells which survive the injury induced by DSP-4 exhibit greater DBH immunocytochemical reaction (Fritschy and Grzanna 1991b).

There are contradictory studies concerning the DSP-4 influence on tyrosine hydroxylase, a synthesizing enzyme that is localized in noradrenergic neurons and regulated independently (Fig. 2). It is proposed that DSP-4 treatment induces a decrease of basal tyrosine hydroxylase activity in the hippocampus and parietal cortex, whose axon terminals arise exclusively from LC, but not in the hypothalamus which is innervated by noradrenergic terminals originating from the tegmental lateral

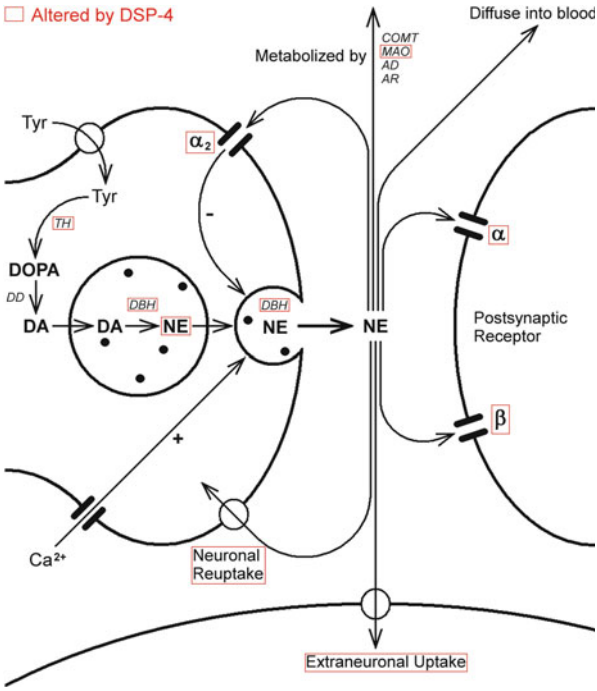

Fig. 2 DSP-4-induced alterations in noradrenergic nerve terminals. DSP-4 directly or indirectly affects norepinephrine synthesis, release, transport and metabolism. Abbreviations are shown in the abbreviation list

nuclei (Prieto and Giralt 2001). However, other studies show that DSP-4 exerts a long-lasting NE depletion with compensatory increased accumulation of tyrosine hydroxylase in noradrenergic neurons (Booze et al. 1988; Dudley et al. 1990).

It was suggested that DSP-4 may also interfere with some enzymes involved in NE metabolism. First, DSP-4 inhibits monoamine oxidase (MAO) activity in peripheral tissues (Lyles and Callingham 1981). Furthermore, the MAO-B inhibitor deprenyl effectively blocks the neurotoxic action of DSP-4 on noradrenergic neurons. However, MAO-B inhibition of DSP-4 toxicity is unrelated to NE metabolism, because MAO-A plays a central role in metabolizing endogenous monoamines including NE (Gibson 1987). Finnegan et al. (1990) have proposed that deprenyl neuroprotective effects are unrelated to MAO-B inhibition but to other properties such as the effects on the monoamine release, reuptake and metabolism, as well as the effects on other central neurotransmitter systems. Indeed, DSP-4 or aziridinium ion binds to the active catalytic site of MAO-B but is not further metabolized. Therefore, the neuroprotective deprenyl action depends on the blockade of DSP-4 reuptake into noradrenergic neurons (Dudley et al. 1990; Finnegan et al. 1990). However, the neuroprotective action of highly potent and selective MAO-B inhibitors, that do not interfere with DSP-4 reuptake within NE terminals (as it is observed with deprenyl), likewise fully prevents DSP-4-induced NE depletion (Yu et al. 1994; Zhang et al. 1996). Conversely, some selective

MAO-B inhibitors are inactive against DSP-4-induced neurotoxicity (Bertocci et al. 1988; Finnegan et al. 1990), and the absence of MAO-B (i.e., MAO-B knockout mice) does not influence the mechanism of neurotoxic DSP-4 action (Fornai et al. 2001). Therefore, the neuroprotective and/or neurorescue effects of some MAO-B inhibitors are unclear.

3.4 Electrophysiological Responsiveness

DSP-4 increases NE turnover in brain regions in which NE depletion is greater than 85 % (cerebral cortex and hippocampus), but has no effect on NE turnover when NE is depleted by only 30–60 % (cerebellum, hypothalamus, brainstem, and LC). Firing rates of monoaminergic neurons increase only after 75 % NE depletion. Thus, increased firing represents a compensatory response to the injury, resulting in increased transmitter release from the terminals spared by the lesion (Chiodo et al. 1983; Logue et al. 1985). Olpe et al. (1983) reported that DSP-4 decreases the mean neuronal LC firing rate without altering the structural appearance of LC perikarya. Precisely, DSP-4 does not affect the basal activity of LC noradrenergic neurons but increases the irregularity and the burst firing of noradrenergic neurons 2 weeks after DSP-4 treatment (Szot et al. 2010).

4 DSP-4 Doses and Routes of Administration

Systemic administration (s.c., i.p. or i.v.) of DSP-4 has a rapid dose-related effect on tissue levels of NE (Table 1). Effects are achieved with a DSP-4 dose of 20–100 mg/kg i.p. by 0.5 h after treatment (Hallman and Jonsson 1984; Jaim-Etcheverry 1998). Newborn rats are typically treated s.c., while adult rats are routinely given i.p or i.v. injections. Notably, NE depletion is much greater after i.v. than after i.p. injection of adult rats (Jaim-Etcheverry and Zieher 1980); however, i.p. administration is sufficient to produce pronounced NE depletion (up to 95 %). A dose of 50 mg/kg of DSP-4 is the most efficient in producing pronounced, long-lasting reduction of NE reuptake in the CNS without causing any lethal effects in rats (Jonsson et al. 1981) and mice (Dailly et al. 2006).

Neonatal DSP-4 treatment leads to dose-dependent adulthood changes of NE concentrations in assorted brain regions. The lowest tested DSP-4 dose (10 mg/kg s.c.) produces about 50 % NE reduction in cerebral cortex and spinal cord and small changes in cerebellum and pons-medulla. A large DSP-4 dose (50 mg/kg s.c.) produces near complete NE depletion in cerebral cortex and spinal cord and associated increases in NE concentration, both in the cerebellum and pons-medulla (Jonsson et al. 1982). The latter effect is reflective of reactive collateral sprouting of bi- or multipolar NE neurons of LC, following DSP-4 injury of NE terminals and/or axons projecting to cerebral cortex and hippocampus – as was found in similar studies with the neurotoxins 6-hydroxydopamine (Sachs and Jonsson 1975) and 6-hydroxydopa (Kostrzewa and Garey 1977).

Table 1 Dose-dependent changes of NE tissue levels in different brain areas observed 6 weeks after DSP-4 treatment (Based on Jaim-Etcheverry and Zieher 1980)

DSP-4 doses	<25 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Cortex	–	–	X	X
NE degeneration				
Spinal cord	–	X	X	X
NE degeneration				
Brainstem	–	X	X	–
NE degeneration (A)				
NE hyperinnervation (N)				
Cerebellum	X	X	X	–
NE degeneration (A)				
NE hyperinnervation (N)				

(*A*) NE degeneration observed after DSP-4 injection to adult animals, and (*N*) NE hyperinnervation after DSP-4 injection to newborn animals

5 The Nature and Extension of DSP-4-Induced Neurotoxicity in Adulthood

DSP-4 treatment does not have significant sex differences with respect to the regional alterations in the monoamine levels (Jonsson et al. 1981, 1982). Also, body weight gain in both sexes is virtually identical after DSP-4 and similar to that of control litters. As adults, the weight of neonatally treated rats of both sexes is approximately 95 % of the weight of age-matched controls (Jonsson et al. 1981, 1982). The age of rats at the time of DSP-4 treatment is critical in determining the nature and extent of the long-term changes in noradrenergic neurons (Jaim-Etcheverry 1998).

5.1 DSP-4 Treatment of Adult Rats

5.1.1 Noradrenergic System

DSP-4 treatment of adult rats generally causes a decrease of tissue levels of NE and its two principal metabolites: 3,4-dihydroxyphenylethylene glycol (DHPG) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) (Dudley et al. 1990; Hallman et al. 1984; Logue et al. 1985). However, this process is not long lasting, and after several months a regeneration of NE nerve terminals is observed (Hallman et al. 1984). DSP-4 neurotoxic effects are rapid, being maximal 2 h after DSP-4 injection for NE reuptake in vitro and by 4–6 h for endogenous NE levels. Maximal NE depletion is achieved most rapidly in the occipital cortex, hippocampus, hypothalamus, pons-medulla, cerebellum, and spinal cord, but later, in the frontal cortex, striatum, and mesencephalon. Acutely, DSP-4 has a profound NE-depleting effect in the cortex and cerebellum, but only a modest effect in ventral forebrain and hypothalamus (Grzanna et al. 1989). Simultaneously, a profound reduction in NE

axon staining is found in the cerebral cortex and cerebellum after DSP-4 treatment, whereas the ventral forebrain and hypothalamus appear to be unaffected (Grzanna et al. 1989). Ten days after DSP-4 treatment, NE concentrations are decreased by about 90 % in the cerebral cortex, hippocampus, spinal cord, and cerebellum. Less affected are the hypothalamus and pons-medulla, known to have a mixed NE innervation (both from LC and other noradrenergic nuclei in brainstem). DSP-4 decreases endogenous tissue levels of NE, not only at noradrenergic nerve terminals but also at both noradrenergic and serotoninergic cell body levels. Seven days after DSP-4, NE concentrations are decreased in LC perikarya and dorsal raphe nucleus (Cassano et al. 2009). Thus, DSP-4 neurotoxicity is exerted on the LC via retrograde degeneration (Fritschy and Grzanna 1991a) as well as from direct DSP-4 action at the LC itself, where NET is highly expressed (Cassano et al. 2009). Alterations in NE reuptake in assorted brain areas are compatible with observed changes in tissue levels of NE after DSP-4 (Jonsson et al. 1981).

NE depletion induces profound adaptive changes in other neuronal phenotypes. DSP-4 does not modify endogenous levels of brain acetylcholine, γ-aminobutyric acid (GABA), glutamic acid, glycine, or aspartic acid. However, DSP-4 depletes tissue levels of 5-HT and slightly depletes DA (Jaim-Etcheverry and Zieher 1980; Jonsson et al. 1981; Fornai et al. 1996b).

5.1.2 Serotoninergic System

DSP-4 induces an acute decrease of 5-HT concentrations in the cerebral cortex, mesencephalon, and spinal cord (Jackisch et al. 2008), and at 8 weeks 5-HT is depleted as much as 85 % in the cerebral cortex (Jonsson et al. 1981). DSP-4 does not alter the level of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in hippocampus (Jackisch et al. 2008) and dorsal raphe nucleus (Cassano et al. 2009). However, one day after DSP-4 treatment, a prominent increase in 5-HIAA is observed in the cortex, hippocampus, and hypothalamus (Theron et al. 1993). Because NE normally inhibits presynaptic 5-HT release (Vizi and Kiss 1998), noradrenergic denervation of hippocampus is associated with an increase in basal and evoked 5-HT release and reduced 5-HT accumulation in the hippocampal slices (Cassano et al. 2009). The neurotoxic effect of DSP-4 on 5-HT neurons can be prevented by pretreatment with the SERT blocker (zimelidine) (Jonsson et al. 1981).

5.1.3 Dopaminergic System

DSP-4 alters DA levels in several brain regions (Jonsson et al. 1982). Thus, DSP-4-induced noradrenergic lesioning reduces DA levels in hippocampus, cerebellum, prefrontal cortex, LC, and dorsal raphe nucleus (Jonsson et al. 1981; Cassano et al. 2009). Moreover, DSP-4 treatment decreases (Lategan et al. 1992) or does not change (Hatip-al-Khatib et al. 2001; Häidkind et al. 2002) the extracellular DA concentration and reduces the extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) level in nucleus accumbens (Lategan et al. 1992; Hatip-al-Khatib et al. 2001). A reduction in extracellular DA concentration is attributable to a reduction in basal DA release (Lategan et al. 1992; Weinshenker et al. 2008).

5.1.4 Other Neuronal Phenotypic Systems

The hippocampal endogenous acetylcholine level (Hörtnagl et al. 1989) and activity of the acetylcholine transporter (Harrell et al. 2005) are unaltered following DSP-4 treatment. However, DSP-4-induced lesion of noradrenergic neurons facilitates cholinergic transmission in the hippocampus (Jackisch et al. 2008). DSP-4 treatment likewise induces an upregulation of the turnover and activity of cholinergic neurons. Acetylcholine accumulation is enhanced in the hippocampus, while basal and evoked acetylcholine efflux are increased in DSP-4-treated rats (Jackisch et al. 2008).

5.1.5 Peripheral Sympathetic Neurons

Related DSP-4 effects are observed in peripheral sympathetic neurons as compared with central NE neurons. However, a complete recovery of peripheral adrenergic nerves is observed 1 week after DSP-4, and a gradual recovery of decreased NE tissue levels and reuptake occurs by 2–4 weeks after DPS-4 treatment (Jaim-Etcheverry et al. 1980; Jonsson et al. 1981).

5.2 DSP-4 Treatment of Newborn Rats

5.2.1 Noradrenergic System

Systemic DSP-4 treatment of newborn rats results in an alteration in postnatal development of the noradrenergic system, with a permanent denervation of distal NE projection regions and associated NE hyperinnervation in regions proximal to LC cell bodies. The total number of NE nerve terminals is considered to be unaltered (Jaim-Etcheverry and Zieher 1980). NE hyperinnervation is considered to be a consequence of collateral NE accumulation and/or sprouting of noradrenergic neurons (Jaim-Etcheverry and Zieher 1980). Neonatal DSP-4 treatment leads to adulthood changes of tissue NE levels in several brain regions. In adulthood NE is depleted approximately 90 % in cerebral cortex, olfactory bulb, hippocampus, and spinal cord of rats treated as neonates with DSP-4 (Jonsson et al. 1982; Nowak et al. 2006; Bortel et al. 2008b), with less pronounced NE effects in hypothalamus and septum. No significant effects are noted in the striatum, olfactory tubercle, and medulla oblongata (Jonsson et al. 1982; Nowak et al. 2006), while a pronounced increase in NE concentrations are present in the cerebellum, brainstem, mesencephalon, and pons-medulla after DSP-4 (Jonsson et al. 1982; Bortel et al. 2008b). It is notable that NE concentrations are decreased in the cerebellum, mesencephalon, and pons-medulla 6 h after DSP-4 administration, in contrast to the marked increase in NE tissue levels in these regions in adulthood (Jonsson et al. 1982).

When administered at birth, DSP-4 decreases in vitro NE reuptake in cerebral cortex and spinal cord by approximately 90 %, but does not alter NE reuptake in the striatum and increases NE reuptake in the cerebellum (Jonsson et al. 1982).

Permanent NE denervation in cerebral cortex and spinal cord is present at all developmental stages, although more pronounced when animals are treated with DSP-4 up to the 5th day after birth. However, NE hyperinnervation in the cerebellum

and pons-medulla is only observed when DSP-4 is administered to 1 and 3 days old rats (Jonsson et al. 1982; Bortel et al. 2008b). By the age of 8 days, permanent NE depletion is present in these structures of the brain of animals treated at birth with DSP-4 (Jonsson et al. 1982). Acutely (1 day after DSP-4 treatment) NE depletion is observed in animals of all ages (Jonsson et al. 1982).

5.2.2 Serotoninergic System

DSP-4 induces dose-dependent changes of 5-HT tissue levels in several brain regions. DSP-4 reduces the endogenous 5-HT concentrations in the cerebral cortex and spinal cord and reduces 5-HT reuptake in the occipital cortex, hippocampus, and cerebellum. No significant effects are noted in other brain regions (Jonsson et al. 1981).

Neonatal DSP-4 treatment does not induce any change in 5-HT levels and 5-HT reuptake when whole brain is analyzed (Jonsson et al. 1982). SERT blocker zimelidine attenuates the effects of DSP-4 on 5-HT and 5-HIAA in the frontal cortex, hippocampus, striatum, and hypothalamus (Jonsson et al. 1981; Dabrowska et al. 2007).

5.2.3 Dopaminergic System

Although neonatal DSP-4 treatment does not acutely (i.e., directly) affect DA and DOPAC levels in the hippocampus and striatum (Nowak et al. 2006), there is an adaptive decrease in DA content of occipital cortex, hippocampus, cerebellum, mesencephalon (Jonsson et al. 1982), and dorsal raphe nuclei (Cassano et al. 2009) in adulthood.

5.2.4 GABAergic System

Neonatal DSP-4 treatment of rats does not alter GABA tissue levels in prefrontal cortex, hippocampus, brainstem, and cerebellum (Bortel et al. 2008b) and does not alter the extracellular GABA concentration in prefrontal cortex (Bortel et al. 2008a). However, DSP-4-induced noradrenergic denervation of the cerebral cortex reduces the number of $GABA_A$ receptors that are localized on the presynaptic axons and nerve terminals of noradrenergic neurons (Medina and Novas 1983; Suzdak and Gianutsos 1985). This significant decrease in density of benzodiazepine receptors persists even 1 year after DSP-4 treatment (Wolfman et al. 1994). Moreover, DSP-4 increases the number of benzodiazepine receptors in the cerebellum and brainstem (Medina and Novas 1983).

5.2.5 Peripheral Sympathetic Neurons

The effect of DSP-4 on the peripheral sympathetic nervous system is minor. DSP-4 fails to permanently alter the NE concentration in peripheral organs (Jaim-Etcheverry and Zieher 1980; Jonsson et al. 1982). At 1 month after DSP-4 treatment, NE tissue levels approximate control values (Jonsson et al. 1982). Neonatal DSP-4 treatment does not produce long-lasting changes in NE content of heart, salivary glands, and spleen (Jaim-Etcheverry and Zieher 1980; Jonsson et al. 1981).

5.3 Prenatal DSP-4 Treatment

Prenatal DSP-4 administration (at the end of gestation; twice 25 mk/kg i.v.) modifies NE tissue levels to the same extent as when DSP-4 is administered to newborn animals (Jaim-Etcheverry and Zieher 1980). DSP-4 prenatal treatment permanently decreases NE levels in cerebral cortex and spinal cord, demonstrating that the neurotoxin passes the blood-placenta barrier and has a potent neurotoxic effect in the prenatal stage. Interestingly, there is no ensuing increase in NE concentration in the cerebellum and pons-medulla of treated prenates when observed as adults – in contrast to effects after DSP-4 treatment of newborn animals (Jonsson et al. 1981).

6 Rodent Differences in the Susceptibility to DSP-4

DSP-4-induced neurodegenerative changes in noradrenergic neurons in the CNS are qualitatively similar in both rats and mice (Jonsson et al. 1981). However, NE depletion in forebrain regions such as cortex and hippocampus appears to be more pronounced and persistent in mice versus rats (Fornai et al. 1996a; Dailly et al. 2006; Cassano et al. 2009; Szot et al. 2010). As well, sympathetic adrenergic nerves of the mouse are more sensitive than rats to the neurotoxic action of DSP-4. This effect is likely due to the differences in diffusion conditions or size of NE varicosities (Jonsson 1980). It is notable that various mouse and rat strains show a different sensitivity in the CNS to the neurotoxic DSP-4 action, as exemplified by the finding that Long-Evans rats are less sensitive than Sprague–Dawley rats to DSP-4 (Schuerger and Balaban 1995).

7 Conclusion

DSP-4 is an alkylating agent with high specificity for NE and 5-HT neurons in vivo. The neurotoxin transiently alters sympathetic neurons in the periphery and produces persistent alterations in the central noradrenergic system. DSP-4, having high affinity for the NET, and being accumulated within NE nerve endings, depletes monoamines by reducing mitochondrial function and ultimately destroying noradrenergic neurons in the periphery and brain (Jaim-Etcheverry and Zieher 1980; Dudley et al. 1990). Moreover, DSP-4 is able to cross the blood-placenta barrier in pregnant animals, thereby enabling DSP-4 to alter noradrenergic neuronal development in early stages of ontogeny.

DSP-4 has the capacity to produce destruction of NE axons in brain regions innervated by LC, without affecting non-coerulean NE axons (Fritschy and Grzanna 1989). Therefore, DSP-4 appears to be a useful denervation tool to study the projections of non-coerulean NE neurons in isolation. Additionally, DSP-4 may serve as a useful experimental tool to discern LC neuron degeneration and recovery. The long-term NE depletion and irreversible inhibition of neuronal reuptake induced by DSP-4 can be used in pharmacological investigations. Thus, the

alterations in NE tissue levels and release may be used to investigate synaptic biochemistry including mechanisms of noradrenergic receptor up- or downregulation. The NE-depleting DSP-4 effects can be used to study behavioral disorders. Until now, we know that DSP-4 treatment decreases exploratory activity in rats that results from increased anxiety (Delini-Stula et al. 1984; Harro et al. 1995). DSP-4-induced NE depletion exerts deficits in working memory without affecting reference memory (DSP-4 did not affect the previously learned pattern) (Ohno et al. 1993; Sontag et al. 2008). Moreover, NE possesses an anticonvulsant effect; therefore, central NE depletion facilitates induction of seizures evoked by bicuculline, pentylenetetrazol, or maximal electroshock (Mishra et al. 1994; Giorgi et al. 2003, 2006).

The noradrenergic lesion obtained with DSP-4 is remarkably reproducible and the usefulness and impact of DSP-4-lesioned models have enormous pharmacological potential. Despite these obvious successes, limitation still exists for DSP-4 because of unspecificity in its mechanism of action. DSP-4 interacts not only with NE reuptake systems, but alters, as well, other monoamine reuptake systems as well as enzymes involved in NE synthesis. Adaptive changes in noradrenergic receptors, relative to NE depletion, further complicate the actions of DSP-4. Therefore, all experiments based on DSP-4 should be carefully interpreted.

References

Bertocci, B., Gill, G., & Da Prada, M. (1988). Prevention of the DSP-4-induced noradrenergic neurotoxicity by irreversible, not by reversible MAO-B inhibitors. *Pharmacogical Research Communications, 20*, 131–132.

Booze, R. M., Hall, J. A., Cress, N. M., Miller, G. D., & Davis, J. N. (1988). DSP-4 treatment produces abnormal tyrosine hydroxylase immunoreactive fibers in rat hippocampus. *Experimental Neurology, 101*, 75–86.

Bortel, A., Nowak, P., & Brus, R. (2008a). Neonatal DSP-4 treatment modifies GABAergic neurotransmission in the prefrontal cortex of adult rats. *Neurotoxicity Research, 13*, 247–252.

Bortel, A., Slomian, L., Nitka, D., Swierszcz, M., Jaksz, M., Adamus-Sitkiewicz, B., Nowak, P., Josko, J., Kostrzewa, R. M., & Brus, R. (2008b). Neonatal N-(−2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) treatment modifies the vulnerability to phenobarbital- and ethanol-evoked sedative-hypnotic effects in adult rats. *Pharmacological Reports: PR, 60*, 331–338.

Cassano, T., Gaetani, S., Morgese, M. G., Macheda, T., Laconca, L., Dipasquale, P., Taltavull, J., Shippenberg, T. S., Cuomo, V., & Gobbi, G. (2009). Monoaminergic changes in locus coeruleus and dorsal raphe nucleus following noradrenaline depletion. *Neurochemical Research, 34*, 1417–1426.

Chiodo, L. A., Acheson, A. L., Zigmond, M. J., & Stricker, E. M. (1983). Subtotal destruction of central noradrenergic projections increases the firing rate of locus coeruleus cells. *Brain Research, 264*, 123–126.

Dabrowska, J., Nowak, P., & Brus, R. (2007). Desensitization of 5-HT(1A) autoreceptors induced by neonatal DSP-4 treatment. *European Neuropsychopharmacology, 17*, 129–137.

Dailly, E., Chenu, F., Petit-Demoulière, B., & Bourin, M. (2006). Specificity and efficacy of noradrenaline, serotonin depletion in discrete brain areas of Swiss mice by neurotoxins. *Journal of Neuroscience Methods, 150*, 111–115.

Delini-Stula, A., Mogilnicka, E., Hunn, C., & Dooley, D. J. (1984). Novelty-oriented behavior in the rat after selective damage of locus coeruleus projections by DSP-4, a new noradrenergic neurotoxin. *Pharmacology Biochemistry and Behavior, 20*, 613–618.

Dooley, D. J., Heal, D. J., & Goodwin, G. M. (1987). Repeated electroconvulsive shock prevents increased neocortical beta 1-adrenoceptor binding after DSP-4 treatment in rats. *European Journal of Pharmacology, 134*, 333–337.

Dudley, M. W., Howard, B. D., & Cho, A. K. (1990). The interaction of the beta-haloethyl benzylamines, xylamine, and DSP-4 with catecholaminergic neurons. *Annual Review of Pharmacology and Toxicology, 30*, 387–403.

Finnegan, K. T., Skratt, J. J., Irwin, I., DeLanney, L. E., & Langston, J. W. (1990). Protection against DSP-4-induced neurotoxicity by deprenyl is not related to its inhibition of MAO-B. *European Journal of Pharmacology, 184*, 119–126.

Fornai, F., Bassi, L., Torracca, M. T., Alessandrì, M. G., Scalori, V., & Corsini, G. U. (1996a). Region- and neurotransmitter-dependent species and strain differences in DSP-4-induced monoamine depletion in rodents. *Neurodegeneration, 5*, 241–249.

Fornai, F., Torracca, M. T., Bassi, L., D'Errigo, D. A., Scalori, V., & Corsini, G. U. (1996b). Norepinephrine loss selectively enhances chronic nigrostriatal dopamine depletion in mice and rats. *Brain Research, 735*, 349–353.

Fornai, F., Giorgi, F. S., Gesi, M., Chen, K., Alessrì, M. G., & Shih, J. C. (2001). Biochemical effects of the monoamine neurotoxins DSP-4 and MDMA in specific brain regions of MAO-B-deficient mice. *Synapse, 39*, 213–221.

Fritschy, J. M., & Grzanna, R. (1989). Immunohistochemical analysis of the neurotoxic effects of DSP-4 identifies two populations of noradrenergic axon terminals. *Neuroscience, 30*, 181–197.

Fritschy, J. M., & Grzanna, R. (1991a). Experimentally-induced neuron loss in the locus coeruleus of adult rats. *Experimental Neurology, 111*, 123–127.

Fritschy, J. M., & Grzanna, R. (1991b). Selective effects of DSP-4 on locus coeruleus axons: Are there pharmacologically different types of noradrenergic axons in the central nervous system? *Progress in Brain Research, 88*, 257–268.

Fritschy, J. M., Geffard, M., & Grzanna, R. (1990). The response of noradrenergic axons to systemically administered DSP-4 in the rat: An immunohistochemical study using antibodies to noradrenaline and dopamine-beta-hydroxylase. *Journal of Chemical Neuroanatomy, 3*, 309–321.

Ghraf, R., Michel, M., Hiemke, C., & Knuppen, R. (1983). Competition by monophenolic estrogens and catecholestrogens for high-affinity uptake of [^{3}H](−)-norepinephrine into synaptosomes from rat cerebral cortex and hypothalamus. *Brain Research, 277*, 163–168.

Gibson, C. J. (1987). Inhibition of MAO B, but not MAO A, blocks DSP-4 toxicity on central NE neurons. *European Journal of Pharmacology, 141*, 135–138.

Giorgi, F. S., Ferrucci, M., Lazzeri, G., Pizzanelli, C., Lenzi, P., Alessandri, M. G., Murri, L., & Fornai, F. (2003). A damage to locus coeruleus neurons converts sporadic seizures into self-sustaining limbic status epilepticus. *European Journal of Neuroscience, 17*, 2593–2601.

Giorgi, F. S., Mauceli, G., Blandini, F., Ruggieri, S., Paparelli, A., Murri, L., & Fornai, F. (2006). Locus coeruleus and neuronal plasticity in a model of focal limbic epilepsy. *Epilepsia, 47*, 21–25.

Grzanna, R., Berger, U., Fritschy, J. M., & Geffard, M. (1989). Acute action of DSP-4 on central norepinephrine axons: Biochemical and immunohistochemical evidence for differential effects. *Journal of Histochemistry and Cytochemistry, 37*, 1435–1442.

Häidkind, R., Kivastik, T., Eller, M., Kolts, I., Oreland, L., & Harro, J. (2002). Denervation of the locus coeruleus projections by treatment with the selective neurotoxin DSP-4 [N (2-chloroethyl)-N-ethyl-2-bromobenzylamine] reduces dopamine release potential in the nucleus accumbens shell in conscious rats. *Neuroscience Letters, 332*, 79–82.

Hallman, H., & Jonsson, G. (1984). Pharmacological modifications of the neurotoxic action of the noradrenaline neurotoxin DSP-4 on central noradrenaline neurons. *European Journal of Pharmacology, 103*, 269–278.

Hallman, H., Sundström, E., & Jonsson, G. (1984). Effects of the noradrenaline neurotoxin DSP-4 on monoamine neurons and their transmitter turnover in rat CNS. *Journal of Neural Transmission, 60*, 89–102.

Harrell, L. E., Parsons, D. S., & Kolasa, K. (2005). The effect of central cholinergic and noradrenergic denervation on hippocampal sympathetic ingrowth and apoptosis-like reactivity in the rat. *Brain Research, 1033*, 68–77.

Harro, J., Oreland, L., Vasar, E., & Bradwejn, J. (1995). Impaired exploratory behaviour after DSP-4 treatment in rats: Implications for the increased anxiety after noradrenergic denervation. *European Neuropsychopharmacology, 5*, 447–455.

Harro, J., Häidkind, R., Harro, M., Modiri, A. R., Gillberg, P. G., Pähkla, R., Matto, V., & Oreland, L. (1999). Chronic mild unpredictable stress after noradrenergic denervation: Attenuation of behavioural and biochemical effects of DSP-4 treatment. *European Neuropsychopharmacology, 10*, 5–16.

Hatip-Al-Khatib, I., Mishima, K., Iwasaki, K., & Fujiwara, M. (2001). Microdialysates of amines and metabolites from core nucleus accumbens of freely moving rats are altered by dizocilpine. *Brain Research, 902*, 108–118.

Heal, D. J., Butler, S. A., Prow, M. R., & Buckett, W. R. (1993). Quantification of presynaptic alpha 2- adrenoceptors in rat brain after short-term DSP-4 lesioning. *European Journal of Pharmacology, 249*, 37–41.

Hörtnagl, H., Potter, P. E., Kindel, G., & Hanin, I. (1989). Noradrenaline depletion protects cholinergic neurons in rat hippocampus against AF64A-induced damage. *Journal of Neuroscience Methods, 27*, 103–108.

Howard, B. D., Cho, A. K., Zhang, M. B., Koide, M., & Lin, S. (1990). Covalent labeling of the cocaine-sensitive catecholamine transporter. *Journal of Neuroscience Research, 26*, 149–158.

Hughes, Z. A., & Stanford, S. C. (1998a). A partial noradrenergic lesion induced by DSP-4 increases extracellular noradrenaline concentration in rat frontal cortex: A microdialysis study in vivo. *Psychopharmacology, 136*, 299–303.

Hughes, Z. A., & Stanford, S. C. (1998b). Evidence from microdialysis and synaptosomal studies of rat cortex for noradrenaline uptake sites with different sensitivities to SSRIs. *British Journal of Pharmacology, 124*, 1141–1148.

Jackisch, R., Gansser, S., & Cassel, J. C. (2008). Noradrenergic denervation facilitates the release of acetylcholine and serotonin in the hippocampus: Towards a mechanism underlying upregulations described in MCI patients? *Experimental Neurology, 213*, 345–353.

Jaim-Etcheverry, G. (1998). 2-Chloroethylamines (DSP-4 and Xylamine). In R. M. Kostrzewa (Ed.), *Highly selective neurotoxins: Basic and clinical applications* (pp. 131–140). Totowa: Humana Press.

Jaim-Etcheverry, G., & Zieher, L. M. (1980). DSP-4: A novel compound with neurotoxic effects on noradrenergic neurons of adult and developing rats. *Brain Research, 188*, 513–523.

Jonsson, G. (1980). Chemical neurotoxins as denervation tools in neurobiology. *Annual Review of Neuroscience, 3*, 169–187.

Jonsson, G., Hallman, H., Ponzio, F., & Ross, S. (1981). DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) – A useful denervation tool for central and peripheral noradrenaline neurons. *European Journal of Pharmacology, 72*, 173–188.

Jonsson, G., Hallman, H., & Sundström, E. (1982). Effects of the noradrenaline neurotoxin DSP-4 on the postnatal development of central noradrenaline neurons in the rat. *Neuroscience, 7*, 2895–2907.

Kask, A., Harro, J., Tuomaine, P., Rägo, L., & Männistö, P. T. (1997). Overflow of noradrenaline and dopamine in frontal cortex after [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] (DSP-4) treatment: In vivo microdialysis study in anaesthetized rats. *Naunyn-Schmiedeberg's Archives of Pharmacology, 355*, 267–272.

Kostrzewa, R. M., & Garey, R. E. (1977). Sprouting of noradrenergic terminals in rat cerebellum following neonatal treatment with 6-hydroxydopa. *Brain Research, 124*, 385–391.

Landa, M. E., Rubio, M. C., & Jaim-Etcheverry, G. (1984). The neurotoxic compound N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) depletes endogenous norepinephrine and enhances release of [^{3}H]norepinephrine from rat cortical slices. *Journal of Pharmacology and Experimental Therapeutics, 231*, 131–136.

Lategan, A. J., Marien, M. R., & Colpaert, F. C. (1992). Suppression of nigrostriatal and mesolimbic dopamine release in vivo following noradrenaline depletion by DSP-4: A microdialysis study. *Life Sciences, 50*, 995–999.

Lee, C. M., Javitch, J. A., & Snyder, S. H. (1982). Characterization of [3H]desipramine binding associated with neuronal norepinephrine uptake sites in rat brain membranes. *Journal of Neuroscience, 10*, 1515–1525.

Logue, M. P., Growdon, J. H., Coviella, I. L., & Wurtman, R. J. (1985). Differential effects of DSP-4 administration on regional brain norepinephrine turnover in rats. *Life Sciences, 37*, 403–409.

Lyles, G. A., & Callingham, B. A. (1981). The effect of DSP-4 on monoamine oxidase activities in tissues of the rat. *Journal of Pharmacy and Pharmacology, 33*, 632–638.

Medina, J. H., & Novas, M. L. (1983). Parallel changes in brain flunitrazepam binding and density of noradrenergic innervation. *European Journal of Pharmacology, 88*, 377–382.

Mishra, P. K., Burger, R. L., Bettendorf, A. F., Browning, R. A., & Jobe, P. C. (1994). Role of norepinephrine in forebrain and brainstem seizures: Chemical lesioning of locus ceruleus with DSP-4. *Experimental Neurology, 125*, 58–64.

Nowak, P., Labus, Ł., Kostrzewa, R. M., & Brus, R. (2006). DSP-4 prevents dopamine receptor priming by quinpirole. *Pharmacology Biochemistry and Behavior, 84*, 3–7.

Ohno, M., Yamamoto, T., Kobayashi, M., & Watanabe, S. (1993). Impairment of working memory induced by scopolamine in rats with noradrenergic DSP-4 lesions. *European Journal of Pharmacology, 238*, 117–120.

Olpe, H. R., Laszlo, J., Dooley, D. J., Heid, T., & Steinmann, M. W. (1983). Decreased activity of locus coeruleus neurons in the rat after DSP-4 treatment. *Neuroscience Letters, 40*, 81–84.

Prieto, M., & Giralt, M. T. (2001). Effects of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) on alpha2-adrenoceptors which regulate the synthesis and release of noradrenaline in the rat brain. *Pharmacology and Toxicology, 88*, 152–158.

Ransom, R. W., Kammerer, R. C., & Cho, A. K. (1982). Chemical transformations of xylamine (N-2′-chloroethyl-N-ethyl-2-methylbenzylamine) in solution. Pharmacological activity of the species derived from this irreversible norepinephrine uptake inhibitor. *Molecular Pharmacology, 21*, 380–386.

Ransom, R. W., Waggaman, L. A., & Cho, A. K. (1985). Interaction of xylamine with peripheral sympathetic neurons. *Life Sciences, 37*, 1177–1182.

Ross, S. B. (1976). Long-term effects of N-2-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride on noradrenergic neurones in the rat brain and heart. *British Journal of Pharmacology, 58*, 521–527.

Sachs, C., & Jonsson, G. (1975). Effects of 6-hydroxydopamine on central noradrenaline neurons during ontogeny. *Brain Research, 99*, 277–291.

Schuerger, R. J., & Balaban, C. D. (1995). N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) has differential efficacy for causing central noradrenergic lesions in two different rat strains: Comparison between Long-Evans and Sprague–Dawley rats. *Journal of Neuroscience Methods, 58*, 95–101.

Snyder, S. H., & Coyle, J. T. (1969). Regional differences in ^{3}Hnorepinephrine and ^{3}H-dopamine uptake into rat brain homogenates. *Journal of Pharmacology and Experimental Therapeutics, 165*, 78–86.

Sontag, T. A., Hauser, J., Kaunzinger, I., Gerlach, M., Tucha, O., & Lange, K. W. (2008). Effects of the noradrenergic neurotoxin DSP-4 on spatial memory in the rat. *Journal of Neural Transmission, 115*, 299–303.

Suzdak, P. D., & Gianutsos, G. (1985). GABA-noradrenergic interaction: Evidence for differential sites of action for GABA-A and GABA-B receptors. *Journal of Neural Transmission, 64*, 163–172.

Szot, P., Miguelez, C., White, S. S., Franklin, A., Sikkema, C., Wilkinson, C. W., Ugedo, L., & Raskind, M. A. (2010). A comprehensive analysis of the effect of DSP-4 on the locus coeruleus noradrenergic system in the rat. *Neuroscience, 166*, 279–291.

Theron, C. N., de Villiers, A. S., & Taljaard, J. J. (1993). Effects of DSP-4 on monoamine and monoamine metabolite levels and on beta adrenoceptor binding kinetics in rat brain at different times after administration. *Neurochemical Research, 18*, 1321–1327.

Vizi, E. S., & Kiss, J. P. (1998). Neurochemistry and pharmacology of the major hippocampal transmitter systems: Synaptic and nonsynaptic interactions. *Hippocampus, 8*, 566–607.

Weinshenker, D., Ferrucci, M., Busceti, C. L., Biagioni, F., Lazzeri, G., Liles, L. C., Lenzi, P., Pasquali, L., Murri, L., Paparelli, A., & Fornai, F. (2008). Genetic or pharmacological blockade of noradrenaline synthesis enhances the neurochemical, behavioral, and neurotoxic effects of methamphetamine. *Journal of Neurochemistry, 105*, 471–483.

Wenge, B., & Bönisch, H. (2009). Interference of the noradrenergic neurotoxin DSP-4 with neuronal and nonneuronal monoamine transporters. *Naunyn-Schmiedeberg's Archives of Pharmacology, 380*, 523–529.

Wolfman, C., Abó, V., Calvo, D., Medina, J., Dajas, F., & Silveira, R. (1994). Recovery of central noradrenergic neurons one year after the administration of the neurotoxin DSP-4. *Neurochemistry International, 25*, 395–400.

Yu, P. H., Davis, B. A., Fang, J., & Boulton, A. A. (1994). Neuroprotective effects of some monoamine oxidase B inhibitors against DSP-4-induced noradrenaline depletion in the mouse hippocampus. *Journal of Neurochemistry, 63*, 1820–1828.

Zaczek, R., Fritschy, J. M., Culp, S., De Souza, E. B., & Grzanna, R. (1990). Differential effects of DSP-4 on noradrenaline axons in cerebral cortex and hypothalamus may reflect heterogeneity of noradrenaline uptake sites. *Brain Research, 522*, 308–314.

Zahniser, N. R., Weiner, G. R., Worth, T., Philpott, K., Yasuda, R. P., Jonsson, G., & Dunwiddie, T. V. (1986). DSP-4-induced noradrenergic lesions increase beta-adrenergic receptors and hippocampal electrophysiological responsiveness. *Pharmacology Biochemistry and Behavior, 24*, 1397–1402.

Zhang, X., Zuo, D. M., Davis, B. A., Boulton, A. A., & Yu, P. H. (1996). Immunohistochemical evidence of neuroprotection by R(2)-deprenyl and N-(2-hexyl)-N-methylpropargylamine on DSP-4-induced degeneration of rat brain noradrenergic axons and terminals. *Journal of Neuroscience Research, 43*, 482–489.

MPTP Neurotoxicity: Actions, Mechanisms, and Animal Modeling of Parkinson's Disease

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Contents

Abstract

The study of neurotoxicity induced by MPTP led to drastically change the perspective on Parkinson's Disease. In fact the selective neurotoxicity induced by MPTP rejuvenated PD research and generated a number of studies aimed at

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elucidating the mechanisms of action of MPTP. Remarkably, these molecular mechanisms turned out to be critical also for the survival of DA neurons in idiopathic PD. In this chapter we report the main concepts developed over the last three decades to understand key molecular steps which are pivotal in MPTP toxicity. This is the case of the role played by DAT and VMAT-2 in conditioning the sensitivity to MPTP neurotoxicity. Similarly, the mitochondria as targets of MPTP toxicity appear similarly affected by selective mutation of genes leading to PD. Again, the fate of mitochondria and the ability to clear these organelles when being dysfunctional is key in the modulation of MPTP toxicity. This also applies for misfolded proteins such as alpha synuclein. Again, multiple brain areas as well as peripheral sites are increasingly recognized to be affected both during MPTP toxicity and sporadic PD patients. Nowadays it seems that MPTP per se did not lead to discovery of the environmental compound which causes PD, nonetheless the study of MPTP did disclose several molecular and cellular pathways which are critical in the genesis of PD. This latter point fairly corresponds to what we enthusiastically expected from MPTP when it was identified as a causal agent of what it remains, a toxic form of environmental parkinsonism.

Keywords

Animal models • MPP+ • Parkinsonism

List of Abbreviations

AMPA	Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
ATG	Autophagy
ATP	Adenosine triphosphate
BBB	Blood–brain barrier
COX	Cyclooxygenase
DA	Dopamine
DAT	DA transporter
DSP-4	*N*-(−2-chloroethyl)-*N*-ethyl-2-bromobenzylamine
EAA	Excitatory amino acids
ENS	Enteric nervous system
GI	Gastrointestinal
GSH	Glutathione
i.c.v.	Intracerebroventricular
IP_3	Inositol(1,4,5)trisphosphate
JNK	c-Jun N-terminal kinase
KO	Knockout
LC	Locus coeruleus
MAO-B	Monoamine oxidase type B
METH	Methamphetamine
MK-801	Dizocilpine
Mn-SOD	Manganese superoxide dismutase
$MPDP^+$	1-Methyl-4-phenyl-2,3-dihydropyridine
MPP^+	1-Methyl-4-phenylpyridinium

MPPP	1-Methyl-4-phenyl-4-propionoxy-piperidine
MPT	Mitochondrial permeability transition
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAD	Nicotinamide adenine dinucleotide
NE	Norepinephrine
NET	NE transporter
NMDA	*N*-methyl-D-aspartate
NO	Nitric oxide
PD	Parkinson's diseases
ROS	Reactive oxygen species
SC	Spinal cord
SNpc	Substantia nigra pars compacta
SOD	Superoxide dismutase
TH	Tyrosine hydroxylase
UP	Ubiquitin–proteasome
VMAT-2	Vesicular monoamine transporter type 2

1 Introduction

The history of MPTP began in 1947, when researchers from a North American pharmaceutical company named Hoffmann-La Roche while developing compounds as painkillers synthesized a novel opioid derivative named meperidine (Demerol$^{(R)}$). In this context, Dr. Ziering synthesized a meperidine derivative, 1-methyl-4-phenyl-4-propionoxy-piperidine (MPPP, Fig. 1), which possesses the meperidine structure, containing an esteric group (Ziering et al. 1947). Since MPPP turned out not to be more effective than Demerol, it was never marketed. In 1976, a 23-year-old Maryland chemistry graduate named Barry Kidston, in the attempt to synthesize MPPP to explore its potential as a recreational drug, esterified the intermediate tertiary alcohol of MPPP with propionic anhydride at a drastically high temperature. This led to the synthesis of 3 % of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as a by-product of MPPP (Fig. 1). Within 3 days following MPTP intake, Kidston developed parkinsonism, which was successfully treated with L-DOPA. However, he died 18 months later due to cocaine overdosing. The neuropathological exam was in line with neurological symptoms showing dopamine (DA) neuron loss in the substantia nigra *pars compacta* (SNpc) (Davis et al. 1979). Kidston's case remained unique for a few years; in 1982, in Northern California during a very short time, a sort of epidemic parkinsonism was reported in Santa Clara Valley Medical Center in San Jose (CA) by Dr. J. William Langston, the hospital's chief neurologist. Dr. Langston initially described seven cases, but this number increased soon after, when several cases of severe parkinsonism occurring in young adults were described in the same area. It was evident that these patients were drug addicts and all of them injected *intravenously* an illicit street drug called "Super Demerol"

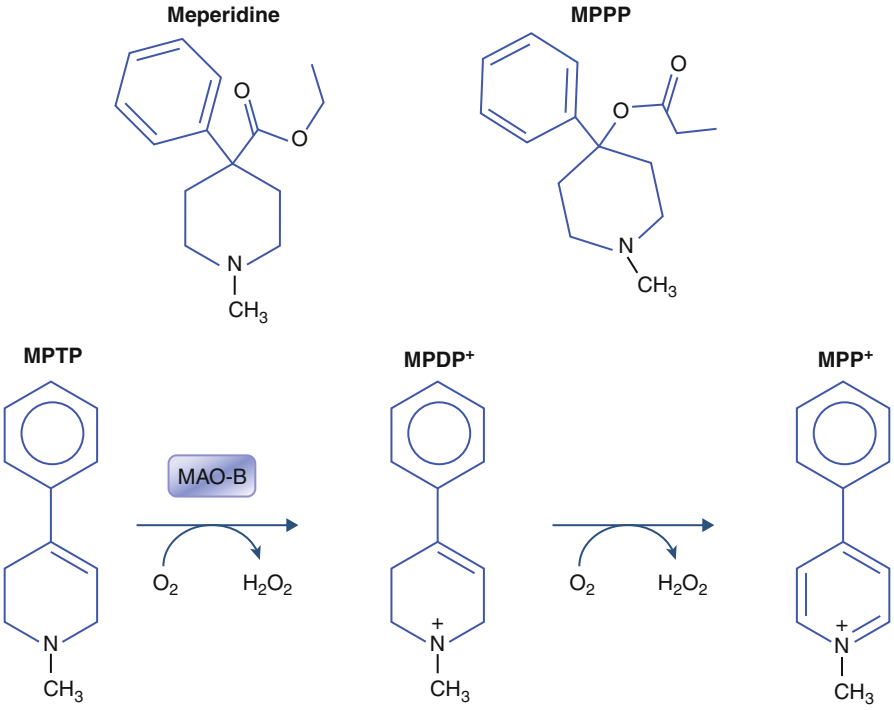

Fig. 1 Structural formula of meperidine analogues. The cartoon shows the structural affinity between MPTP and its metabolites with meperidine and MPPP. MPTP was produced as by-product in the effort to synthesize MPPP. Occurrence of MPTP and administration of the compound to a variety of animal species lead to the enzymatic oxidation by monoamine oxidase (*MAO*) type B to produce the intermediate $MPDP^+$ which, in turn, undergoes spontaneous oxidation to MPP^+

which turned out to contain significant amount of MPTP (Langston et al. 1983). Also these patients showed the clinical features of idiopathic Parkinson's disease (PD), including bradykinesia, rigidity, and, in some cases, resting tremor (Ballard et al. 1985). Again, these symptoms were relieved by the intake of oral L-DOPA, while postmortem analysis confirmed the occurrence of massive cell loss in the SNpc (Ballard et al. 1985). Only later on the occurrence of Lewy bodies in these patients was demonstrated thus adding on to the remarkable overlapping with idiopathic PD (Langston et al. 1999).

The complete story of these patients was reported by Dr. William Langston in his monograph "The Case of the Frozen Addicts" (Langston and Palfreman 1996).

After identification of MPTP as the causal agent for the epidemic environmental parkinsonism, neuroscientists involved in PD research evaluated its neurotoxic effects in a variety of animal species including nonhuman primates, rodents, cats, pigs, dogs, sheep, rats, and goldfishes (Kopin and Markey 1988; Gerlach and Riederer 1996; Przedborski et al. 2001). It was soon evident that the neurotoxic properties of MPTP vary depending to which animal it was administered. In fact,

when MPTP is administered to monkeys, most clinical and pathological hallmarks of PD can be reproduced (Langston et al. 1984a; Chiueh et al. 1984; Doudet et al. 1985; Crossman et al. 1985; Nomoto et al. 1985; Vezoli et al. 2011), while rats are quite resistant to MPTP toxicity (Chiueh et al. 1984).

The discovery of selective neurotoxicity induced by MPTP rejuvenated PD research and generated a number of studies aimed at elucidating the mechanisms of action of MPTP.

In fact, it was postulated that MPTP analogues such as pesticides may represent environmental neurotoxins responsible for the etiology of idiopathic PD. Moreover, for the first time, an experimental model mimicking both neurological symptoms and key neuropathological features of PD was available. This experimental setting appeared to be ideal to dissect the most intimate molecular mechanisms generating selective neurodegeneration underlying PD. A bulk of studies now demonstrate that MPTP analogues are not primarily involved in PD etiology. Moreover, now we are aware that PD possesses a strong genetic background which limits the role of environmental factors. Again, even in primates, some discrepancies between PD and MPTP-induced parkinsonism do exist. For instance, the striatal pattern of DA loss in PD involves more the putamen than caudate nucleus (as witnessed by pathobiochemistry, neuropathology, and more recently brain imaging, Kish et al. 1988; Jellinger 2002; Brooks and Pavese 2011), while MPTP does not discriminate between DA innervations of various neostriatal regions (Pifl et al. 1988). Nonetheless, MPTP intoxication still represents the best experimental tool to mimic very closely the selective and specific features which characterize PD. This extends also to those neuropathological features which were not immediately evident in MPTP-intoxicated patients, such as the occurrence of neuronal inclusions. In fact, neuronal inclusions known as Lewy bodies which are considered a hallmark of PD were not described at first following sporadic injections of MPTP. Now we are aware that in MPTP-intoxicated patients, Lewy bodies do occur (Langston et al. 1999).

Again, clinical and pathological research on PD in the last three decades demonstrated that despite the multiple etiology (various genetic forms or idiopathic), the disease is constantly characterized by degeneration of multiple areas both within the CNS (Braak et al. 2003) and outside the brain, the spinal cord, and periphery. Accordingly, research on MPTP intoxication cops quite perfectly with such novel findings in PD. In fact, nowadays we are aware that by selecting the appropriate animal species and MPTP dosing regimen, we can reproduce most of these extra-nigral degenerations in multiple brain and peripheral areas. Thus, apart from discrepancies in the topography of nigral cell loss and nigrostriatal denervation, at present we can firmly establish the occurrence of neuronal inclusions and the involvement of multiple brain sites and peripheral regions following MPTP intoxication. This renders the MPTP model constantly updated in following progress in PD research. In fact, some potential discrepancies were resolved progressively adapting in-depth studies on MPTP intoxication to the extended degeneration which was increasingly described in PD.

In order to analyze MPTP toxicity, it is mandatory to dissect those molecular steps which characterize MPTP metabolism and the dynamics of MPTP in target cells. At the same time, after reviewing main concepts which characterize MPTP

toxicity at neuronal level, we will mention briefly those neurotransmitter and biochemical pathways which either contribute to detrimental effects or mediate compensatory mechanisms which counteract MPTP intoxication.

2 MPTP Metabolism

MPTP is a highly lipophilic substance (Fig. 1) that easily crosses the blood–brain barrier (BBB) (Fig. 2). In the brain, MPTP is metabolized to 1-methyl-4-phenylpyridinium (MPP^+) (Figs. 1 and 2) via the formation of the intermediate 1-methyl-4-phenyl-2,3-dihydropyridine ($MPDP^+$, Fig. 1), this oxidation being catalyzed by monoamine oxidase type B (MAO-B) (Chiba et al. 1984; Kopin 1987) (Figs. 1 and 2). Once formed, MPP^+, which is a polar compound, cannot cross back the BBB. MPP^+ is the real neurotoxin at cellular level; in fact MAO inhibitors (which impede the generation of MPP^+ from MPTP) provide complete protection against MPTP toxicity (Heikkila et al. 1984a; Langston et al. 1984b). When MPP^+ is administered systemically, there is no damage within the CNS due to the high polarity of the MPP^+ ion which impedes the crossing of the BBB. On the other hand, when MPP^+ is administered intracerebroventricularly (*i.c.v.*), toxicity in the CNS does occur (Sundström et al. 1986; Giovanni et al. 1994a, b; Sonsalla et al. 2008). Once crossed the BBB, MPTP can interact with MAO-B placed in several cell types (including astrocytes and serotonergic neurons). Since glial cells are abundant in the CNS, MAO-B in astrocytes are thought to be mainly involved in MPTP metabolism (Brooks et al. 1989), while a damage to serotonin neurons fails to protect from MPTP toxicity (Melamed et al. 1986). Once formed MPP^+ is released from glia in the extracellular space, and it enters within DA neurons via the high-affinity DA transporter (DAT, Javitch et al. 1984) (Fig. 2). This was first demonstrated by preventing MPTP-induced DA toxicity using selective DAT inhibitors (Pileblad and Carlsson 1985; Ricaurte et al. 1985; Sundstrom & Jonsson 1985). Uptake selectivity contributes to the selective toxicity of MPP^+. In fact, MPP^+ is also taken up by norepinephrine (NE) neurons through the NE transporter (NET). Indeed, the uptake within NE neurons exceeds that occurring in DA cells in spite of the fact that NE neurons were originally considered not to be involved in MPP^+ toxicity (Herkenham et al. 1991). Now we are aware that the dosing protocol of MPTP is critical in producing a damage which also involves NE neurons (Seniuk et al. 1990). Remarkably, this point provides a further convergence between MPTP and PD since the damage to NE neurons mostly placed in the nucleus locus coeruleus (LC) is a typical feature of PD patients (Gesi et al. 2000; Zarow et al. 2003, see also in this chapter the Sect. 8). Once entered the DA/NE axons, MPP^+ is accumulated in the cytoplasm where it forms a complex with neuromelanin (D'Amato et al. 1987), being otherwise stored within DA/NE vesicles through the vesicular monoamine transporter type 2 (VMAT-2) (Fig. 2). From these storage sites, MPP^+ is released into the cytoplasm where it may enter the mitochondrion (Ramsay and Singer 1986), which is believed to be the ultimate target to produce neurotoxicity (Fig. 2). Therefore, it is expected that reducing the entry of MPP^+ into

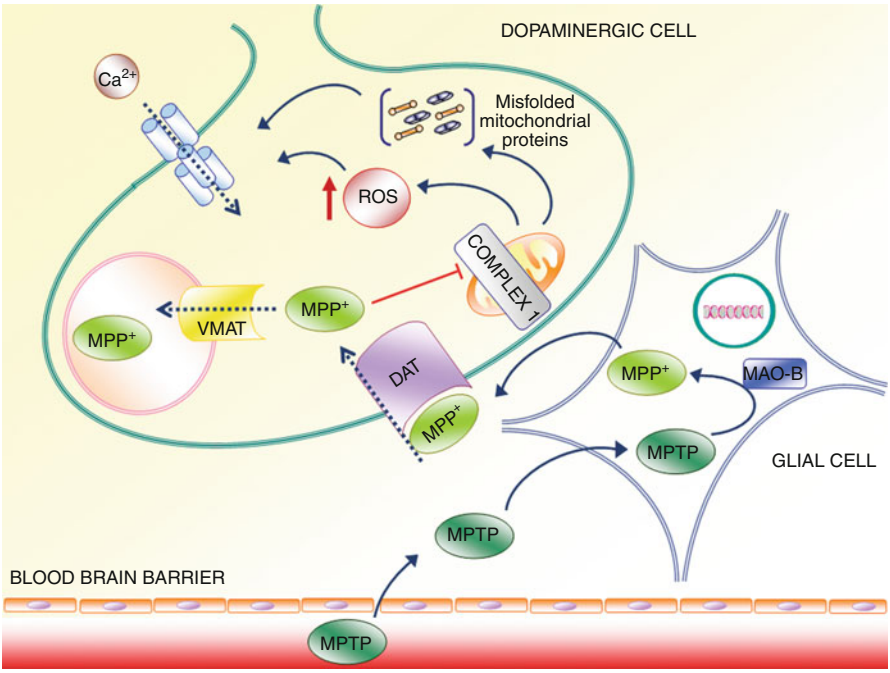

Fig. 2 Kinetics and dynamics of MPTP in the CNS. Once injected MPTP, which is a lipophilic compound, easily crosses the blood–brain barrier. This allows MPTP to enter within the CNS, where it is converted by monoamine oxidase (*MAO*)-B into MPP^+ which is actively secreted from glia in the extracellular space. MPP^+ is taken up quite selectively mostly by catecholamine neurons through the high-affinity DA transporter (DAT as shown in the cartoon) by also the NE transporter (*NET*, not shown). This allows MPP^+ to access DA axons where it can be stored either within synaptic vesicles through the vesicular monoamine transporter (*VMAT*) or within mitochondria. MPP^+ within mitochondria binds to complex I to inhibit NADH dehydrogenase. The production of reactive oxygen species (*ROS*) and other free radicals may be the consequence of mitochondrial impairment and the direct oxidative effects of MPP^+ on specific proteins and the oxidative effects of DA which is released by MPP^+ from synaptic vesicles on specific targets. Increased amount of oxidative species produced by all these mechanisms is expected to alter the folding of a variety of cell proteins. This leads to the accumulation of misfolded proteins which mostly consist of co-chaperones such as alpha-synuclein. The amount of oxidative stress is increased by the concomitant opening of calcium (Ca^{2+}) channels which may derive from the overactivation of glutamate receptors

the axons or increasing the storage into the vesicles should protect from MPTP toxicity (Staal and Sonsalla 2000). In fact, MPTP toxicity does not occur in DAT knockout (KO) mice (Gainetdinov et al. 1997), while MPTP toxicity is increased in VMAT-2 heterozygote KO mice (Gainetdinov et al. 1998). In line with this, mice with a 50 % depletion of VMAT show increased vulnerability to MPTP (Takahashi et al. 1997). Thus, synaptic vesicles represent storage sites which buffer MPP^+ and prevent its interaction with mitochondria and other cytosolic targets of toxicity (i.e., chaperone proteins). Intracellular compartmentalization of MPP^+ is critical to understand species differences in MPTP toxicity (Fig. 2).

3 MPP^+ and Mitochondria

Although the ultimate mechanisms of cell death induced by MPP^+ remain unclear, strong evidence indicates that MPP^+ inhibits mitochondrial function. MPP^+ enters into mitochondria to inhibit NADH dehydrogenase within mitochondrial respiratory chain (complex I, Fig. 2, Nicklas et al. 1985; Ramsay et al. 1986), thus binding to the same site as rotenone, which is a classic complex I inhibitor (Heikkila et al. 1985; Ramsay and Singer 1986; Krueger et al. 1990). This leads to morphological damage of mitochondria as shown in Fig. 3. As shown in Fig. 4, impairment of mitochondrial respiration by MPP^+ is believed to decrease the levels of oxidized nicotinamide adenine dinucleotide (NAD) (Sonsalla et al. 1992). In line with this, MPP^+ neurotoxicity is related to the failure of energy supplies in a number of in vitro and in vivo experimental settings (Di Monte et al. 1986; Denton and Howard 1987). For instance, within synaptosome preparations, MPP^+ dose-dependently decreases adenosine triphosphate (ATP) content (Scotchner et al. 1990). These effects produce a failure of electrogenic pumps such as $3Na^+$-$2K^+$ ATPases (Storey et al. 1992), which determines depolarization which leads to a sudden release of neurotransmitters. In fact, acute DA depletion caused by MPTP is preceded by a decrease in ATP levels in vivo within mouse striatum (Chan et al. 1993a). Consistently, the depletion of energy stores occurs more in those regions which take up selectively MPP^+ such as the nigrostriatal DA system (and other catecholamine-containing areas both in the CNS and periphery). This is

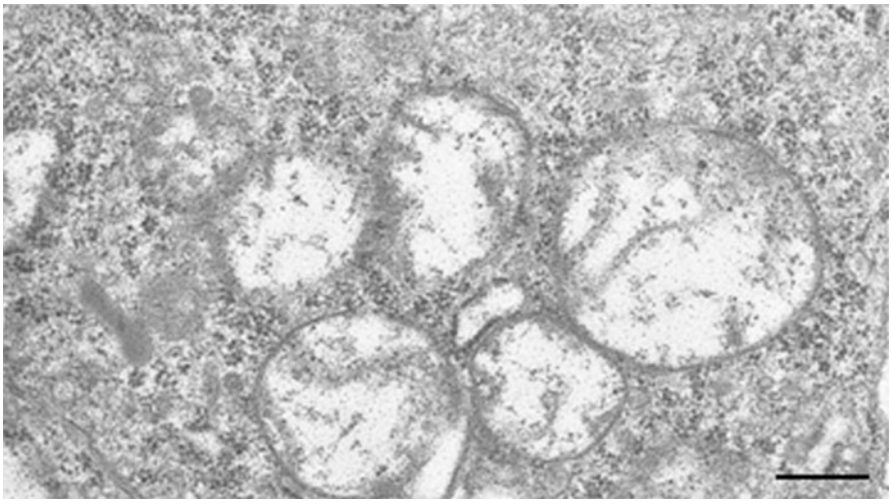

Fig. 3 Electron micrograph of mitochondria following MPTP exposure. MPTP exposure targets mitochondria which are functionally impaired but also deranged in their fine structure. This electron micrograph shows mitochondria within DA neurons from a C57 black mouse treated with MPTP (20 mg/Kg, X4, 2 h apart). Mitochondria lose their crests and undergo severe matrix dilution. Bar=0.24μm (Unpublished data, courtesy of Dr. P Lenzi, University of Pisa)

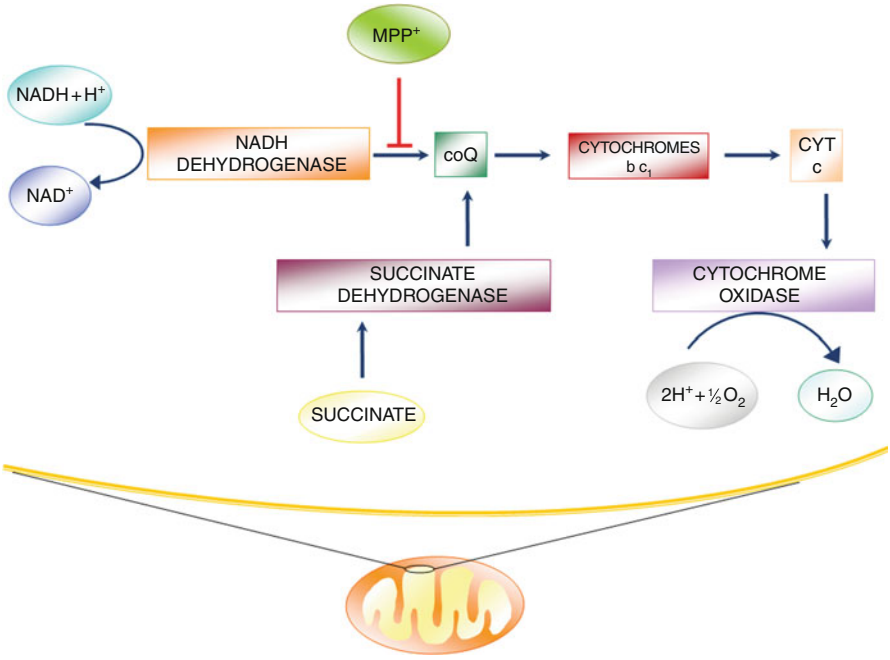

Fig. 4 The effects of MPP^+ on mitochondrial respiratory chain. The cartoon shows the molecular target of MPP^+ within the mitochondrial respiratory chain at the level of complex I. This corresponds mainly to the same site targeted by rotenone (see "▶ Rotenone as Preclinical Model Compound in Parkinson Disease" in this book) and it affects the enzyme NADH dehydrogenase. Thus, MPP^+ blocks NADH dehydrogenase, which produces a variety of downstream effects consisting of ATP loss, formation of ROS, and induction of apoptotic proteins

why, apart from DA toxicity, even the transient DA depletion, which occurs suddenly following MPTP administration, can be prevented by DA uptake inhibitors (Chan et al. 1991).

The identification of mitochondria as the primary target of MPTP-induced toxicity cops well with mitochondrial defects occurring in idiopathic parkinsonian patients as well as the mutations in mitochondrial proteins described in genetic forms of PD (Gu et al. 1998; Dodson & Guo 2007; Büeler 2009).

4 MPP^+ and Free Radicals

In physiological conditions free radicals, including reactive oxygen species (ROS), are constantly produced as the consequence of several chemical reactions in the cell including mitochondrial metabolism (Figs. 2 and 4). Routinely, this is balanced by antioxidants, free radical scavengers, and a variety of chaperones, which lead to either innocuous end-products or detrimental compounds. Antioxidants include

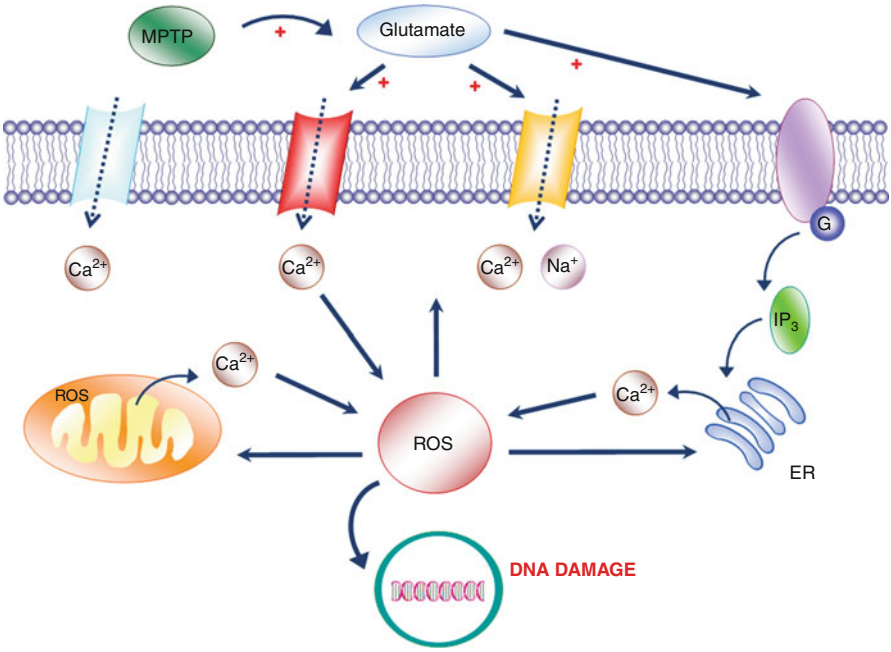

Fig. 5 Glutamate binds to ionotropic and metabotropic receptors. MPTP exposure increases the level of extracellular amino acid transmitters. In the case of this cartoon, glutamate overactivity stimulates both ionotropic (NMDA, kainate and AMPA) receptors and several subtypes of metabotropic receptors. This glutamate overactivity leads to the entry of a variety of ions. In particular, calcium entry, which is also modulated by metabotropic receptors acting on IP_3, is a strong candidate to contribute to free radical and ROS increase in DA neurons

superoxide dismutase (SOD), glutathione peroxidase, catalase, glutathione (GSH) and coenzyme Q10, essential nutrients (vitamin C, vitamin E, selenium, etc.), and dietary compounds (such as bioflavonoids). Oxidative stress results from an imbalance between formation and neutralization of pro-oxidants.

Mitochondrial impairment increases the production of free radicals (Fig. 2), and these effects may be enhanced by glutamate overactivity (Fig. 5; see also the Sect. 6, in this chapter), being one the consequence of the other. Impairment of mitochondrial function and decrease of cellular ATP may increase free radicals and mostly ROS (Cleeter et al. 1992).

Mitochondrial ROS react with several targets, including manganese SOD (Mn-SOD, SOD2), nitric oxide (NO), and susceptible iron–sulfide (Fe–S)-containing proteins. Mn-SOD is an antioxidant enzyme which protects against ROS produced by mitochondria. In fact, overexpression of Mn-SOD in transgenic mice protects against MPP^+ toxicity (Klivenyi et al. 1998; Maragos et al. 2000). On the other hand, heterozygous Mn-SOD KO mice show higher DA depletion and significantly larger striatal lesions after systemic MPTP treatment compared with controls (Andreassen et al. 2001). Again, overexpression of copper–zinc SOD

(Cu–Zn SOD, SOD1) in transgenic mice protects against MPTP neurotoxicity (Przedborski et al. 1992), and similar effects occur in mice overexpressing either human wild-type SOD1 or human mutant SOD1-G93A (Fornai et al. 2002). This may be due to the interaction of SOD1 with mitochondrial complex I activity thus competing with MPP^+ (Browne et al. 1998; Fornai et al. 2002).

It is puzzling that, apart from conferring protection against MPTP toxicity, the mutation in the gene coding for the human SOD1 leads to a spontaneous loss of nigral DA neurons (Andreassen et al. 2001).

5 MPTP, Protein Clearing Mechanisms, and Apoptosis

When acutely administered, MPTP does not affect much protein clearance, but directly targets the mitochondria. In contrast, when MPTP intoxication is continuously protracted for a few weeks, it impairs protein clearing mechanisms (Fornai et al. 2005). This is due mainly to the oxidation of specific proteins such as alpha-synuclein which works as co-chaperone (Fig. 6, Chandra et al. 2004, 2005). In fact following MPTP administration, there is an increased expression of alpha-synuclein in the cytoplasm (Fornai et al. 2005; Purisai et al. 2005) which may induce the formation of toxic alpha-synuclein oligomers (Stefanis et al. 2001; Tanaka et al. 2001; Bucciantini et al. 2002; Walsh et al. 2002; Giorgi et al. 2006), thus leading to neuronal toxicity (Fig. 6).

In this way, despite of its role as co-chaperone (Chandra et al. 2005), alpha-synuclein may become a detrimental compound for the survival of both DA and NE neurons once oxidized in excess during MPTP intoxication (Figs. 6 and 7, Conway et al. 2000; Fornai et al. 2005). In fact, chronic or continuous exposure to MPTP (Fornai et al. 2005; Meredith et al. 2008) is accompanied by pathological protein aggregates containing ubiquitin and alpha-synuclein since free radicals and ROS react with alpha-synuclein producing toxic oligomers which cannot be metabolized (Conway et al. 2000; Sulzer 2001) (Figs. 6, 7 and 8).

In order to counteract the formation of detrimental protein adducts, the proper activity of protein clearing pathways is critical. This explains why, in recent years, a variety of research studies both on MPTP toxicity and PD focussed on protein clearing systems. The ubiquitin–proteasome (UP) system (Fig. 7) and autophagy (ATG, Fig. 8) are the major systems that degrade intracellular components in eukaryotic cells. The UP system (Fig. 7) mainly serves to degrade short-lived misfolded proteins (Ciechanover 2006). These proteins are targeted by polyubiquitin conjugation, which addresses them to proteolytic degradation within the proteasome core. The ATG pathway is involved in the degradation of long-lived proteins, protein aggregates, and organelles, such as mitochondria, which are delivered to lysosomes for digestion (Fig. 8, Mariño and López-Otín 2004). ATG was found to be increased within the substantia nigra of PD patients by Anglade and collaborators (1997). These authors found the coexistence of ATG vacuoles and apoptosis within melanized neurons of the human substantia nigra. The neurons undergoing an excess of ATG possess condensation of chromatin, moderate vacuolation of endoplasmic

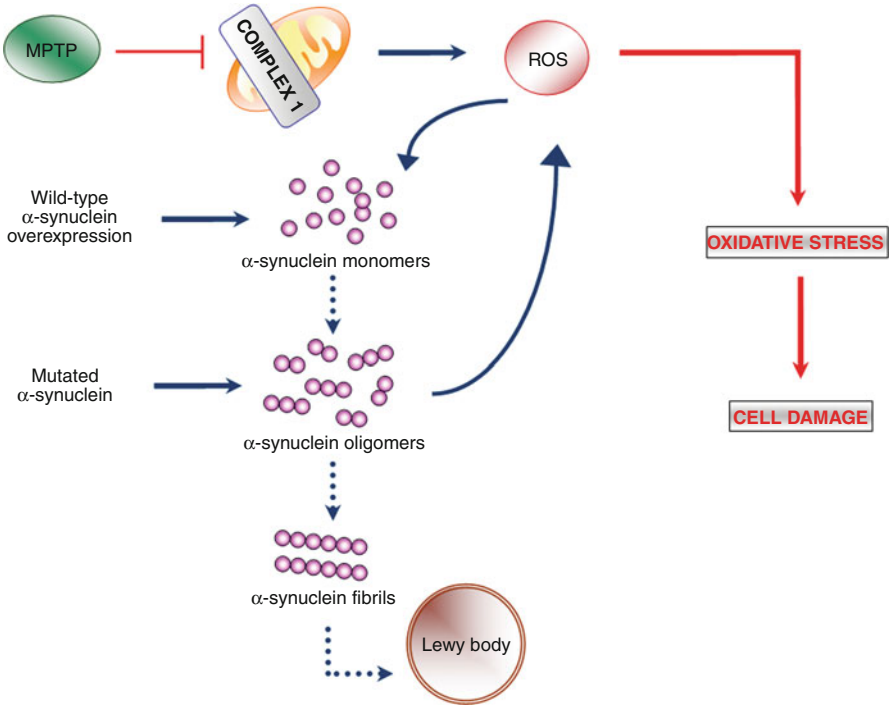

Fig. 6 MPTP recapitulates protein misfolding which may primarily occur in genetic PD. MPTP administration, mainly via inhibition of mitochondria, or directly, or via DA release, produces high amount of oxidative species which can interact with co-chaperones such as alpha-synuclein to stabilize this protein in oligomeric forms. These oligomers are also produced as a consequence of genetic mutations in alpha-synuclein gene as occurs in a few cases of inherited PD. In all cases alpha-synuclein adducts are toxic for the cells and can either precipitate to form Lewy bodies or spread to specific cell compartments to impair key biochemical pathways thus contributing to DA toxicity

reticulum, and lysosome-like vacuoles containing cytoplasmic material. The existence of dual neuronal death suggests the occurrence of both ATG and apoptosis at different time intervals during degeneration of DAergic cells, with apoptosis being the final stage. This is in line with the recent evidence showing that in DAergic cells dysfunctional ATG triggers apoptosis (Fig. 9, Castino et al. 2008).

A defective protein clearance is supposed to lead to protein accumulation even when misfolded proteins are not produced in great amount. This is in line with the fact that MPTP intoxication, just like PD, produces both ATG alterations and apoptosis (Fig. 9). This is witnessed by the accumulation of altered mitochondria (Fig. 3) and large ATG vacuoles which follows MPTP intoxication. This explains why rapamycin, by activating ATG and promoting lysosomal activity (Fig. 10), attenuates MPTP-induced DA neurodegeneration, thus promoting neuroprotection (Dehay et al. 2010). In addition, in MPTP-treated mice rapamycin has been reported to block the induction of RTP801 gene that promotes neuronal death,

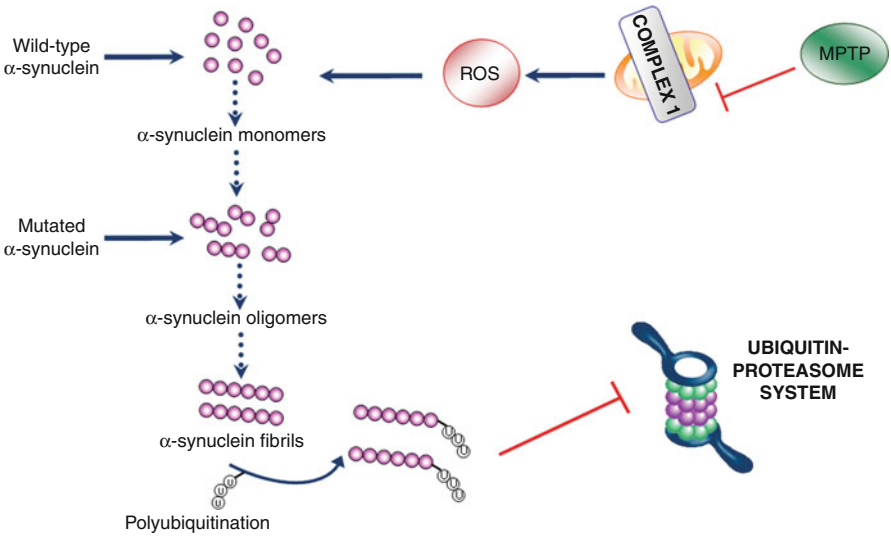

Fig. 7 During MPTP intoxication alpha-synuclein inhibits the proteasome. Among a variety of molecular targets (see Fig. 6 legend), alpha-synuclein can interact with physiological mechanisms which clear short-lived misfolded proteins named ubiquitin–proteasome (*UP*) system. When alpha-synuclein toxic oligomers (which represent a misfolded form of alpha-synuclein) are produced in excess, this saturates the UP system and leads to UP inhibition

which is highly expressed in DA neurons of the substantia nigra both in MPTP-treated mice and PD patients (Malagelada et al. 2006).

Remarkably, Liu et al. (2013) found that rapamycin rescues the number of DA neurons and reduces the increase of alpha-synuclein immunoreactivity induced by MPTP. These studies demonstrate that rapamycin via activation of ATG/lysosome pathways both prevents and reverts MPTP toxicity.

Thus, most evidence indicates that MPTP-induced neuronal death is the consequence of energy failure and oxidative stress that occur early on after MPTP administration. In turn, these phenomena are pivotal in triggering molecular pathways which, once activated, rapidly lead to the death of intoxicated neurons (Przedborski and Vila 2003). In fact, the removal of damaged mitochondria and misfolded proteins occurring following MPTP administration is critical in modulating MPTP toxicity. In fact, the occurrence of misfolded proteins in neuronal bodies following prolonged MPTP administration is accompanied by the impairment of the UP as a protein clearing system (Fig. 7, Fornai et al. 2005). Similarly, defective clearance of damaged mitochondria and long-lived proteins cleared by ATG is the hallmark of cell pathology in PD and following MPTP as well (Fig. 8). The abundance of altered mitochondria and toxic protein species is pivotal in triggering apoptotic cell death (Fig. 9). This is in line with the concomitant occurrence of altered ATG and apoptosis both in PD patients (Anglade et al. 1997) and following MPTP intoxication (Novikova et al. 2006). Thus, MPTP can induce apoptosis by different mechanisms. In fact oxidative stress and impairment

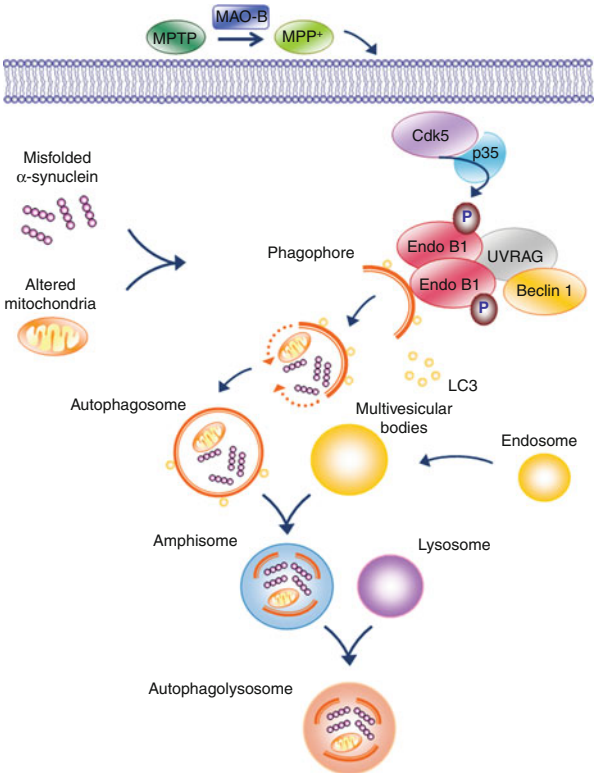

Fig. 8 During MPTP intoxication alpha-synuclein inhibits the autophagy. Among a variety of molecular targets (see Fig. 6 legend) alpha-synuclein can interact with physiological mechanisms which clear long-lived misfolded proteins and organelles named autophagy (*ATG*) system. When alpha-synuclein toxic oligomers (which represent a misfolded form of alpha-synuclein) are produced in excess, this saturates the ATG system and leads to ATG inhibition. On the other hand, during MPTP intoxication the variety of ROS produced and the toxicological effects of MPTP increase ATG activity via the Cdk5 pathway. This compensatory increase in ATG may be overcome by the overproduction of alpha-synuclein

of mitochondrial respiration, following MPTP treatment, can initiate apoptosis (Hartley et al. 1994), and MPTP also induces mitochondrial permeability transition (MPT), which determines mitochondrial swelling and cytochrome c release, with subsequent caspase-dependent apoptosis (Cassarino et al. 1999; Lee et al. 2006). MPTP-induced apoptosis involves different molecules and pathways, including DNA strand breaks, the c-Jun N-terminal kinase (JNK) pathway, caspases, Par-4 protein, p53, Apaf-1, and Bax (Eberhardt and Schulz 2003) (Fig. 9).

6 MPTP and Glutamate

Other sources of free radicals during MPP^+ toxicity besides mitochondria are glutamate and DA itself. Robust experimental evidence indicates that glutamate and other excitatory amino acids (EAA) play a role in MPTP toxicity since chronic MPTP increases extracellular glutamate in the substantia nigra (Meredith et al. 2009) and MPP^+ increases extracellular levels of glutamate in the striatum (Carboni et al. 1990).

The acidic amino acids glutamate, aspartate, and their analogues bind to three main types of ionotropic and also to metabotropic receptors (Fig. 5). These amino acids are well known to be responsible for "excitotoxicity" (Olney et al. 1980),

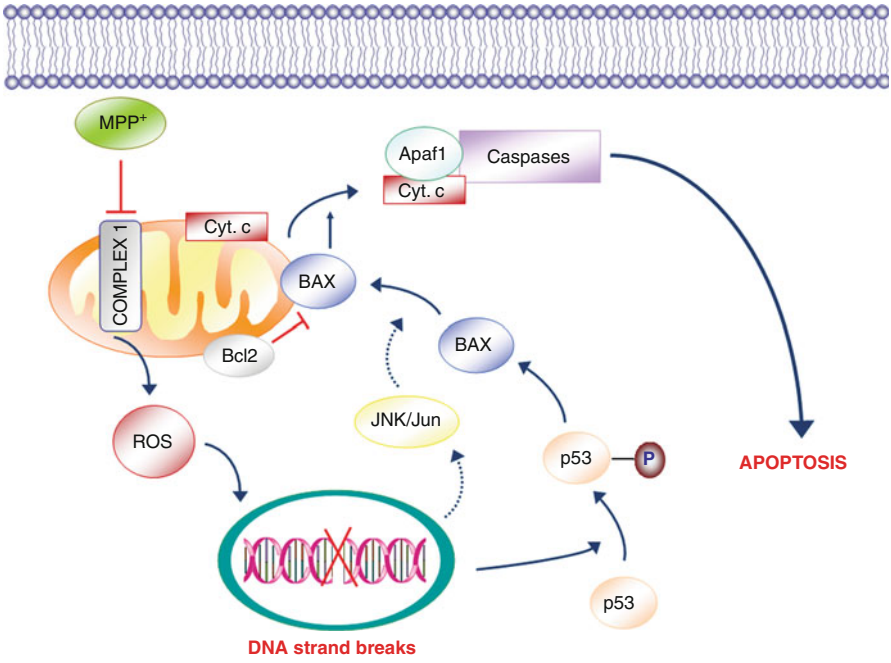

Fig. 9 The effects of MPP^+ on mitochondria trigger apoptotic proteins. The cartoon shows the molecular target of MPP^+ within the mitochondrial respiratory chain at the level of complex I. This inhibition leads to production of a variety of downstream effects consisting of ROS formation and induction of apoptotic proteins such as BAX and Bcl-2

which indicates how endogenous glutamate overactivity becomes frankly toxicant. These conditions include excessive and/or prolonged release of glutamate into the synaptic cleft. In these cases, neuronal loss progresses downstream along synaptically joined neurons, both after repeated acute insults and in neurodegenerative diseases in which the "systemic degeneration" consists of spreading cell loss to neurons interconnected within functional circuits. This might be the case of glutamatergic pathways from the neocortex to the SNpc and striatum (Cotman et al. 1987). In keeping with these concepts, the study of toxicity progression along the circuitries enrolled during acute insults (MPTP) provided the pathophysiological basis to depict the regional impairment occurring also in neurodegenerative diseases (PD). In fact the nigrostriatal pathway receives synaptic terminals from glutamatergic neurons and possesses EAA receptors. Furthermore interrupting the excitatory circuit by lesioning upstream neurons or trans-sectioning the glutamatergic input to the nigrostriatal pathway attenuates the toxic insult. This is in line with the fact that a noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist MK-801 (Chan et al. 1993b) protects against MPTP toxicity in rodents (Turski et al. 1991; Storey et al. 1992) and primates (Zuddas et al. 1992). An

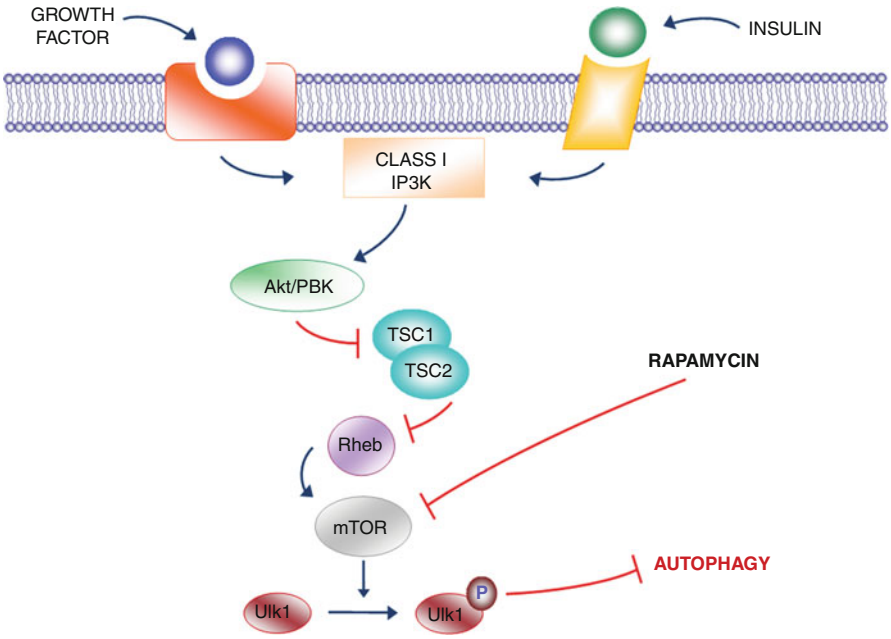

Fig. 10 Effects of rapamycin on autophagy. The ATG pathway can be modulated by a number of compounds among which growth factors and insulin are shown in the cartoon. Independently by the way of inducing ATG, rapamycin is a strong ATG inducer which promotes ATG downstream in the regulatory chain at the level of mTOR which is a strong ATG inhibitor. Rapamycin, by inhibiting mTOR, powerfully activates ATG

analogous protection was obtained by damaging glutamatergic corticostriatal fibers (Storey et al. 1992) suggesting that endogenous excitotoxicity contributes to MPTP toxicity (Brouillet and Beal 1993; Lange et al. 1993; Srivastava et al. 1993; Lange and Riederer 1994; Loschmann et al. 1994; Ossowska 1994; Vaglini et al. 1994; Kanthasamy et al. 1997; Sonsalla et al. 1998; Araki et al. 2001).

Species differences play a major role in the effects of glutamate in MPTP toxicity (Fornai et al. 1997b) leaving under debate the relevance of this point for PD patients.

As mentioned above, some authors have shown an involvement of EAA in the toxicity of MPP^+ injected directly into the substantia nigra (Turski et al. 1991) or the striatum (Storey et al. 1992) which was counteracted by the simultaneous administration of a competitive or noncompetitive NMDA receptor antagonist to the substantia nigra. Other authors could not confirm these results (Sonsalla et al. 1992) nor did they observe any involvement of EAA after systemic injection of MPTP in mice (Sonsalla et al. 1989, 1992; Kupsch et al. 1992). The way of administering MPTP also appears to be crucial. This concept is in line with the results of Storey et al. (1992), who showed that MPP^+ administered within the rat striatum produces long-lasting effects on neuronal energy metabolism, thus causing a three- to fourfold decrease in ATP levels, which persists for over 48 h.

Although rodent data are contradictory, studies performed on MPTP-treated primates, both old- and new-world monkeys: (Zuddas et al. 1992; Lange et al. 1993), respectively, demonstrated a clear protective role of NMDA and non-NMDA ionotropic receptor antagonists (both competitive and noncompetitive) in MPTP-induced parkinsonism (Crossman et al. 1989; Close et al. 1990).

The role of glutamate is not limited to its effects on ionotropic receptors and involves the role of metabotropic receptors which might be a therapeutic target with less side effects. In detail it was demonstrated that mGlu5 receptor agonists exacerbate, while antagonists may counteract MPTP-induced nigrostriatal damage (Battaglia et al. 2004). Nowadays it seems likely that glutamate per se is not the mediator of MPTP toxicity. The neurotoxic mechanisms rather rely on other pathways, and this brings the role of glutamate in the sideshow along the mainstream leading to MPTP toxicity.

7 MPTP and Dopamine

As reported, MPP^+ provokes a sudden, quite generalized neurotransmitter release. This phenomenon is more intense for catecholamine-containing neurons including DA cells. At this level, MPP^+ induces massive DA release from storage vesicles into the cytosol, where DA itself produces free radicals via auto-oxidation or MAO-mediated oxidative deamination, resulting in the formation of hydrogen peroxide, superoxide, and reactive quinone species (Lotharius and O'Malley 2000). The latter are known as DA quinones which also derive by activating cyclooxygenase (COX)-2 (Teismann et al. 2003). This may explain the neuroprotective effects of COX-2 inhibitors against MPTP toxicity. In addition, the activity of tyrosine hydroxylase (TH) may produce ROS as a normal by-product.

To emphasize the detrimental effects induced by DA within DA neurons in experimental parkinsonism induced by MPTP, it is useful to mention the toxic mechanisms induced by methamphetamine (METH), a neurotoxic agent which releases DA even more powerfully than MPTP (refer to specific chapters on ▶ Methamphetamine and MDMA Neurotoxicity: Biochemical and Molecular Mechanisms, this volume). Administration of METH produces experimental parkinsonism by inducing the loss of nigrostriatal DA axons (Ricaurte et al. 1982; Woolverton et al. 1989; Ricaurte and McCann 1992; McCann et al. 1998; Villemagne et al. 1998; Volkow et al. 2001a, b; Battaglia et al. 2002) and DA perikarya (Sonsalla et al. 1996; Castino et al. 2008; Granado et al. 2011). As for MPTP, METH abuse was recently shown to facilitate PD (Callaghan et al. 2010, 2012). As MPTP, METH is transported into DA terminals where it interacts with DAT and VMAT-2. In this way, METH causes massive DA release (O'Dell et al. 1991; Cubells et al. 1994). This reference to METH is key in the context of understanding the toxic potential of DA-releasing neurotoxicants. In fact, a high DA release occurs also during MPTP intoxication. High DA levels promote DA oxidation which is toxic to a variety of key proteins and organelles within DA neurons (Castino et al. 2008). The role of DA toxicity can lead to an understanding of some critical questions about MPTP such as species differences. In fact, rats are

resistant to MPTP toxicity, while mice are more sensitive. When looking at the kinetics and dynamics of MPTP in mice and rats, no difference between mice and rats appears but a remarkable discrepancy in MPTP-induced DA release. Mice, which are sensitive to MPTP toxicity, undergo massive MPTP-induced DA release, while rats which are almost resistant to MPTP toxicity undergo a minimal DA release following MPTP administration. This concept is extensively reviewed in the paragraph titled "Species Differences to MPTP Toxicity" but is useful to be mentioned here to understand the role of DA in MPTP toxicity. It is quite intriguing that DA as a neurotransmitter possesses such a great potential to produce neurotoxicity. This was already demonstrated by pioneer studies injecting DA directly in the striatum and reviewing the potential toxicity which follows DA release. The ability of DA to act as a neurotoxicant relates to the chemical reactivity reviewed so far. In contrast, NE does not produce toxicity but it is rather neuroprotectant against MPTP toxicity (Mavridis et al. 1991; Marien et al. 1993; Fornai et al. 1995, 1996; 1997a).

8 MPTP Toxicity and Norepinephrine

Damage to the NE nucleus LC is known to worsen the onset and progression of experimental parkinsonism induced by MPTP both in rodents and monkeys (Mavridis et al. 1991; Marien et al. 1993; Bing et al. 1994; Fornai et al. 1995; Gesi et al. 2000). Damage to NE axon terminals, induced by the selective NE neurotoxin *N*-(−2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) (Fornai et al. 1996) before MPTP administration, increases the sudden MPTP-induced DA release and enhances MPTP toxicity (Fornai et al. 1997a). The role of NE in MPTP toxicity directly applies to human PD patients where analogous effects were described (Rye and DeLong 2003).

In fact, the loss of NE is thought to facilitate the onset and progression of PD (Tong et al. 2006; Rye and DeLong 2003). Actually, the loss of LC neurons also represents a hallmark of PD since extensive neuropathological analysis demonstrated that LC neurons are lost even more than SNpc cells in PD (Zarow et al. 2003). The loss of LC neurons in PD represents a pioneer finding dating back at the beginning of the last century (Tretiakoff 1919). It remained a smoldering concept up to experimental studies using MPTP and showing a causal link between LC damage and the loss of DA-containing neurons. Mavridis et al. (1991) developed a primate model demonstrating that previous damage to LC NE-containing neurons may impair the recovery of nigrostriatal DA pathway following MPTP intoxication. Further studies demonstrated that a loss of NE innervation causes an increased susceptibility of DA neurons to MPTP (Fornai et al. 1997a). It is worth to be mentioned that such a reduced threshold within DA neurons to MPTP neurotoxicity is not due to a prolonged persistence of MPP^+ within the CNS. In fact, since MPP^+ is taken up within NE neurons at a rate which is faster compared with DA neurons (Herkenham et al. 1991), it is expected that a damage to NE cells would have produced a loss of what may be considered a sort of buffering activity. Paradoxically, LC-damaged brains retain MPP^+ for a longer time compared with LC-intact

brain. This indicates that variations in toxin kinetics are not causing enhancement of MPTP toxicity in NE-damaged brains (Fornai et al. 1997a). It is rather the occurrence of NE or the absence of such a neurotransmitter which occludes or sensitizes, respectively, MPTP-induced DA toxicity. Enhancement of MPTP toxicity occurs also by preventing NE synthesis using a DA beta-hydroxylase inhibitor such as fusaric acid or disulfiram (Corsini et al. 1985; Weinshenker et al. 2008). Thus, the protective effects of NE-containing neurons on MPTP toxicity are demonstrated throughout a variety of experimental approaches and animal species (Fornai et al. 2007a). These findings pose the need for well-preserved LC neurons in order to prevent the occurrence of PD. In fact, in human patients Tong et al. (2006) demonstrated a tight correlation between early loss of NE- and the later damage to DA-containing neurons within specific brain areas of PD patients. These authors suggested that a loss of NE innervations anticipates subsequent damage to DA-containing cells. On the other hand, if PD is characterized by the concomitant loss of LC NE- and SNpc DA-containing neurons, a neurotoxin which is able to mimic PD is also expected to reproduce such a combined neurotoxicity. This poses the following question: Is there a neurotoxic effect of MPTP for NE neurons? The study of Seniuk et al. (1990) indicates that small reiterated doses of MPTP were effective in damaging NE more than DA neurons (Seniuk et al. 1990). Pathobiochemical (Hornykiewicz 1975, 1998; Tong et al. 2006) and pathological findings (Braak et al. 2000; Zarow et al. 2003) clearly established the concomitant NE and DA damage in PD. A number of studies on MPTP toxicity up to the recent findings in mice (Meredith et al. 2002; Fornai et al. 2005) and nonhuman primates by Masilamoni et al. (2011) confirm that prolonged exposure to MPTP does produce a concomitant damage to DA and NE neurons as demonstrated in PD.

These findings introduce the issue of whether we should consider prolonged MPTP exposure more reliable than acute administration to produce toxic effects which mimic PD. This point extends to the evaluation of various protocols of MPTP administration which were developed during the last decades.

9 Acute, Chronic, and Continuous MPTP Administration

In mice a wide range of MPTP administrations were experimented. These vary with respect to the total amount of MPTP administered, the route (intraperitoneally versus subcutaneously), and time between injections, including the acute (usually four doses within 6 h), the subacute (usually 5 days), and the chronic administration (weeks), the latter often accompanied by an adjuvant drug, probenecid (Petroske et al. 2001; Kurosaki et al. 2004; Lau et al. 2005; Anderson et al. 2006). Very recently (Prediger et al. 2011) mice have been treated with intranasal MPTP, showing impairments in olfactory, cognitive, emotional, and motor functions, similarly to those observed in PD. This was accompanied by TH loss in the olfactory bulb and SNpc, with significant DA depletion in different brain areas (Prediger et al. 2011).

The different MPTP administrations result in different amount of DA depletion, motor deficits, and time course of recovery (Sonsalla and Heikkila 1986;

Jackson-Lewis et al. 1995; Bezard et al. 1997a, b; Kühn et al. 2003). When injected into mice, MPTP causes a PD-like syndrome with massive loss of nigral DAergic neurons and striatal DA. Acute administration of MPTP leads to striatal loss that can range from 40 % (when MPTP is given at 14 mg/kg per dose four times a day) to approximately 90 % (20 mg/kg per dose four times, 2 h apart in a single day) 7 days after the last MPTP dose and at least 40–50 % loss of nigrostriatal DAergic neurons in young adult C57BL/6 mice by 7 days after MPTP administration (Jackson-Lewis and Przedborski 2007). A subchronic regimen consisting of administration of systemic MPTP 30 mg/kg per day for 5 consecutive days in Swiss mice leads to morphologic features resembling apoptotic cell death occurring on day 6 after the last MPTP administration, with tissue DA depletion of 59 % 1 day after MPTP last administration (Tatton and Kish 1997) and a progressive recovery of DA depletion (about 28 %) from day 3 to day 14 after MPTP discontinuation (Serra et al. 2002). During the acute MPTP administration, necrotic rather than apoptotic features of the neurons of the SNpc were observed (Jackson-Lewis and Przedborski 2007); this suggests that the schedule of MPTP administration does not only influence the time course of nigrostriatal damage but may also provide insights into the underlying mechanisms of PD pathogenesis, such as the induction and manifestation of neuronal death (necrotic or apoptotic), which appears to be correlated to different stages of the human PD (presymptomatic, immediate onset, progressive, and final stage).

Shall we expect that, in order to better reproduce PD, MPTP needs to be administered once in high doses or rather being reiterated in small amount during long time intervals or even administered continuously?

In this context when considering the effects of acute MPTP administration to a variety of animal species, it is evident that despite being able to reproduce a massive neuronal loss in the SNpc, such an experimental protocol leaves NE neurons intact and does not produce the occurrence of neuronal inclusions neither in mice nor in primates. In fact, although intraneural inclusions containing eosinophilic materials and alpha-synuclein aggregates have been described in MPTP-treated monkeys (Forno et al. 1986; Kowall et al. 2000), the classic Lewy bodies, a typical feature of PD, have not been convincingly observed (Forno et al. 1993). The intraneural inclusions containing eosinophilic material and alpha-synuclein that are reminiscent of Lewy bodies have not been observed in young monkeys (Forno et al. 1995). In fact, following acute administrations the time course of MPTP-induced neurotoxicity is rapid, and therefore it does not activate long-acting neuronal mechanisms which are required to build neuronal bodies. In fact, it is likely that neuronal inclusions represent a compromise between long-acting slight neurotoxic mechanisms and the recruitment of compensatory mechanisms. This is what is thought to happen during PD which possesses a chronic progressive disease course.

On the other hand, when MPTP is administered chronically or continuously in mice apart from NE damage, also neuronal bodies can be described (Meredith et al. 2008; Fornai et al. 2005; Gibrat et al. 2009; Beal 2011). Sometimes chronic models based on repetitive small doses remain subthreshold to induce toxicity as reported in one study (Alvarez-Fischer et al. 2008), while most studies found that chronic

(Meredith et al. 2008) or continuous (Fornai et al. 2005; Cleren et al. 2008; Gibrat et al. 2009; Yasuda et al. 2011) MPTP administration protracted for a few weeks does produce alpha-synuclein-positive neuronal bodies in addition to massive striatal DA depletion and SNpc neuronal loss. The amount of MPTP and the time needed to produce inclusions may vary between different studies; this is likely to depend on experimental variability such as purchaser and housing which are well known to modulate the threshold for MPTP toxicity (Heikkila et al. 1984b; see also the Sect. 14).

Prolonged neurotoxin administration is effective also for the active metabolite MPP^+ when this is delivered continuously across the blood–brain barrier both in rodents and primates (Imai et al. 1988; Sirinathsinghji et al. 1988). In the rat, continuous *i.c.v* MPP^+ administration produces striatal DA loss and SNpc cell death as well as neuronal bodies staining for ubiquitin and alpha-synuclein (Yazdani et al. 2006).

10 Spinal Cord and MPTP

The classic pathological features of sporadic PD include the degeneration of DA neurons in the SNpc and NE neurons in the LC. Besides these areas, also the spinal cord (SC) seems to be affected in PD in the presymptomatic stage. This was already demonstrated by significant motor neuron loss in various PD models (Bloch et al. 2006; Klos et al. 2006; Samantaray et al. 2008a), including the A53T mutant mice that express the mutant alpha-synuclein gene of familial PD (Martin et al. 2006). Following MPTP administration in the SC, specific apoptotic proteins are highly expressed (Chera et al. 2002, 2004; Samantaray et al. 2006, 2007). In a very recent paper, it was found that in MPTP-treated mice there is a significant motor neuron loss in the lumbar and cervical SC (Vivacqua et al. 2012). Samantaray and colleagues (2008b) showed that MAO-B are expressed in the SC and DAT is expressed in motor neurons thus suggesting that MPTP can be converted to MPP^+ which in turn might be taken up by motor neurons to induce cell death.

11 MPTP, the Heart, and the Gut

While MPTP toxicity in the CNS is extensively investigated, peripheral autonomic dysfunction following MPTP intoxication received much less attention. Only recent studies demonstrated a parallelism between PD and MPTP in producing cardiac denervation due to a loss of NE axons in the heart (the so-called Parkinsonian heart; Fukumitsu et al. 2006; Fornai et al. 2007b). Similarly, MPTP intoxication was recently reported to reproduce the decrease in plasma testosterone levels observed in PD, and this was related with alteration produced by MPTP in Leydig cells (Ruffoli et al. 2008). Gastrointestinal (GI) dysfunction is a well-recognized feature of PD (Natale et al. 2008) and the entire GI tract may be affected (Edwards et al. 1991; Jost 1997; Pfeiffer 2003). A few studies suggest that GI symptoms in PD may be caused by a neurodegenerative process in the enteric

nervous system (ENS), similar to what occurs in the CNS (Singaram et al. 1995; Wakabayashi et al. 1988), and it is hypothesized that degeneration in the ENS mirrors and precedes analogous pathological events in the brain or SC (Braak et al. 2003, 2006, 2007; Natale et al. 2011).

Only a few experimental studies using MPTP addressed whether such intoxication involves the gut. In rats exposed to MPTP, Szabo et al. (1985) described inhibition of duodenal spike activity. Decreased DA levels were found in the jejunal myenteric plexus, although this occurred only for high doses (Szabo et al. 1985; Eaker et al. 1987).

The effects of MPTP in the mouse colon have been evaluated in vivo, following a total dose of 60 mg/kg of MPTP. This produced a 40 % reduction of TH-positive neurons (Anderson et al. 2007). Whether the loss of TH-positive neurons following MPTP administration was due to DA or NA or both was recently demonstrated by Natale and collaborators (2010). This appears to be due to the selective loss of enteric DAergic neurons, as confirmed by the selective damage to DAT-containing cells and the specific loss of DA in the homogenates from the gut. In the same studies, it was found that MPTP increases alpha-synuclein thus reproducing alterations occurring in the digestive system of PD patients.

12 Species Differences in MPTP Toxicity

A variety of animal species are sensitive to MPTP toxicity including monkeys, rodents, cats, pigs, dogs, sheep, rats, and goldfishes (Kopin and Markey 1988; Gerlach and Riederer 1996; Przedborski et al. 2001); however, different effects are produced by MPTP in different species ranging from massive (primates) to minimal (rats) toxicity.

In monkeys, MPTP administration reproduces most of the clinical and pathological hallmarks of PD (Langston et al. 1984a; Chiueh et al. 1984; Doudet et al. 1985; Crossman et al. 1985; Nomoto et al. 1985; Vezoli et al. 2011) but not in rats, which were found to be resistant to MPTP (Chiueh et al. 1984). In detail, rats can be damaged by MPTP only in the periphery, being necessary to administer MPP^+ at very high doses intracerebroventricularly to produce a damage within CNS to DA neurons (Yazdani et al. 2006). Mice are sensitive to MPTP but require higher amount than primates. In detail, in mice MPTP needs to be administered acutely at doses ranging from 15 to 80 mg/Kg to produce a progressively massive loss of nigral DA neurons, while in monkeys the same effects are induced at doses at least 100-fold lower. Such a discrepancy is not related with species differences in the bioavailability of MPP^+ within the striatum following systemic MPTP (Zuddas et al. 1994; Vaglini et al. 1996); it is likely that species differences in the toxic effects of comparable amounts of nigrostriatal MPP^+ may relate to species differences in the pharmacodynamics of MPTP. In fact, it is well known that comparable amounts of MPP^+ are formed within the striatum of mice and rats following similar doses of systemic MPTP (Zuddas et al. 1994; Vaglini et al. 1996). This rules out the chance that MAO-B works differently in these species to convert MPTP into MPP^+. However, if one

considers that MPP^+ is selectively taken up by DA terminals, this process could differ significantly. In a careful series of experiments, Giovanni et al. (1994a, b) found no species difference in the uptake of MPP^+, ruling out also the difference in DAT efficacy. On the other hand, MPP^+ apart from damaging the mitochondrial respiratory chain is also able to produce a robust DA release (see Sect. 7). It was shown that equimolar amount of MPP^+ produces a pronounced DA release in mice versus a modest effect in rats (Giovanni et al. 1994a, b). This point which remained not further investigated sheds a new light on the potential role of intracellular DA joined with the impairment of mitochondrial respiratory chain in sustaining MPTP neurotoxicity. It would be interesting to test the effects of MPP^+ on striatal DA release in primates.

These latter experiments to our knowledge were never carried out. Nonetheless, it is worth to mention that MPTP toxicity is positively related to the amount of DAT (Watanabe et al. 2005) while is negatively related to the amount of VMAT-2 (Chen et al. 2005). In this scenario, we already reported that MPP^+ is sequestered into synaptic vesicles by VMAT-2, and this mechanism appears to protect rat DA neurons from MPP^+ toxicity (Staal and Sonsalla 2000). In fact, in primates MPTP sensitivity is bound to the lower expression of VMAT-2 compared with other species (Chen et al. 2008). Again, if one looks at the site specificity of DA neuron loss in the substantia nigra following MPTP, there is a negative correlation with the expression of VMAT-2 and MPTP-damaged cells. Conversely, the amount of DAT is higher in those subsets of neurons of the substantia nigra which are more vulnerable to MPTP (Haber et al. 1995).

Among the different animal species, mice are commonly used in MPTP experiments. This is due to easy handling, less ethical restrictions, and lower costs compared with monkeys. Nonetheless, mice are significantly less sensitive to MPTP than primates and do not convincingly develop persistent and progressive motor symptoms unless they are treated following sophisticated protocols (Sundström et al. 1990; Przedborski et al. 2001; Fornai et al. 2005).

13 MPTP in Nonhuman Primates

MPTP administration to monkeys is considered the best model to reproduce PD due to similarities in neuropathology and behavior. This model was routinely applied in preclinical studies aimed to test pharmacological and surgical treatments of PD. The first MPTP administration in common marmosets was described by Jenner and colleagues (1984), who observed profound akinesia, rigidity of the trunk and limbs, postural abnormalities, loss of vocalization, and, occasionally, whole body tremor (Jenner et al. 1984). MPTP can be administered to monkey using different routes, such as gavage or stereotactical injection. One of the most common consists in the parenteral administration (subcutaneous, intravenous, intra-arterial, intraperitoneal, or intramuscular; Przedborski et al. 2001). Among systemic routes, MPTP is administered through multiple i.p. and and i.m. injections which induce a progressive generalized parkinsonism or through unilateral intracarotid infusion

that determines hemiparkinsonism in the injected side. This is critical since bilateral parkinsonism requires L-DOPA to keep the animal alive (Petzinger and Langston 1998). In contrast, unilateral intracarotideal infusions, which determine a chronic model of hemiparkinsonism, enable the monkeys to feed without supporting therapeutics (Bankiewicz et al. 1986; Przedborski et al. 1991).

MPTP can be delivered acutely or chronically, the former being particularly useful in the assessment of treatments aimed at understanding the mechanisms and reducing the amount of L-DOPA-induced dyskinesias (Boraud et al. 2001; Samadi et al. 2008; Johnston et al. 2010; Riahi et al. 2011; Huot et al. 2011; Koprich et al. 2011; Grégoire et al. 2011).

Although both acute and chronic MPTP monkey models are appropriate for testing novel therapies, the chronic model is more appropriate to test neuroprotective drugs.

Even within primates a strong variability to MPTP toxicity exists. For instance old-world monkeys such as macaques are much more sensitive to MPTP than new-world monkeys such as marmosets.

14 MPTP in Mice

In mice, MPTP determines degeneration of DA neurons which again varies depending on the specific mouse strain (Heikkila et al. 1984a). Motor impairment is difficult to be detected in mice although evidence exists showing a correlation between behavioral and pathological findings in MPTP-treated mice (Goldberg et al. 2011). Within mouse strains there is a wide variation in their sensitivity to the MPTP, the C57BL/6 mouse being the most sensitive, whereas CD-1 and BALB/c appear more resistant and Swiss Webster are very resistant to MPTP toxicity (Sonsalla and Heikkila 1988; Muthane et al. 1994; Hamre et al. 1999; Sedelis et al. 2000). Thus, in order to obtain subtotal nigrostriatal damage, it is much better to use C57BL/6 mice.

Sensitivity of mice to MPTP increases with age independently of the strain. A mechanism of strain-dependent sensitivity to MPTP has been hypothesized by Boyd and collaborators (2007), who reported a divergence in the inflammatory response, by examining JNK and COX-2 signaling induction in C57BL/6 J and Swiss Webster mice. A different sensitivity to MPTP within each strain has been reported depending on the purchaser. Thus, Swiss Webster mice from three different commercial suppliers possess different sensitivity to MPTP (Heikkila 1985). Therefore, it is important for investigators to consider both the strain and the vendor used when developing new treatment protocols for MPTP-induced neurotoxicity.

15 MPTP in Rats

Rats injected with MPTP doses comparable to those used in mice do not exhibit any significant DA degeneration. The MPTP toxic doses in rats would be incompatible with rat life if injected systemically; therefore, it is necessary to protect against the peripheral effects of MPTP by coadministering a peripheral adrenergic ganglionic

blocker like guanethidine (Giovanni et al. 1994a). Alternatively one might administer MPP^+ at high doses directly within the CNS. The relative resistance of rats to MPTP toxicity was initially attributed to species differences in MPTP metabolism and/or sequestration of MPP^+. In fact, monkeys have a much longer striatal retention of MPP^+ (half-life about 10 days) compared with mice (half-life about 4 h) (Johannessen 1991). However, Giovanni et al. (1994a, b) found that although the rat is relatively insensitive to MPTP toxicity, the in vitro MPP^+ uptake into DA synaptosomes, the in vivo striatal extracellular MPP^+ concentrations, and the amount of postmortem striatal MPP^+ levels did not correlate with the higher sensitivity to MPTP of mice compared with rats. Unexpectedly, postmortem striatal MPP^+ levels were higher in rats than in mice (Giovanni et al. 1994a, b; Zuddas et al. 1994). Additionally, previous studies showed no difference in mice compared with rats for striatal synaptosomal MAO-B activity and striatal synaptosomal MPP^+ uptake (Giovanni et al. 1991). Zuddas et al. (1994) confirmed the absence of any significant difference between C57 black mouse and various rat strains in striatal MPP^+ kinetics following systemic MPTP administration. Staal and collaborators (2000) confirmed that there was no significant species difference (mice versus rats) in striatal DAT numbers, suggesting a similar accumulation of MPP^+ within the DA neurons in both species. Thus, lower vulnerability to MPP^+ in rats versus mice could not be explained by lower intracellular levels of MPP^+ in rat DA neurons. MPP^+ is actively accumulated into vesicles by VMAT-2 (Del Zompo et al. 1993; Moriyama et al. 1993), and striatal vesicles isolated from rats have a higher density of VMAT-2 compared with mice and a greater ability to sequester MPP^+, suggesting a larger storage capacity for MPP^+ in rat vesicles (Staal and Sonsalla 2000). In turn this produces lower amount of DA release following MPTP in rats compared with mice. Pretreatment of mice and rats with a VMAT-2 inhibitor, Ro 4–1,284 (10 mg/kg i.p.), produces a fivefold leftward shift in the MPP^+ dose–response curve and a significant lowering of the EC_{50} concentration for MPP^+-induced damage in rats. This provides evidence for a substantial accumulation of MPP^+ in vivo in the rat striatum and explains why enhanced MPP^+ toxicity occurs when blocking VMAT-2. In mice VMAT-2 inhibition fails to enhance MPP^+ toxicity in the striata, suggesting that vesicular sequestration may not be as significant in providing protection in this species as it occurs in rats. Although VMAT-2 inhibition increases MPP^+ toxicity to striatal DA nerve terminals in rats, the potency of MPP^+ toxicity in the striatum of rats in which VMAT-2 is inhibited is still less pronounced than that in mouse striata exposed to MPP^+. This implies that mechanisms other than the sole MPP^+ sequestration protect against MPP^+ toxicity to DA neurons in different species (Staal and Sonsalla 2000).

16 Conclusion

The study of neurotoxicity induced by MPTP is a seminal research field to understand PD. In this chapter we reported the main concepts developed over the last three decades which led us to understand key molecular steps which are pivotal in

mediating MPTP toxicity. Remarkably, these molecular mechanisms turned out to be critical also for the survival of DA neurons in idiopathic PD. This is the case of the role played by DAT and VMAT-2 in conditioning the sensitivity to MPTP neurotoxicity and the tendency of DA neurons to undergo progressive degeneration in PD. Similarly, the mitochondria as targets of MPTP toxicity appear similarly affected by selective mutation of genes leading to PD. Again, the fate of mitochondria and the ability to clear these organelles when being dysfunctional are key in the modulation of MPTP toxicity as well as in the course of PD, where certain gene mutations impair the clearance of mitochondria. This is similar for what we discovered about the role of specific misfolded proteins such as alpha-synuclein.

Our perspective on PD is drastically changed in the past three decades, when novel brain areas as well as peripheral sites are increasingly recognized to be affected in PD patients. It is remarkable that progress on MPTP research led to mimic PD even for these additional areas underlying specific additional symptoms. PD is no longer viewed as a highly specific degeneration which involves solely the DA nigrostriatal pathway; similarly MPTP intoxication covers most of the additional features now evident in PD patients. Nowadays, after three decades, we can say that if MPTP per se did not lead to discovery of the environmental compound which causes PD, indeed the study of MPTP did disclose several molecular and cellular pathways which are critical in the genesis of PD. This latter point corresponds fairly to what we enthusiastically expected from MPTP when it was identified as a causal agent of what it remains, a toxic form of environmental parkinsonism.

References

Alvarez-Fischer, D., Guerreiro, S., Hunot, S., Saurini, F., Marien, M., Sokoloff, P., Hirsch, E. C., Hartmann, A., & Michel, P. P. (2008). Modelling Parkinson-like neurodegeneration via osmotic minipump delivery of MPTP and probenecid. *Journal of Neurochemistry, 107*(3), 701–711.

Anderson, D. W., Bradbury, K. A., & Schneider, J. S. (2006). Neuroprotection in Parkinson models varies with toxin administration protocol. *The European Journal of Neuroscience, 24*(11), 3174–3182.

Anderson, G., Noorian, A. R., Taylor, G., Anitha, M., Bernhard, D., Srinivasan, S., & Greene, J. G. (2007). Loss of enteric dopaminergic neurons and associated changes in colon motility in an MPTP mouse model of Parkinson's disease. *Experimental Neurology, 207*(1), 4–12.

Andreassen, O. A., Ferrante, R. J., Dedeoglu, A., Albers, D. W., Klivenyi, P., Carlson, E. J., Epstein, C. J., & Beal, M. F. (2001). Mice with a partial deficiency of manganese superoxide dismutase show increased vulnerability to the mitochondrial toxins malonate, 3-nitropropionic acid, and MPTP. *Experimental Neurology, 167*(1), 189–195.

Anglade, P., Vyas, S., Hirsch, E. C., & Agid, Y. (1997). Apoptosis in dopaminergic neurons of the human substantia nigra during normal aging. *Histology and Histopathology, 12*, 603–610.

Araki, T., Kumagai, T., Tanaka, K., Matsubara, M., Kato, H., Itoyama, Y., & Imai, Y. (2001). Neuroprotective effect of riluzole in MPTP-treated mice. *Brain Research, 918*, 176–181.

Ballard, P. A., Tetrud, J. W., & Langston, J. W. (1985). Permanent human parkinsonism due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): Seven cases. *Neurology, 35*(7), 949–956.

Bankiewicz, K. S., Oldfield, E. H., Chiueh, C. C., Doppman, J. L., Jacobowitz, D. M., & Kopin, I. J. (1986). Hemiparkinsonism in monkeys after unilateral internal carotid artery infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Life Sciences, 39*(1), 7–16.

Battaglia, G., Fornai, F., Busceti, L. C., Aloisi, G., Cerrito, F., De Blasi, A., Melchiorri, D., & Nicoletti, F. (2002). Selective blockade of mGlu5 metabotropic glutamate receptors is protective against methamphetamine neurotoxicity. *The Journal of Neuroscience, 22*, 2135–2141.

Battaglia, G., Busceti, C. L., Molinaro, G., Biagioni, F., Storto, M., Fornai, F., Nicoletti, F., & Bruno, V. (2004). Endogenous activation of mGlu5 metabotropic glutamate receptors contributes to the development of nigro-striatal damage induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. *The Journal of Neuroscience, 24*(4), 828–835.

Beal, M. F. (2011). Neuroprotective effects of creatine. *Amino Acids, 40*(5), 1305–1313.

Bezard, E., Imbert, C., Deloire, X., Bioulac, B., & Gross, C. E. (1997a). A chronic MPTP model reproducing the slow evolution of Parkinson's disease: Evolution of motor symptoms in the monkey. *Brain Research, 766*(1–2), 107–112.

Bezard, E., Dovero, S., Bioulac, B., & Gross, C. (1997b). Effects of different schedules of MPTP administration on dopaminergic neurodegeneration in mice. *Experimental Neurology, 148*(1), 288–292.

Bing, G., Zhang, Y., Watanabe, Y., Mcewen, B. S., & Stone, E. A. (1994). Locus coeruleus lesions potentiate neurotoxic effects of MPTP in dopaminergic neurons in the substantia nigra. *Brain Research, 668*, 261–265.

Bloch, A., Probst, A., Bissig, H., Adams, H., & Tolnay, M. (2006). Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. *Neuropathology and Applied Neurobiology, 32*(3), 284–295.

Boraud, T., Bezard, E., Bioulac, B., & Gross, C. E. (2001). Dopamine agonist-induced dyskinesias are correlated to both firing pattern and frequency alterations of pallidal neurones in the MPTP-treated monkey. *Brain, 124*(Pt 3), 546–557.

Boyd, J. D., Jang, H., Shepherd, K. R., Faherty, C., Slack, S., Jiao, Y., & Smeyne, R. J. (2007). Response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) differs in mouse strains and reveals a divergence in JNK signaling and COX-2 induction prior to loss of neurons in the substantia nigra pars compacta. *Brain Research, 1175*, 107–116.

Braak, H., Rüb, U., Sandmann-Keil, D., Gai, W. P., de Vos, R. A., Jansen Steur, E. N., Arai, K., & Braak, E. (2000). Parkinson's disease: Affection of brain stem nuclei controlling premotor and motor neurons of the somatomotor system. *Acta Neuropathologica, 99*(5), 489–495.

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging, 24*, 197–211.

Braak, H., de Vos, R. A., Bohl, J., & Del Tredici, K. (2006). Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease related brain pathology. *Neuroscience Letters, 396*, 67–72.

Braak, H., Sastre, M., Bohl, J. R., de Vos, R. A., & Del Tredici, K. (2007). Parkinson's disease: Lesions in dorsal horn layer I, involvement of parasympathetic and sympathetic pre- and postganglionic neurons. *Acta Neuropathologica, 113*, 421–429.

Brooks, D. J., & Pavese, N. (2011). Imaging biomarkers in Parkinson's disease. *Progress in Neurobiology, 95*(4), 614–628.

Brooks, W. J., Jarvis, M. F., & Wagner, G. C. (1989). Astrocytes as a primary locus for the conversion MPTP into MPP^+. *Journal of Neural Transmission, 76*(1), 1–12.

Brouillet, E., & Beal, M. F. (1993). NMDA antagonists partially protect against MPTP-induced neurotoxicity in mice. *Neuroreport, 4*, 387–390.

Browne, S. E., Bowling, A. C., Baik, M. J., Gurney, M., Brown, R. H., Jr., & Beal, M. F. (1998). Metabolic dysfunction in familial, but not sporadic, amyotrophic lateral sclerosis. *Journal of Neurochemistry, 71*, 281–287.

Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C. M., & Stefani, M. (2002). Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature, 416*, 507–511.

Büeler, H. (2009). Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease. *Experimental Neurology, 218*(2), 235–246.

Callaghan, R. C., Cunningham, J. K., Sajeev, G., & Kish, S. J. (2010). Incidence of Parkinson's disease among hospital patients with methamphetamine-use disorders. *Movement Disorders, 25*(14), 2333–2339.

Callaghan, R. C., Cunningham, J. K., Sykes, J., & Kish, S. J. (2012). Increased risk of Parkinson's disease in individuals hospitalized with conditions related to the use of methamphetamine or other amphetamine-type drugs. *Drug and Alcohol Dependence, 120*(1–3), 35–40.

Carboni, S., Melis, F., Pani, L., Hadgiconstantinou, M., & Rossetti, Z. (1990). Non-competitive NMDA receptor antagonist MK-801 prevents the massive release of glutamate and aspartate from rat striatum induced by MPP^+. *Neuroscience Letters, 117*, 129–133.

Cassarino, D. S., Parks, J. K., Parker, W. D., Jr., & Bennett, J. P., Jr. (1999). The parkinsonian neurotoxin MPP^+ opens the mitochondrial permeability transition pore and releases cytochrome c in isolated mitochondria via an oxidative mechanism. *Biochimica et Biophysica Acta, 1453*, 49–62.

Castino, R., Lazzeri, G., Lenzi, P., Bellio, N., Follo, C., Ferrucci, M., Fornai, F., & Isidoro, C. (2008). Suppression of autophagy precipitates neuronal cell death following low doses of methamphetamine. *Journal of Neurochemistry, 106*(3), 1426–1439.

Chan, P., DeLanney, L. E., Irwin, I., Langston, J. W., & Di Monte, D. (1991). Rapid ATP loss caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mouse brain. *Journal of Neurochemistry, 57*, 348–351.

Chan, P., Langston, J. W., Irwin, I., DeLanney, L. E., & Di Monte, D. A. (1993a). 2-Deoxyglucose enhances 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine-induced ATP loss in the mouse brain. *Journal of Neurochemistry, 61*, 610–616.

Chan, P., Langston, J. W., & Di Monte, D. A. (1993b). MK-801 temporarily prevents MPTP-induced acute dopamine depletion and MPP^+ elimination in the mouse striatum. *The Journal of Pharmacology and Experimental Therapeutics, 267*, 1515–1520.

Chandra, S., Fornai, F., Kwon, H. B., Yazdani, U., Atasoy, D., Liu, X., Hammer, R. E., Battaglia, G., German, D. C., Castillo, P. E., & Südhof, T. C. (2004). Double-knockout mice for alpha- and beta-synucleins: Effect on synaptic functions. *Proceedings of the National Academy of Sciences of the United States of America, 101*(41), 14966–14971.

Chandra, S., Gallardo, G., Fernández-Chacón, R., Schlüter, O. M., & Südhof, T. C. (2005). Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. *Cell, 123*(3), 383–396.

Chen, C. X., Huang, S. Y., Zhang, L., & Liu, Y. J. (2005). Synaptophysin enhances the neuroprotection of VMAT2 in MPP^+ −induced toxicity in MN9D cells. *Neurobiology of Disease, 19*(3), 419–426.

Chen, M. K., Kuwabara, H., Zhou, Y., Adams, R. J., Brasię, J. R., McGlothan, J. L., Verina, T., Burton, N. C., Alexander, M., Kumar, A., Wong, D. F., & Guilarte, T. R. (2008). VMAT2 and dopamine neuron loss in a primate model of Parkinson's disease. *Journal of Neurochemistry, 105*(1), 78–90.

Chera, B., Schaecher, K. E., Rocchini, A., Imam, S. Z., Ray, S. K., Ali, S. F., & Banik, N. L. (2002). Calpain upregulation and neuron death in spinal cord of MPTP-induced parkinsonism in mice. *Annals of the New York Academy of Sciences, 965*, 274–280.

Chera, B., Schaecher, K. E., Rocchini, A., Imam, S. Z., Sribnick, E. A., Ray, S. K., Ali, S. F., & Banik, N. L. (2004). Immunofluorescent labeling of increased calpain expression and neuronal death in the spinal cord of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice. *Brain Research, 1006*(2), 150–156.

Chiba, K., Trevor, A., & Castagnoli, N., Jr. (1984). Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochemical and Biophysical Research Communications, 120*(2), 574–578.

Chiueh, C. C., Markey, S. D., Burns, R. S., Johannessen, J. N., Jacobowitz, D. M., & Kopin, I. J. (1984). Neurochemical and behavioral effects of MPTP in rat, guinea pig and monkey. *Psychopharmacology Bulletin, 20*, 548–553.

Ciechanover, A. (2006). The ubiquitin proteolytic system: From a vague idea, through basic mechanisms, and onto human diseases and drug targeting. *Neurology, 66*(2 supp 1), 7–19.

Cleeter, M. W., Cooper, J. M., & Schapira, A. H. (1992). Irreversible inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium: Evidence for free radical involvement. *Journal of Neurochemistry, 58*, 786–789.

Cleren, C., Yang, L., Lorenzo, B., Calingasan, N. Y., Schomer, A., Sireci, A., Wille, E. J., & Beal, M. F. (2008). Therapeutic effects of coenzyme Q10 (CoQ10) and reduced CoQ10 in the MPTP model of Parkinsonism. *Journal of Neurochemistry, 104*(6), 1613–1621.

Close, S. P., Elliot, P. J., Hayes, A. G., & Marriott, A. S. (1990). Effects of classical and novel agent in a MPTP-induced reversible model of Parkinson's disease. *Psychopharmacology, 102*, 295–300.

Conway, K. A., Lee, S. J., Rochet, J. C., Ding, T. T., Harper, J. D., Williamson, R. E., & Lansbury, P. T., Jr. (2000). Accelerated oligomerization by Parkinson's disease linked alpha-synuclein mutants. *Annals of the New York Academy of Sciences, 920*, 42–45.

Corsini, G. U., Pintus, S., Chiueh, C. C., Weiss, J. F., & Kopin, I. J. (1985). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity in mice is enhanced by pretreatment with diethyldithiocarbamate. *European Journal of Pharmacology, 119*(1–2), 127–128.

Cotman, C. W., Monaghan, D. T., Ottersen, O. P., & Storm-Mathisen, J. (1987). Anatomical organization of excitatory amino acid receptors and their pathways. *Trends in Neurosciences, 10*, 273–280.

Crossman, A. R., Mitchell, I. J., & Sambrook, M. A. (1985). Regional brain uptake of 2-deoxyglucose in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the macaque monkey. *Neuropharmacology, 24*, 587–591.

Crossman, A. R., Peggs, D., Boyce, S., Luquin, M. R., & Sambrook, M. A. (1989). Effect of the NMDA antagonist MK-801 on MPTP-induced parkinsonism in the monkey. *Neuropharmacology, 28*, 1271–1273.

Cubells, J. F., Rayport, S., Rajendran, G., & Sulzer, D. (1994). Methamphetamine neurotoxicity involves vacuolation of endocytic organelles and dopamine-dependent intracellular oxidative stress. *The Journal of Neuroscience, 14*, 2260–2271.

D'Amato, R. J., Alexander, G. M., Schwartzman, R. J., Kitt, C. A., Price, D. L., & Snyder, S. H. (1987). Evidence for neuromelanin involvement in MPTP-induced neurotoxicity. *Nature, 327*(6120), 324–326.

Davis, G. C., Williams, A. C., Markey, S. P., Ebert, M. H., Caine, E. D., Reichert, C. M., & Kopin, I. J. (1979). Chronic parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Research, 1*, 249–254.

Dehay, B., Bové, J., Rodríguez-Muela, N., Perier, C., Recasens, A., Boya, P., & Vila, M. (2010). Pathogenic lysosomal depletion in Parkinson's disease. *The Journal of Neuroscience, 30*(37), 12535–12544.

Del Zompo, M., Piccardi, M. P., Ruiu, S., Quartu, M., Gessa, G. L., & Vaccari, A. (1993). Selective uptake into synaptic dopamine vesicles: Possible involvement in MPTP neurotoxicity. *British Journal of Pharmacology, 109*, 411–414.

Denton, T., & Howard, B. D. (1987). A dopaminergic cell line variant resistant to the neurotoxin 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine. *Journal of Neurochemistry, 49*, 622–629.

Di Monte, D., Jewell, S. A., Ekstromm, G., Sandy, M. S., & Smith, M. T. (1986). 1-Methyl-4-phenyl-l,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridine (MPP^+) cause rapid ATP depletion in isolated hepatocytes. *Biochemical and Biophysical Research Communications, 137*, 310–315.

Dodson, M. W., & Guo, M. (2007). Pink1, Parkin, DJ-1 and mitochondrial dysfunction in Parkinson's disease. *Current Opinion in Neurobiology, 17*(3), 331–337.

Doudet, D., Gross, C., Lebrun-Grandie, P., & Bioulac, B. (1985). MPTP primate model of Parkinson's disease: A mechanographic and electromyographic study. *Brain Research, 335*, 194–199.

Eaker, E. Y., Bixler, G. B., Dunn, A. J., Moreshead, W. V., & Mathias, J. R. (1987). Chronic alterations in jejunal myoelectric activity in rats due to MPTP. *The American Journal of Physiology, 253*, 809–815.

Eberhardt, O., & Schulz, J. B. (2003). Apoptotic mechanisms and antiapoptotic therapy in the MPTP model of Parkinson's disease. *Toxicology Letters, 139*(2–3), 135–151.

Edwards, L. L., Pfeiffer, R. F., Quigley, E. M., Hofman, R., & Balluff, M. (1991). Gastrointestinal symptoms in Parkinson's disease. *Movement Disorders, 6*(2), 151–156.

Fornai, F., Bassi, L., Torracca, M. T., Scalori, V., & Corsini, G. U. (1995). Norepinephrine loss exacerbates methamphetamine induced striatal dopamine depletion in mice. *European Journal of Pharmacology, 283*, 99–102.

Fornai, F., Torracca, M. T., Bassi, L., D'Errigo, D. A., Scalori, V., & Corsini, G. U. (1996). Norepinephrine loss selectively enhances chronic nigrostriatal dopamine depletion in mice and rats. *Brain Research, 735*(2), 349–353.

Fornai, F., Alessandrì, M. G., Torracca, M. T., Bassi, L., & Corsini, G. U. (1997a). Effects of noradrenergic lesions on MPTP/MPP^+ kinetics and MPTP-induced nigrostriatal dopamine depletions. *The Journal of Pharmacology and Experimental Therapeutics, 283*(1), 100–107.

Fornai, F., Vaglini, F., Maggio, R., Bonuccelli, U., & Corsini, G. U. (1997b). Species differences in the role of excitatory amino acids in experimental parkinsonism. *Neuroscience and Biobehavioral Reviews, 21*(4), 401–415.

Fornai, F., Carrì, M. T., Ferri, A., Paolucci, E., Prisco, S., Bernardi, G., Rotilio, G., & Mercuri, N. B. (2002). Resistance to striatal dopamine depletion induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice expressing human mutant Cu, Zn superoxide dismutase. *Neuroscience Letters, 325*(2), 124–128.

Fornai, F., Schlüter, O. M., Lenzi, P., Gesi, M., Ruffoli, R., Ferrucci, M., Lazzeri, G., Busceti, C. L., Pontarelli, F., Battaglia, G., Pellegrini, A., Nicoletti, F., Ruggieri, S., Paparelli, A., & Südhof, T. C. (2005). Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein. *Proceedings of the National Academy of Sciences of the United States of America, 102*(9), 3413–3418.

Fornai, F., Di Poggio, A. B., Pellegrini, A., Ruggieri, S., & Paparelli, A. (2007a). Noradrenaline in Parkinson's disease: From disease progression to current therapeutics. *Current Medicinal Chemistry, 14*(22), 2330–2334.

Fornai, F., Ruffoli, R., Soldani, P., Ruggieri, S., & Paparelli, A. (2007b). The "parkinsonian heart": From novel vistas to advanced therapeutic approaches in Parkinson's disease. *Current Medicinal Chemistry, 14*(23), 2421–2428.

Forno, L. S., Langston, J. W., DeLanney, L. E., Irwin, I., & Ricaurte, G. A. (1986). Locus ceruleus lesions and eosinophilic inclusions in MPTP-treated monkeys. *Annals of Neurology, 20*(4), 449–455.

Forno, L. S., DeLanney, L. E., Irwin, I., & Langston, J. W. (1993). Similarities and differences between MPTP-induced parkinsonsim and Parkinson's disease. Neuropathologic considerations. *Advances in Neurology, 60*, 600–608.

Forno, L. S., DeLanney, L. E., Irwin, I., & Langston, J. W. (1995). Ultrastructure of eosinophilic inclusion bodies in the amygdala-parahippocampal region of aged squirrel monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a dopaminergic neurotoxin. *Neuroscience Letters, 184*(1), 44–47.

Fukumitsu, N., Suzuki, M., Fukuda, T., Kiyono, Y., Kajiyama, S., & Saji, H. (2006). Reduced 125I-meta-iodobenzylguanidine uptake and norepinephrine transporter density in the hearts of mice with MPTP-induced parkinsonism. *Nuclear Medicine and Biology, 33*, 37–42.

Gainetdinov, R. R., Fumagalli, F., Jones, S. R., & Caron, M. G. (1997). Dopamine transporter is required for in vivo MPTP neurotoxicity: Evidence from mice lacking the transporter. *Journal of Neurochemistry, 69*(3), 1322–1325.

Gainetdinov, R. R., Fumagalli, F., Wang, Y. M., Jones, S. R., Levey, A. I., Miller, G. W., & Caron, M. G. (1998). Increased MPTP neurotoxicity in vesicular monoamine transporter 2 heterozygote knockout mice. *Journal of Neurochemistry, 70*(5), 1973–1978.

Gerlach, M., & Riederer, P. (1996). Animal models of Parkinson's disease: An empirical comparison with the phenomenology of the disease in man. *Journal of Neural Transmission. Supplementum, 103*, 987–1041.

Gesi, M., Soldani, P., Giorgi, F. S., Santinami, A., Bonaccorsi, I., & Fornai, F. (2000). The role of locus coeruleus in the development of Parkinson's disease. *Neuroscience and Biobehavioral Reviews, 24*, 655–658.

Gibrat, C., Saint-Pierre, M., Bousquet, M., Lévesque, D., Rouillard, C., & Cicchetti, F. (2009). Differences between subacute and chronic MPTP mice models: Investigation of dopaminergic neuronal degeneration and alpha-synuclein inclusions. *Journal of Neurochemistry, 109*(5), 1469–1482.

Giorgi, F. S., Bandettini di Poggio, A., Battaglia, G., Pellegrini, A., Murri, L., Ruggieri, S., Paparelli, A., & Fornai, F. (2006). A short overview on the role of alpha-synuclein and proteasome in experimental models of Parkinson's disease. *Journal of Neural Transmission. Supplementum, 70*, 105–109.

Giovanni, A., Sieber, B. A., Heikkila, R. E., & Sonsalla, P. K. (1991). Correlation between the neostriatal content of the 1-methyl-4-phenylpyridinium species and dopaminergic neurotoxicity following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration to several strains of mice. *The Journal of Pharmacology and Experimental Therapeutics, 257*(2), 691–697.

Giovanni, A., Sieber, B. A., Heikkila, R. E., & Sonsalla, P. K. (1994a). Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine part 1: Systemic administration. *The Journal of Pharmacology and Experimental Therapeutics, 270*, 1000–1007.

Giovanni, A., Sonsalla, P. K., & Heikkila, R. E. (1994b). Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine Part 2: Central administration of 1-methyl-4-phenylpyridinium. *The Journal of Pharmacology and Experimental Therapeutics, 270*, 1008–1014.

Goldberg, N. R., Haack, A. K., Lim, N. S., Janson, O. K., & Meshul, C. K. (2011). Dopaminergic and behavioral correlates of progressive lesioning of the nigrostriatal pathway with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neuroscience, 180*, 256–271.

Granado, N., Ares-Santos, S., Oliva, I., O'Shea, E., Martin, E. D., Colado, M. I., & Moratalla, R. (2011). Dopamine D2-receptor knockout mice are protected against dopaminergic neurotoxicity induced by methamphetamine or MDMA. *Neurobiology of Disease, 42*(3), 391–403.

Grégoire, L., Morin, N., Ouattara, B., Gasparini, F., Bilbe, G., Johns, D., Vranesic, I., Sahasranaman, S., Gomez-Mancilla, B., & Di Paolo, T. (2011). The acute antiparkinsonian and antidyskinetic effect of AFQ056, a novel metabotropic glutamate receptor type 5 antagonist, in L-Dopa-treated parkinsonian monkeys. *Parkinsonism & Related Disorders, 17*(4), 270–276.

Gu, M., Cooper, J. M., Taanman, J. W., & Schapira, A. H. (1998). Mitochondrial DNA transmission of the mitochondrial defect in Parkinson's disease. *Annals of Neurology, 44*(2), 177–186.

Haber, S. N., Ryoo, H., Cox, C., & Lu, W. (1995). Subsets of midbrain dopaminergic neurons in monkeys are distinguished by different levels of mRNA for the dopamine transporter: Comparison with the mRNA for the D2 receptor, tyrosine hydroxylase and calbindin immunoreactivity. *The Journal of Comparative Neurology, 362*(3), 400–410.

Hamre, K., Tharp, R., Poon, K., Xiong, X., & Smeyne, R. J. (1999). Differential strain susceptibility following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration acts in an autosomal dominant fashion: Quantitative analysis in seven strains of Mus musculus. *Brain Research, 828*, 91–103.

Hartley, A., Stone, J. M., Heron, C., Cooper, J. M., & Schapira, A. H. (1994). Complex I inhibitors induce dose-dependent apoptosis in PC12 cells: Relevance to Parkinson's disease. *Journal of Neurochemistry, 63*(5), 1987–1990.

Heikkila, R. E. (1985). Differential neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in Swiss-Webster mice from different sources. *European Journal of Pharmacology, 117*(1), 131–133.

Heikkila, R. E., Manzino, L., Cabbat, F. S., & Duvoisin, R. C. (1984a). Protection against the dopaminergic toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by monoamine oxidase inhibitors. *Nature, 311*, 467–469.

Heikkila, R. E., Hess, A., & Duvoisin, R. C. (1984b). Dopaminergic neurotoxicity of MPTP in mice. *Science, 224*, 1451–1453.

Heikkila, R. E., Nicklas, W. J., Vyas, I., & Duvoisin, R. C. (1985). Dopaminergic toxicity of rotenone and the 1-methyl-4-phenylpyridinium ion after their stereotaxic administration to rats: Implication for the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity. *Neuroscience Letters, 62*(3), 389–394.

Herkenham, M., Little, M. D., Bankiewicz, K., Yang, S. C., Markey, S. P., & Johannessen, J. N. (1991). Selective retention of MPP^+ within the monoaminergic systems of the primate brain following MPTP administration: An in vivo autoradiographic study. *Neuroscience, 40*(1), 133–158.

Hornykiewicz, O. (1975). Brain monoamines and parkinsonism. *National Institute on Drug Abuse Research Monograph Series, 3*, 13–21.

Hornykiewicz, O. (1998). Biochemical aspects of Parkinson's disease. *Neurology, 51*(2 Suppl 2), 2–9.

Huot, P., Johnston, T. H., Lewis, K. D., Koprich, J. B., Reyes, M. G., Fox, S. H., Piggott, M. J., & Brotchie, J. M. (2011). Characterization of 3,4-methylenedioxymethamphetamine (MDMA) enantiomers in vitro and in the MPTP-lesioned primate: R-MDMA reduces severity of dyskinesia, whereas S-MDMA extends duration of ON-time. *The Journal of Neuroscience, 31*(19), 7190–7198.

Imai, H., Nakamura, T., Endo, K., & Narabayashi, H. (1988). Hemiparkinsonism in monkeys after unilateral caudate nucleus infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): Behavior and histology. *Brain Research, 474*(2), 327–332.

Jackson-Lewis, V., & Przedborski, S. (2007). Protocol for the MPTP mouse model of Parkinson's disease. *Nature Protocols, 2*(1), 141–151.

Jackson-Lewis, V., Jakowec, M., Burke, R. E., & Przedborski, S. (1995). Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurodegeneration, 4*(3), 257–269.

Javitch, J. A., Uhl, G. R., & Snyder, S. H. (1984). Parkinsonism-inducing neurotoxin, *N*-methyl-4-phenyl-1,2,3,6 -tetrahydropyridine: Characterization and localization of receptor binding sites in rat and human brain. *Proceedings of the National Academy of Sciences of the United States of America, 81*(14), 4591–4595.

Jellinger, K. A. (2002). Recent developments in the pathology of Parkinson's disease. *Journal of Neural Transmission. Supplementum, 62*, 347–376.

Jenner, P., Rupniak, N. M., Rose, S., Kelly, E., Kilpatrick, G., Lees, A., & Marsden, C. D. (1984). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the common marmoset. *Neuroscience Letters, 50*(1–3), 85–90.

Johannessen, J. N. (1991). A model for chronic neurotoxicity: Long-term retention of the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) within catecholaminergic neurons. *Neurotoxicology, 12*, 285–302.

Johnston, T. H., Fox, S. H., McIldowie, M. J., Piggott, M. J., & Brotchie, J. M. (2010). Reduction of L-DOPA-induced dyskinesia by the selective metabotropic glutamate receptor 5 antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. *The Journal of Pharmacology and Experimental Therapeutics, 333*(3), 865–873.

Jost, W. H. (1997). Gastrointestinal motility problems in patients with Parkinson's disease: Effects of antiparkinsonian treatment and guidelines for management. *Drugs & Aging, 10*, 249–258.

Kanthasamy, A. G., Kanthasamy, A., Matsumoto, R. R., Vu, T. Q., & Truong, D. D. (1997). Neuroprotective effects of the strychnine-insensitive glycine site NMDA antagonist (R)-HA-966 in an experimental model of Parkinson's disease. *Brain Research, 759*, 1–8.

Kish, S. J., Shannak, K., & Hornykiewicz, O. (1988). Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. *The New England Journal of Medicine, 318*(14), 876–880.

Klivenyi, P., St Clair, D., Wermer, M., Yen, H. C., Oberley, T., Yang, L., & Flint Beal, M. (1998). Manganese superoxide dismutase overexpression attenuates MPTP toxicity. *Neurobiology of Disease, 5*(4), 253–258.

Klos, K. J., Ahlskog, J. E., Josephs, K. A., Apaydin, H., Parisi, J. E., Boeve, B. F., DeLucia, M. W., & Dickson, D. W. (2006). Alpha-synuclein pathology in the spinal cords of neurologically asymptomatic aged individuals. *Neurology, 66*(7), 1100–1102.

Kopin, I. J. (1987). MPTP: An industrial chemical and contaminant of illicit narcotics stimulates a new era in research on Parkinson's disease. *Environmental Health Perspectives, 75*, 45–51.

Kopin, I. J., & Markey, S. P. (1988). MPTP toxicity: Implication for research in Parkinson's disease. *Annual Review of Neuroscience, 11*, 81–96.

Koprich, J. B., Fox, S. H., Johnston, T. H., Goodman, A., Le Bourdonnec, B., Dolle, R. E., DeHaven, R. N., DeHaven-Hudkins, D. L., Little, P. J., & Brotchie, J. M. (2011). The selective mu-opioid receptor antagonist ADL5510 reduces levodopa-induced dyskinesia without affecting antiparkinsonian action in MPTP-lesioned macaque model of Parkinson's disease. *Movement Disorders, 26*(7), 1225–1233.

Kowall, N. W., Hantraye, P., Brouillet, E., Beal, M. F., McKee, A. C., & Ferrante, R. J. (2000). MPTP induces alpha-synuclein aggregation in the substantia nigra of baboons. *Neuroreport, 11*(1), 211–213.

Krueger, M. J., Singer, T. P., Casida, J. E., & Ramsay, R. R. (1990). Evidence that the blockade of mitochondrial respiration by the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) involves binding at the same site as the respiratory inhibitor, rotenone. *Biochemical and Biophysical Research Communications, 169*(1), 123–128.

Kühn, K., Wellen, J., Link, N., Maskri, L., Lübbert, H., & Stichel, C. C. (2003). The mouse MPTP model: Gene expression changes in dopaminergic neurons. *The European Journal of Neuroscience, 17*(1), 1–12.

Kupsch, A., Loshmann, P. A., Saner, H., Arnold, G., Renner, P., Pufal, D., Burg, M., Wachtel, H., ten Bruggencate, G., & Oertel, W. H. (1992). Do NMDA receptor antagonists protect against MPTP toxicity? Biochemical and immunocytochemical analysis in black mice. *Brain Research, 592*, 74–83.

Kurosaki, R., Muramatsu, Y., Kato, H., & Araki, T. (2004). Biochemical, behavioral and immunohistochemical alterations in MPTP-treated mouse model of Parkinson's disease. *Pharmacology Biochemistry, and Behavior, 78*(1), 143–153.

Lange, K. W., & Riederer, P. (1994). Glutamatergic drugs in Parkinson's disease. *Life Sciences, 55*, 2067–2075.

Lange, K. W., Loschmann, P. A., Sofic, E., Burg, M., Horowski, R., Kalveram, K. T., Wachtel, H., & Riederer, P. (1993). The competitive NMDA antagonist CPP protects substantia nigra neurons from MPTP- induced degeneration in primates. *Naunyn-Schmiedeberg's Archives of Pharmacology, 348*, 586–592.

Langston, J. W., & Palfreman, J. (1996). *The case of the frozen addicts*. New York: Vintage Books.

Langston, J. W., Ballard, P. A., Tetrud, J. W., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science, 219*, 979–980.

Langston, J. W., Forno, L. S., Rebert, C. S., & Irwin, I. (1984a). Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. *Brain Research, 292*, 390–394.

Langston, J. W., Irwin, I., & Langston, E. B. (1984b). Pargyline prevents MPTP induced parkinsonism in primates. *Science, 225*, 1480–1482.

Langston, J. W., Forno, L. S., Tetrud, J., Reeves, A. G., Kaplan, J. A., & Karluk, D. (1999). Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. *Annals of Neurology, 46*(4), 598–605.

Lau, Y. S., Novikova, L., & Roels, C. (2005). MPTP treatment in mice does not transmit and cause Parkinsonian neurotoxicity in non-treated cagemates through close contact. *Neurosciences Research, 52*(4), 371–378.

Lee, C. S., Park, W. J., Ko, H. H., & Han, E. S. (2006). Differential involvement of mitochondrial permeability transition in cytotoxicity of 1-methyl-4-phenylpyridinium and 6-hydroxydopamine. *Molecular and Cellular Biochemistry, 289*, 193–200.

Liu K., Shi N., Sun Y., Zhang T., Sun X. (2013). Therapeutic effects of rapamycin on MPTP-induced Parkinsonism in mice. *Neurochem Res, 38*(1), 201–207.

Loschmann, P. A., Lange, K. W., Wachtel, H., & Turski, L. (1994). MPTP-induced degeneration: Interference with glutamatergic toxicity. *Journal of Neural Transmission. Supplementum, 43*, 133–143.

Lotharius, J., & O'Malley, K. L. (2000). The parkinsonism-inducing drug 1-methyl-4-phenylpyridinium triggers intracellular dopamine oxidation A novel mechanism of toxicity. *The Journal of Chemical Biology, 275*, 38581–38588.

Malagelada, C., Ryu, E. J., Biswas, S. C., Jackson-Lewis, V., & Greene, L. A. (2006). RTP801 is elevated in Parkinson brain substantia nigral neurons and mediates death in cellular models of Parkinson's disease by a mechanism involving mammalian target of rapamycin inactivation. *The Journal of Neuroscience, 26*(39), 9996–10005.

Maragos, W. F., Jakel, R., Chesnut, D., Pocernich, C. B., Butterfield, D. A., St Clair, D., & Cass, W. A. (2000). Methamphetamine toxicity is attenuated in mice that overexpress human manganese superoxide dismutase. *Brain Research, 878*(1–2), 218–222.

Marien, M., Briley, M., & Colpaert, F. (1993). Noradrenaline depletion exacerbates MPTP-induced striatal dopamine loss in mice. *European Journal of Pharmacology, 236*, 487–489.

Mariño, G., & López-Otín, C. (2004). Autophagy: Molecular mechanisms, physiological functions and relevance in human pathology. *Cellular and Molecular Life Sciences, 61*, 1439–1454.

Martin, L. J., Pan, Y., Price, A. C., Sterling, W., Copeland, N. G., Jenkins, N. A., Price, D. L., & Lee, M. K. (2006). Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. *The Journal of Neuroscience, 26*(1), 41–50.

Masilamoni, G. J., Bogenpohl, J. W., Alagille, D., Delevich, K., Tamagnan, G., Votaw, J. R., Wichmann, T., & Smith, Y. (2011). Metabotropic glutamate receptor 5 antagonist protects dopaminergic and noradrenergic neurons from degeneration in MPTP-treated monkeys. *Brain, 134*, 2057–2073.

Mavridis, M., Degryse, A. D., Lategan, A. J., Marien, M. R., & Colpaert, F. C. (1991). Effects of locus coeruleus lesions on parkinsonian signs, striatal dopamine and substantia nigra cell loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in monkeys: A possible role for the locus coeruleus in the progression of Parkinson's disease. *Neuroscience, 41*, 507–523.

McCann, U. D., Wong, D. F., Yokoi, F., Villemagne, V., Dannals, R. F., & Ricaurte, G. A. (1998). Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: Evidence from positron emission tomography studies with [11C]WIN-35,428. *The Journal of Neuroscience, 18*, 8417–8422.

Melamed, E., Pikarski, E., Goldberg, A., Rosenthal, J., Uzzan, A., & Conforti, N. (1986). Effect of serotonergic, corticostriatal and kainic acid lesions on the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) in mice. *Brain Research, 399*(1), 178–180.

Meredith, G. E., Totterdell, S., Petroske, E., Santa Cruz, K., Callison, R. C., Jr., & Lau, Y. S. (2002). Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease. *Brain Research, 956*(1), 156–165.

Meredith, G. E., Totterdell, S., Potashkin, J. A., & Surmeier, D. J. (2008). Modeling PD pathogenesis in mice: Advantages of a chronic MPTP protocol. *Parkinsonism & Related Disorders, 14*(Suppl 2), 112–115.

Meredith, G. E., Totterdell, S., Beales, M., & Meshul, C. K. (2009). Impaired glutamate homeostasis and programmed cell death in a chronic MPTP mouse model of Parkinson's disease. *Experimental Neurology, 219*(1), 334–340.

Moriyama, Y., Amakatsu, K., & Futai, M. (1993). Uptake of the neurotoxin, 4-methylphenylpyridinium, into chromaffin granules and synaptic vesicles: A proton gradient drives its uptake through monoamine transporter. *Archives of Biochemistry and Biophysics, 305*, 271–277.

Muthane, U., Ramsay, K. A., Jiang, H., Jackson-Lewis, V., Donaldson, D., Fernando, S., Ferreira, M., & Przedborski, S. (1994). Differences in nigral neuron number and sensitivity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium in C57/bl and CD-1 mice. *Experimental Neurology, 126*, 195–204.

Natale, G., Pasquali, L., Ruggieri, S., Paparelli, A., & Fornai, F. (2008). Parkinson's disease and the gut: A well known clinical association in need of an effective cure and explanation. *Neurogastroenterology and Motility, 20*(7), 741–749.

Natale, G., Kastsiushenka, O., Fulceri, F., Ruggieri, S., Paparelli, A., & Fornai, F. (2010). MPTP-induced parkinsonism extends to a subclass of TH-positive neurons in the gut. *Brain Research, 1355*, 195–206.

Natale, G., Pasquali, L., Paparelli, A., & Fornai, F. (2011). Parallel manifestations of neuropathologies in the enteric and central nervous systems. *Neurogastroenterology and Motility, 23*(12), 1056–1065.

Nicklas, W. J., Vyas, I., & Heikkila, R. E. (1985). Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenylpyridine, a metabolite of the neurotoxin 1 -methyl-4-phenyl- 1,2,3,6-tetrahydropyridine. *Life Sciences, 36*, 2503–2508.

Nomoto, M., Jenner, P., & Marsden, C. D. (1985). The dopamine D2 agonist LY 141865, but not the D1 agonist SKF 38393, reverses parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the common marmoset. *Neuroscience Letters, 57*, 37–41.

Novikova, L., Garris, B. L., Garris, D. R., & Lau, Y. S. (2006). Early signs of neuronal apoptosis in the substantia nigra pars compacta of the progressive neurodegenerative mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid model of Parkinson's disease. *Neuroscience, 140*, 67–76.

O'Dell, S. J., Weihmuller, F. B., & Marshall, J. F. (1991). Multiple methamphetamine injections induce marked increases in extracellular striatal dopamine which correlate with subsequent neurotoxicity. *Brain Research, 564*, 256–260.

Olney, J. W., Labruyere, J., & de Gubareff, T. (1980). Brain damage in mice from voluntary ingestion of glutamate and aspartate. *Neurobehavioral Toxicology, 2*(2), 125–129.

Ossowska, K. (1994). The role of excitatory amino acids in experimental models of Parkinson's disease. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 8*, 39–71.

Petroske, E., Meredith, G. E., Callen, S., Totterdell, S., & Lau, Y. S. (2001). Mouse model of Parkinsonism: A comparison between subacute MPTP and chronic MPTP/probenecid treatment. *Neuroscience, 106*(3), 589–601.

Petzinger, G. M., & Langston, J. W. (1998). The MPTP-lesioned nonhuman primate: A model for Parkinson's disease. In J. Marwah & H. Teiltelbaum (Eds.), *Advances in neurodegenerative disorders. Parkinson's disease* (pp. 113–148). Scottsdale: Prominent Press.

Pfeiffer, R. F. (2003). Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurology, 2*, 107–116.

Pifl, C., Schingnitz, G., & Hornykiewicz, O. (1988). The neurotoxin MPTP does not reproduce in the rhesus monkey the interregional pattern of striatal dopamine loss typical of human idiopathic Parkinson's disease. *Neuroscience Letters, 92*(2), 228–233.

Pileblad, E., & Carlsson, A. (1985). Catecholamine-uptake inhibitors prevent the neurotoxicity of 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine (MPTP) in mouse brain. *Neuropharmacology, 24*, 689–692.

Prediger, R. D., Aguiar, A. S., Jr., Moreira, E. L., Matheus, F. C., Castro, A. A., Walz, R., De Bem, A. F., Latini, A., Tasca, C. I., Farina, M., & Raisman-Vozari, R. (2011). The intranasal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): A new rodent model to test palliative and neuroprotective agents for Parkinson's disease. *Current Pharmaceutical Design, 17*(5), 489–507.

Przedborski, S., & Vila, M. (2003). The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model: A tool to explore the pathogenesis of Parkinson's disease. *Annals of the New York Academy of Sciences, 991*, 189–198.

Przedborski, S., Jackson-Lewis, V., Popilskis, S., Kostic, V., Levivier, M., Fahn, S., & Cadet, J. L. (1991). Unilateral MPTP-induced parkinsonism in monkeys. A quantitative autoradiographic study of dopamine D1 and D2 receptors and re-uptake sites. *Neurochirurgie, 37*(6), 377–382.

Przedborski, S., Kostic, V., Jackson-Lewis, V., Naini, A. B., Simonetti, S., Fahn, S., Carlon, E., Epstein, C. J., & Cadet, J. L. (1992). Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. *The Journal of Neuroscience, 12*, 1658–1667.

Przedborski, S., Jackson-Lewis, V., Naini, A. B., Jakowec, M., Petzinger, G., Miller, R., & Akram, M. (2001). The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): A technical review of its utility and safety. *Journal of Neurochemistry, 76*, 1265–1274.

Purisai, M. G., McCormack, A. L., Langston, W. J., Johnston, L. C., & Di Monte, D. A. (2005). Alpha-synuclein expression in the substantia nigra of MPTP-lesioned non- human primates. *Neurobiology of Disease, 20*(3), 898–906.

Ramsay, R. R., & Singer, T. P. (1986). Energy-dependent uptake of 1-methyl-4-phenylpyridinium, the neurotoxic metabolite of 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine, by mitochondria. *The Journal of Biological Chemistry, 261*, 7885–7887.

Ramsay, R. R., Salach, J. I., & Singer, T. P. (1986). Uptake of the neurotoxin 1-methyl-4-phenylpyridine (MPP^+) by mitochondria and its relation to the inhibition of the mitochondrial oxidation of NAD+ −linked substrates by MPP^+. *Biochemical and Biophysical Research Communications, 134*(2), 743–748.

Riahi, G., Morissette, M., Parent, M., & Di Paolo, T. (2011). Brain 5-HT(2A) receptors in MPTP monkeys and levodopa-induced dyskinesias. *The European Journal of Neuroscience, 33*(10), 1823–1831.

Ricaurte, G. A., & McCann, U. D. (1992). Neurotoxic amphetamine analogues: Effects in monkeys and implications for humans. *Annals of the New York Academy of Sciences, 648*, 371–382.

Ricaurte, G. A., Guillery, R. W., Seiden, L. S., Schuster, C. R., & Moore, R. Y. (1982). Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Research, 235*, 93–103.

Ricaurte, G. A., Langston, J. W., DeLanney, L. E., Irwin, I., & Brooks, J. D. (1985). Dopamine uptake blockers protect against the dopamine depleting effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the mouse striatum. *Neuroscience Letters, 59*(3), 259–264.

Ruffoli, R., Soldani, P., Pasquali, L., Ruggieri, S., Paparelli, A., & Fornai, F. (2008). Methamphetamine fails to alter the noradrenergic integrity of the heart. *Annals of the New York Academy of Sciences, 1139*, 337–344.

Rye, D., & DeLong, M. R. (2003). Time to focus on the locus. *Archives of Neurology, 60*(3), 320.

Samadi, P., Grégoire, L., Morissette, M., Calon, F., Hadj Tahar, A., Dridi, M., Belanger, N., Meltzer, L. T., Bédard, P. J., & Di Paolo, T. (2008). mGluR5 metabotropic glutamate receptors and dyskinesias in MPTP monkeys. *Neurobiology of Aging, 29*(7), 1040–1051.

Samantaray, S., Ray, S. K., Ali, S. F., & Banik, N. L. (2006). Calpain activation in apoptosis of motoneurons in cell culture models of experimental parkinsonism. *Annals of the New York Academy of Sciences, 1074*, 349–356.

Samantaray, S., Knaryan, V. H., Guyton, M. K., Matzelle, D. D., Ray, S. K., & Banik, N. L. (2007). The parkinsonian neurotoxin rotenone activates calpain and caspase-3 leading to motoneuron degeneration in spinal cord of Lewis rats. *Neuroscience, 146*(2), 741–755.

Samantaray, S., Butler, J. T., Ray, S. K., & Banik, N. L. (2008a). Extranigral neurodegeneration in Parkinson's disease. *Annals of the New York Academy of Sciences, 1139*, 331–336.

Samantaray, S., Knaryan, V. H., Butler, J. T., Ray, S. K., & Banik, N. L. (2008b). Spinal cord degeneration in C57BL/6N mice following induction of experimental parkinsonism with MPTP. *Journal of Neurochemistry, 104*(5), 1309–1320.

Scotchner, K. P., Irwin, I., DeLanney, L. E., Langston, J. W., & Di Monte, D. (1990). Effects of 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine and 1-methyl-4-phenylpyridinium ion on ATP levels of mouse brain synaptosomes. *Journal of Neurochemistry, 54*, 1295–1301.

Sedelis, M., Hofele, K., Auburger, G. W., Morgan, S., Huston, J. P., & Schwarting, R. K. (2000). MPTP susceptibility in the mouse: Behavioral, neurochemical, and histological analysis of gender and strain differences. *Behavior Genetics, 30*(3), 171–182.

Seniuk, N. A., Tatton, W. G., & Greenwood, C. E. (1990). Dose-dependent destruction of the coeruleus-cortical and nigral-striatal projections by MPTP. *Brain Research, 527*(1), 7–20.

Serra, P. A., Sciola, L., Delogu, M. R., Spano, A., Monaco, G., Miele, E., Rocchitta, G., Miele, M., Migheli, R., & Desole, M. S. (2002). The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induces apoptosis in mouse nigrostriatal glia. Relevance to nigral neuronal death and striatal neurochemical changes. *The Journal of Biological Chemistry, 277*(37), 34451–34461.

Singaram, C., Ashraf, W., Gaumnitz, E. A., Torbey, C., Sengupta, A., Pfeiffer, R., & Quigley, E. M. (1995). Dopaminergic defect of enteric nervous system in Parkinson's disease patients with chronic constipation. *Lancet, 346*(8979), 861–864.

Sirinathsinghji, D. J., Heavens, R. P., Richards, S. J., Beresford, I. J., & Hall, M. D. (1988). Experimental hemiparkinsonism in the rat following chronic unilateral infusion of MPP^+ into the nigrostriatal dopamine pathway. Behavioural, neurochemical and histological characterization of the lesion. *Neuroscience, 27*(1), 117–128.

Sonsalla, P. K., & Heikkila, R. E. (1986). The influence of dose and dosing interval on MPTP-induced dopaminergic neurotoxicity in mice. *European Journal of Pharmacology, 129*(3), 339–345.

Sonsalla, P. K., & Heikkila, R. E. (1988). Neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine in several strains of mice. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 12*(2–3), 345–354.

Sonsalla, P. K., Nicklas, W. J., & Heikkila, R. E. (1989). Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity. *Science, 243*, 398–400.

Sonsalla, P. K., Giovanni, A., Sieber, B. A., Delle Donne, K., & Manzino, L. (1992). Characteristics of dopaminergic neurotoxicity produced by MPTP and methamphetamine. *Annals of the New York Academy of Sciences, 648*, 229–238.

Sonsalla, P. K., Jochnowitz, N. D., Zeevalk, G. D., Oostveen, J. A., & Hall, E. D. (1996). Treatment of mice with methamphetamine produces cell loss in the substantia nigra. *Brain Research, 738*(1), 172–175.

Sonsalla, P. K., Albers, D. S., & Zeevalk, G. D. (1998). Role of glutamate in neurodegeneration of dopamine neurons in several animal models of parkinsonism. *Amino Acids, 14*, 69–74.

Sonsalla, P. K., Zeevalk, G. D., & German, D. C. (2008). Chronic intraventricular administration of 1-methyl-4-phenylpyridinium as a progressive model of Parkinson's disease. *Parkinsonism & Related Disorders, 14*(Suppl 2), 116–118.

Srivastava, R., Brouillet, E., Beal, M. F., Storey, E., & Hyman, B. T. (1993). Blockade of 1-methyl-4-phenylpyridinium ion (MPP) nigral toxicity in the rat by prior decortication or MK-801 treatment: A stereological estimate of neuronal loss. *Neurobiology of Aging, 14*, 295–301.

Staal, R. G., & Sonsalla, P. K. (2000). Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata. *The Journal of Pharmacology and Experimental Therapeutics, 293*(2), 336–342.

Stefanis, L., Larsen, K. E., Rideout, H. J., Sulzer, D., & Greene, L. A. (2001). Expression of A53T mutant but not wild-type alpha-synuclein in PC12 cells induces altera- tions of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death. *The Journal of Neuroscience, 21*, 9549–9560.

Storey, E., Hyman, B. T., Jenkins, B., Brouillet, E., Miller, J. M., Rosen, B. R., & Beal, M. F. (1992). 1-Methyl-4-phenylpyridinium produces excitotoxic lesion in rat striatum as a result of impairment of oxidative metabolism. *Journal of Neurochemistry, 58*, 1975–1978.

Sulzer, D. (2001). Alpha-synuclein and cytosolic dopamine: Stabilizing a bad situation. *Nature Medicine, 7*(12), 1280–1282.

Sundstrom, E., & Jonsson, G. (1985). Pharmacological interference with the neurotoxic action of 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine (MPTP) on central catecholamine neurons in the mouse. *European Journal of Pharmacology, 110*, 293–299.

Sundström, E., Goldstein, M., & Jonsson, G. (1986). Uptake inhibition protects nigro-striatal dopamine neurons from the neurotoxicity of 1-methyl-4-phenylpyridine (MPP^+) in mice. *European Journal of Pharmacology, 131*(2–3), 289–292.

Sundström, E., Fredriksson, A., & Archer, T. (1990). Chronic neurochemical and behavioral changes in MPTP-lesioned C57BL/6 mice: A model for Parkinson's disease. *Brain Research, 528*(2), 181–188.

Szabo, S., Brown, A., Pihan, G., Dali, H., & Neumeyer, J. L. (1985). Duodenal ulcer induced by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine). *Proceedings of the Society for Experimental Biology and Medicine, 180*, 567–571.

Takahashi, N., Miner, L. L., Sora, I., Ujike, H., Revay, R. S., Kostic, V., Jackson-Lewis, V., Przedborski, S., & Uhl, G. R. (1997). VMAT2 knockout mice: Heterozygotes display reduced amphetamine conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 9938–9943.

Tanaka, Y., Engelender, S., Igarashi, S., Rao, R. K., Wanner, T., Tanzi, R. E., Sawa, A. L., Dawson, V., Dawson, T. M., & Ross, C. A. (2001). Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. *Human Molecular Genetics, 10*, 919–926.

Tatton, N. A., & Kish, S. J. (1997). In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labelling and acridine orange staining. *Neuroscience, 77*(4), 1037–1048.

Teismann, P., Tieu, K., Choi, D. K., Wu, D. C., Naini, A., Hunot, S., Vila, M., Jackson-Lewis, V., & Przedborski, S. (2003). Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proceedings of the National Academy of Sciences of the United States of America, 100*(9), 5473–5478.

Tong, J., Hornykiewicz, O., & Kish, S. J. (2006). Inverse relationship between brain noradrenaline level and DA loss in Parkinson disease: A possible neuroprotective role for noradrenaline. *Archives of Neurology, 63*(12), 1724–1728.

Tretiakoff C. (1919). *Contributions a l'etude de l'anatomie pathologique du locus niger de soemmering avec quelques deductions relatives a la pathogenie des troubles de tonus musculaire et de la maladie de Parkinson*. (Thesis, Paris).

Turski, L., Bressler, K., Rettig, K. J., Losbmann, P. A., & Wachtel, H. (1991). Protection of substantia nigra from MPP^+ neurotoxicity by N-methylo-aspartate antagonists. *Nature, 349*, 414–418.

Vaglini, F., Fascetti, F., Fornai, F., Maggio, R., & Corsini, G. U. (1994). (+)MK-801 prevents the DDC induced enhancement of MPTP toxicity in mice. *Brain Research, 668*, 194–203.

Vaglini, F., Fascetti, F., Tedeschi, D., Cavalletti, M., Fornai, F., & Corsini, G. U. (1996). Striatal MPP^+ levels do not necessarily correlate with striatal dopamine levels after MPTP treatment in mice. *Neurodegeneration, 5*(2), 129–136.

Vezoli, J., Fifel, K., Leviel, V., Dehay, C., Kennedy, H., Cooper, H. M., Gronfier, C., & Procyk, E. (2011). Early presymptomatic and long-term changes of rest activity cycles and cognitive behavior in a MPTP-monkey model of Parkinson's disease. *PLoS One, 6*(8), 23952.

Villemagne, V., Yuan, J., Wong, D. F., Dannals, R. F., Hatzidimitriou, G., Mathews, W. B., Ravert, H. T., Musachio, J., McCann, U. D., & Ricaurte, G. A. (1998). Brain dopamine neurotoxicity in baboons treated with doses of methamphetamine comparable to those recreationally abused by humans: Evidence from [^{11}C]WIN-35,428 positron emission tomography studies and direct in vitro determinations. *The Journal of Neuroscience, 18*, 419–427.

Vivacqua, G., Biagioni, F., Yu, S., Casini, A., Bucci, D., D'Este, L., & Fornai, F. (2012). Loss of spinal motor neurons and alteration of alpha-synuclein immunostaining in MPTP induced Parkinsonism in mice. *Journal of Chemical Neuroanatomy, 44*(2), 76–85.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Franceschi, D., Sedler, M., Gatley, S. J., Miller, E., Hitzemann, R., Ding, Y. S., & Logan, J. (2001a). Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *The Journal of Neuroscience, 21*, 9414–9418.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Leonido-Yee, M., Franceschi, D., Sedler, M. J., Gatley, S. J., Hitzemann, R., Ding, Y. S., Logan, J., Wong, C., & Miller, E. N. (2001b). Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *The American Journal of Psychiatry, 158*, 377–382.

Wakabayashi, K., Takahashi, H., Takeda, S., Ohama, E., & Ikuta, F. (1988). Parkinson's disease: The presence of Lewy bodies in Auerbach's and Meissner's plexuses. *Acta Neuropathologica, 76*(3), 217–221.

Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J., & Selkoe, D. J. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature, 416*, 535–539.

Watanabe, Y., Himeda, T., & Araki, T. (2005). Mechanisms of MPTP toxicity and their implications for therapy of Parkinson's disease. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 11*(1), 17–23.

Weinshenker, D., Ferrucci, M., Busceti, C. L., Biagioni, F., Lazzeri, G., Liles, L. C., Lenzi, P., Pasquali, L., Murri, L., Paparelli, A., & Fornai, F. (2008). Genetic or pharmacological blockade of noradrenaline synthesis enhances the neurochemical, behavioral, and neurotoxic effects of methamphetamine. *Journal of Neurochemistry, 105*(2), 471–483.

Woolverton, W. L., Ricaurte, G. A., Forno, L. S., & Seiden, L. S. (1989). Long-term effects of chronic methamphetamine administration in rhesus monkey. *Brain Research, 486*, 73–78.

Yasuda, T., Hayakawa, H., Nihira, T., Ren, Y. R., Nakata, Y., Nagai, M., Hattori, N., Miyake, K., Takada, M., Shimada, T., Mizuno, Y., & Mochizuki, H. (2011). Parkin-mediated protection of dopaminergic neurons in a chronic MPTP-minipump mouse model of Parkinson disease. *Journal of Neuropathology and Experimental Neurology, 70*(8), 686–697.

Yazdani, U., German, D. C., Liang, C. L., Manzino, L., Sonsalla, P. K., & Zeevalk, G. D. (2006). Rat model of Parkinson's disease: Chronic central delivery of 1-methyl-4-phenylpyridinium (MPP^+). *Experimental Neurology, 200*(1), 172–183.

Zarow, C., Lyness, S. A., Mortimer, J. A., & Chui, H. C. (2003). Neuronal loss is greater in the LC than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Archives of Neurology, 60*(3), 337–341.

Ziering, A., Berger, L., Heineman, S. D., & Lee, J. (1947). Piperidine derivatives; 4-arylpiperidines. *The Journal of Organic Chemistry, 12*(6), 894–903.

Zuddas, A., Oberto, G., Vaglini, F., Fascetti, F., Fornai, F., & Corsini, G. U. (1992). MK-801 prevents 1-methyl-4-phenyl-l,2,3,6-tetrahydropyri- dine-induced Parkinsonism in primates. *Journal of Neurochemistry, 59*, 733–739.

Zuddas, A., Fascetti, F., Corsini, G. U., & Piccardi, M. P. (1994). In brown Norway rats, MPP^+ is accumulated in the nigrostriatal dopaminergic terminals but it is not neurotoxic: A model of natural resistance to MPTP Toxicity. *Experimental Neurology, 127*(1), 54–61.

Cocaine as a Neurotoxin

Teresa Cunha-Oliveira, A. Cristina Rego, and Catarina R. Oliveira

Contents

Abstract

Cocaine is a widely abused psychostimulant drug, with sympathomimetic properties and intense euphoric effects. Cocaine and some of its toxic metabolites cross the blood–brain barrier and induce neurologic impairments, affecting primarily the prefrontal cortex and basal ganglia. In this review, we discuss the mechanisms involved in brain dysfunction induced by cocaine, focusing on pre- and postsynaptic changes in dopaminergic and glutamatergic

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neurotransmission, oxidative stress, and mitochondrial dysfunction. Neurotoxic effects of combinations of cocaine with other drugs are also discussed. In summary, cocaine neurotoxicity may underlie brain dysfunction in cocaine and polydrug abusers and may predispose the brain to neurodegeneration.

Keywords
Cocaine • Dopamine • Glutamate • Mitochondria • Neurotoxicity • Oxidative stress

List of Abbreviations

Ca^{2+}_i	Intracellular Ca^{2+} concentration
DAT	Dopamine transporter
DAQ	Dopamine quinone
DARPP32	Dopamine- and cAMP-regulated neuronal phosphoprotein
DOPAC	3,4-dihydroxyphenylacetic acid
DOPAL	3,4-dihydroxyphenylacetaldehyde
ERK	Extracellular-signal-regulated kinase
GPx	Glutathione peroxidase
GSH	Reduced glutathione
H_2O_2	Hydrogen peroxide
MAO	Monoamine oxidase
MAPKK/MEK	Mitogen-activated protein kinase kinase/extracellular signal-regulated kinase kinase
MDA	Malondialdehyde
NMDA	*N*-methyl-D-aspartate
$O_2^{\bullet -}$	Superoxide anion
$^{\bullet}OH$	Hydroxyl radical
PARP	Poly (ADP-ribose) polymerase
PD	Parkinson's disease
PKA	Protein kinase A
PP1	Protein phosphatase 1
Ras-GRF-1	Ras protein-specific guanine nucleotide-releasing factor 1
SOD	Superoxide dismutase
VMAT	Vesicular monoamine transporter

1 Introduction

Cocaine is an alkaloid extracted from the plant *Erythroxylum coca*, cultivated in the South American countries of Bolivia, Colombia, and Peru. This substance is a widely abused psychostimulant drug, with sympathomimetic properties, used recreationally to increase alertness, relieve fatigue, and increase self-confidence and is abused due to its intense euphoric effects. Cocaine is among the most abused

illicit drugs, used annually by 0.3–0.4 % of the world population aged 15–64 years, with higher prevalence in North America (1.6 %), Oceania (1.5–1.9 %) and Western and Central Europe (1.3 %) (United Nations Office on Drugs and Crime 2012).

2 Pharmacology of Cocaine

In the streets, cocaine may be available in water-soluble or insoluble forms. Water-soluble forms include cocaine sulfate and cocaine hydrochloride, which are mainly used intranasally, but may also be administered orally, sublingually, or by injection. Some drug abusers inject cocaine hydrochloride subcutaneously, intramuscularly, or intravenously, alone or with heroin ("speedball") or other drugs. Water-insoluble forms such as free base cocaine or crack are usually smoked. Crack cocaine is abused by inhaling the vapor from cigarettes (usually mixed with tobacco or marijuana) or after heating the drug in a glass pipe. When the drug is consumed in the free base form, thermal degradation leads to the formation of the metabolite anhydroecgonine methyl ester (Fig. 1), recently shown to be neurotoxic (Garcia et al. 2012).

Upon consumption, cocaine rapidly reaches the brain because it is able to cross the blood–brain barrier, due to its high lipophilicity, in contrast to its main metabolites ecgonine methyl ester and benzoylecgonine (Buttner et al. 2003). However, cocaine may be converted into other toxic metabolites, such as norcocaine and cocaethylene (Fig. 1). Norcocaine is formed in the liver, via N-demethylation of cocaine by CYP3A4, and is an active cocaine metabolite that crosses the blood–brain barrier and induces neurotoxicity (Nassogne et al. 1997). In the presence of alcohol, another active metabolite, cocaethylene (Fig. 1), is formed, which is more toxic than cocaine itself (Henry 2007), as discussed in Sect. 6.2.

Cocaine available in the streets is often adulterated with sugars, other central nervous system stimulants and local anesthetics (Karch 2009), to increase profit or to modulate the effects experienced by its users, being an additional factor that may contribute to some of the neuropathology observed in cocaine abusers. Purity of cocaine generally ranges from 20 % to 95 %, for cocaine hydrochloride, and 20 % to 80 %, for crack (Couper and Logan 2004).

3 Neurologic Dysfunction in Cocaine Abusers

Cocaine addiction may be considered a neurological disorder (Majewska 1996) because several neurological impairments are found in cocaine abusers, including seizures, cerebral ischemia, cerebral hemorrhages, infarction, optic neuropathy, cerebral atrophy, cognitive impairment, and mood and movement disorders (Koppel et al. 1996; Majewska 1996). Movement disorders described in cocaine users include choreoathetosis, akathisia, and parkinsonism with tremor (Karch 2009), a phenomenon known as "crack dancing," which may be associated with

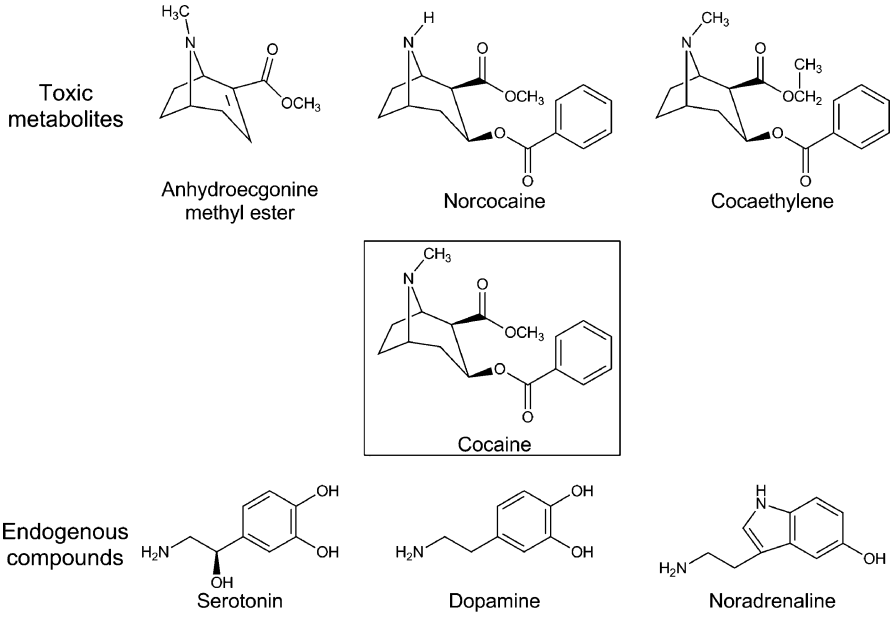

Fig. 1 Chemical structures of cocaine, its toxic metabolites, and monoamine endogenous compounds that share structural similarities with the drug. These similarities are in the basis of many of the drug's effects, due to interference with the same endogenous molecular targets

an increased incidence of basal ganglia abnormalities in cocaine abusers (Bartzokis et al. 1999).

The prefrontal cortex and basal ganglia of cocaine abusers are the brain regions where most brain lesions and cerebral atrophy are observed (Bartzokis et al. 1996; Langendorf et al. 1996). Cocaine abusers also present specific dysfunction of executive functions associated with prefrontal brain regions (Bolla et al. 1998). Cocaine-induced brain dysfunction may be caused by (i) vasoconstriction and consequent hypoxia induced by the drug (Olsen 1995); (ii) impairment of neurotransmission, namely through dopamine and glutamate; and (iii) oxidative stress and mitochondrial dysfunction.

The neurotoxic actions of cocaine in different brain areas involve different mechanisms of action and have specific consequences. Cocaine-induced increase in dopamine activity in the limbic system translates into euphoria, confusion, agitation, and hallucination. Effects in the cortex lead to pressure of speech, excitation, and a reduced feeling of fatigue. Stimulation of lower centers leads to tremor and tonic-clonic convulsions. Cocaine effects in the brain stem lead to stimulation and then depression of the respiratory vasomotor and vomiting centers (International Programme on Chemical Safety 1999). Maternal abuse of cocaine is associated with prenatal brain toxicity, leading to structural, metabolic, and functional brain abnormalities in the offspring (Roussotte et al. 2010).

4 Impairment of Neurotransmission

As most drugs of abuse, cocaine affects neurotransmission in brain areas involved in reward, such as the ventral pallidum; in motivation/drive, such as the orbitofrontal cortex and the subcallosal cortex; in memory and learning, such as the amygdala and the hippocampus; and in impulse control, such as the prefrontal cortex and the anterior cingulate gyrus (Volkow et al. 2003). The addictive effects of cocaine, and virtually all drugs of abuse, are explained by the activation of the brain reward pathway, a neuronal circuit composed of dopaminergic neurons that project from the ventral tegmental area, in the midbrain; to the nucleus accumbens, in the ventral striatum; and to the prefrontal cortex (Hyman et al. 2006).

4.1 Cocaine Effects on Dopamine and Glutamate Signaling

Due to structural similarities with monoamines (Fig. 1), cocaine interferes with the reuptake of these neurotransmitters, particularly dopamine, which is mostly associated with pleasure and movement. Blockade of monoamine reuptake (Ki: 02–0.7 μM) explains most of the effects of cocaine on the central nervous system (Han and Gu 2006). Additionally, at higher concentrations (>5 μM), cocaine may also bind to voltage-gated ion channels and inhibit inward sodium and outward potassium currents, acting as a local anesthetic (Heard et al. 2008). These effects are relevant for the range of cocaine concentrations found in the plasma of cocaine addicts, usually between 0.3 mM and 1 mM, which may even represent higher cocaine concentrations in the brain, because brain cocaine concentrations were reported to exceed the plasma concentration by several times (Heard et al. 2008).

By binding to the dopamine transporter (DAT) (Fig. 2), cocaine blocks its function, leading to increased extracellular dopamine, which results in chronic stimulation of postsynaptic dopamine receptors (Cunha-Oliveira et al. 2008, for review) and leads to the euphoric "rush." Subsequently, dopamine levels fall, resulting in the dysphoric "crash." Cocaine also interferes with the uptake of norepinephrine and serotonin, leading to increased stimulation of postsynaptic receptors for these neurotransmitters (Heard et al. 2008).

Besides inhibiting plasma membrane monoamine transporters, cocaine also interacts with the vesicular monoamine transporter VMAT-2 (Fig. 2), favoring the storage of catecholamines inside synaptic vesicles (Brown et al. 2001). Concomitant inhibition of the DAT and increased vesicular sequestration of dopamine induced by cocaine may cause a shift in the ratio of cytosolic to vesicular dopamine, increasing the amount of neurotransmitter packaged in each vesicle before its release, which may contribute to a greater increase in synaptic dopamine accumulation upon a depolarizing stimulus (Brown et al. 2001).

Several brain structures that receive input from the reward pathway send reciprocal glutamatergic projections back to the ventral tegmental area, which can affect dopamine release (Uys and Reissner 2011). Glutamatergic neurotransmission has been implicated in addiction, in the processes of reinforcement, sensitization, habit

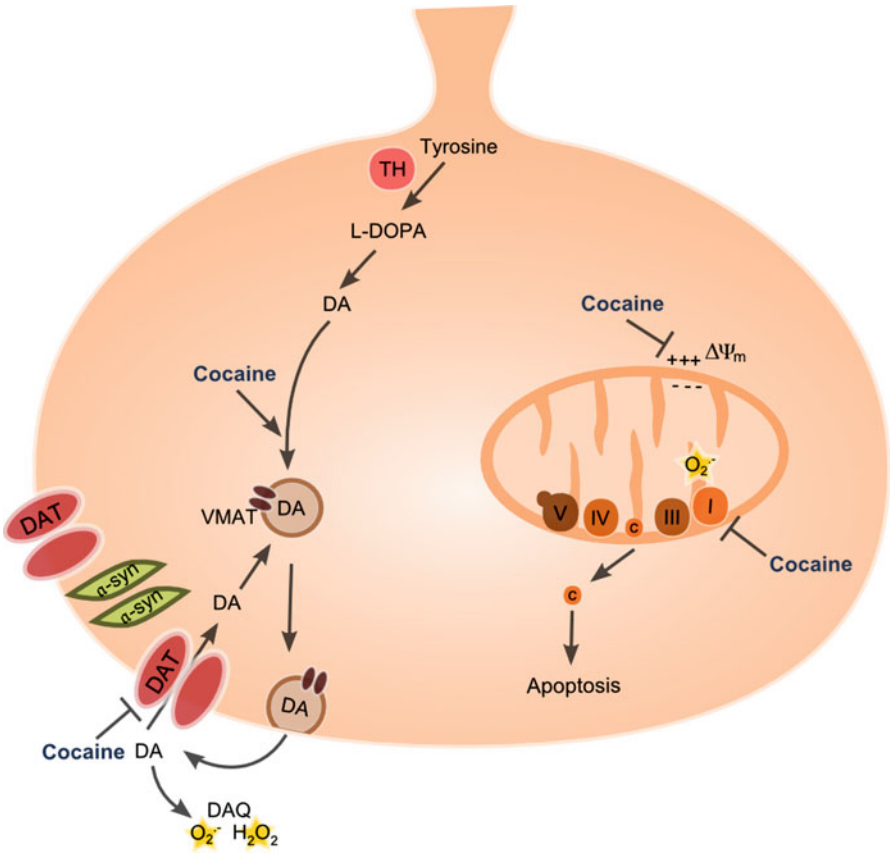

Fig. 2 Presynaptic changes induced by acute cocaine exposure. Cocaine affects neurotransmission in dopaminergic and other monoaminergic nerve terminals. Cocaine inhibits the dopamine (*DA*) transporter (*DAT*), preventing the reuptake of DA into the nerve terminal and prolonging the effects of this neurotransmitter in the synapse. Cocaine also interacts with the vesicular monoamine transporter (*VMAT*)-2, favoring the storage of catecholamines inside synaptic vesicles, which may contribute to a greater increase in synaptic dopamine, upon a depolarizing stimulus. DA is easily auto-oxidized, generating DA quinone (*DAQ*) and reactive oxygen species, such as $O_2^{\bullet-}$ and H_2O_2. Cocaine exposure also affects alpha-synuclein and DAT expression. These two proteins may interact, modulating DA neurotransmission. In addition, since cocaine is positively charged at physiological pH, it may enter into the nerve terminal and interact directly with mitochondria and other intracellular targets, inhibiting complex I-driven mitochondrial respiration, dissipating mitochondrial potential, and activating the mitochondrial apoptotic pathway

learning, context conditioning, craving, and relapse (Tzschentke and Schmidt 2003); moreover, glutamate receptors were shown to play a role in the reinforcement of long-lasting drug-seeking behaviors (Uys and Reissner 2011). Glutamatergic effects of drugs of abuse in the reward pathway may be modulated by dopamine in the nucleus accumbens, which controls the efficacy of glutamatergic corticostriatal synapses (Wickens et al. 2007).

Chronic cocaine exposure induces synaptic plasticity at ventral tegmental area and nucleus accumbens glutamatergic synapses, including changes in structural plasticity (i.e., increase in the number of dendritic spines); in glutamate homeostasis, implicating glial and neuronal impairment; and in postsynaptic glutamate signaling (Uys and Reissner 2011).

Cocaine was shown to increase extracellular glutamate concentrations in brain areas such as the ventral tegmental area, nucleus accumbens, prefrontal cortex, or striatum (Williams and Steketee 2004). An increase in extracellular glutamate has been associated with excitotoxic processes, due to increased activation of *N*-methyl-D-aspartate (NMDA) receptors and subsequent increase in intracellular Ca^{2+} concentration ($Ca^{2+}{}_i$) (Rego and Oliveira 2003, for review), which could contribute to cocaine neurotoxicity. Indeed, an increase in $Ca^{2+}{}_i$ was observed in rat cortical neurons, after cocaine exposure (Cunha-Oliveira et al. 2010).

Plasticity of glutamatergic synaptic transmission in the ventral tegmental area contributes to the development of addictive behaviors in cocaine addicts (Schilstrom et al. 2006; Wolf 2010). Changes in NMDA receptor subunit expression were observed in the brains of cocaine-exposed rats (Huber et al. 2001; Schilstrom et al. 2006; Yamaguchi et al. 2002; Scheggi et al. 2002; Hemby et al. 1999). In neurons from the ventral tegmental area, cocaine was shown to increase the expression of GluN1 and GluN2B (which is mainly extrasynaptic) subunits and their redistribution to synaptic membranes (Schilstrom et al. 2006). GluN2B expression was also shown to increase in the nucleus accumbens and hippocampus of cocaine-exposed rats. This effect was prevented in rats exposed to cocaine and MK-801, an NMDA receptor antagonist (Scheggi et al. 2002). Altered composition of NMDA receptors may increase the susceptibility of cells to the toxicity of drugs of abuse, as demonstrated by the fact that GluN1/GluN2B-transfected HEK293 cells are more susceptible to acute street heroin toxicity when compared to GluN1/GluN2A- and GluN1-transfected cells (Domingues et al. 2006).

The changes in the NMDA receptor subunit expression induced by cocaine may be mediated by extracellular dopamine, via stimulation of dopamine receptors (Schilstrom et al. 2006). Indeed, dopamine receptors D2 and D1 were shown to interact with NMDA receptor subunits, contributing to the stimulant effect of cocaine. Interactions between GluN2B subunits of the NMDA receptor and the D2 dopamine receptor were observed in the neostriatum of cocaine-exposed rats (Liu et al. 2006). In addition, D1 dopamine receptors, NMDA receptors, and extracellular-signal-regulated kinase (ERK) significantly contributed to neuronal morphological changes induced by repeated exposure to cocaine (Fig. 3) (Ren et al. 2010). Activation of NMDA receptors in the striatum was shown to recruit cytoplasmic D1 receptors to the plasma membrane and dendritic spines (Missale et al. 2006; Sun et al. 2008). D1 and NMDA receptors regulate, in opposite directions, the extent of dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP32) phosphorylation, which becomes a potent inhibitor of protein phosphatase 1 (PP1) (Fig. 3). Whereas activation of the D1 receptor leads to increase in cAMP formation and activation of protein kinase A (PKA), which phosphorylates DARPP32, the stimulation of NMDA receptors induces an increase in intracellular calcium, leading

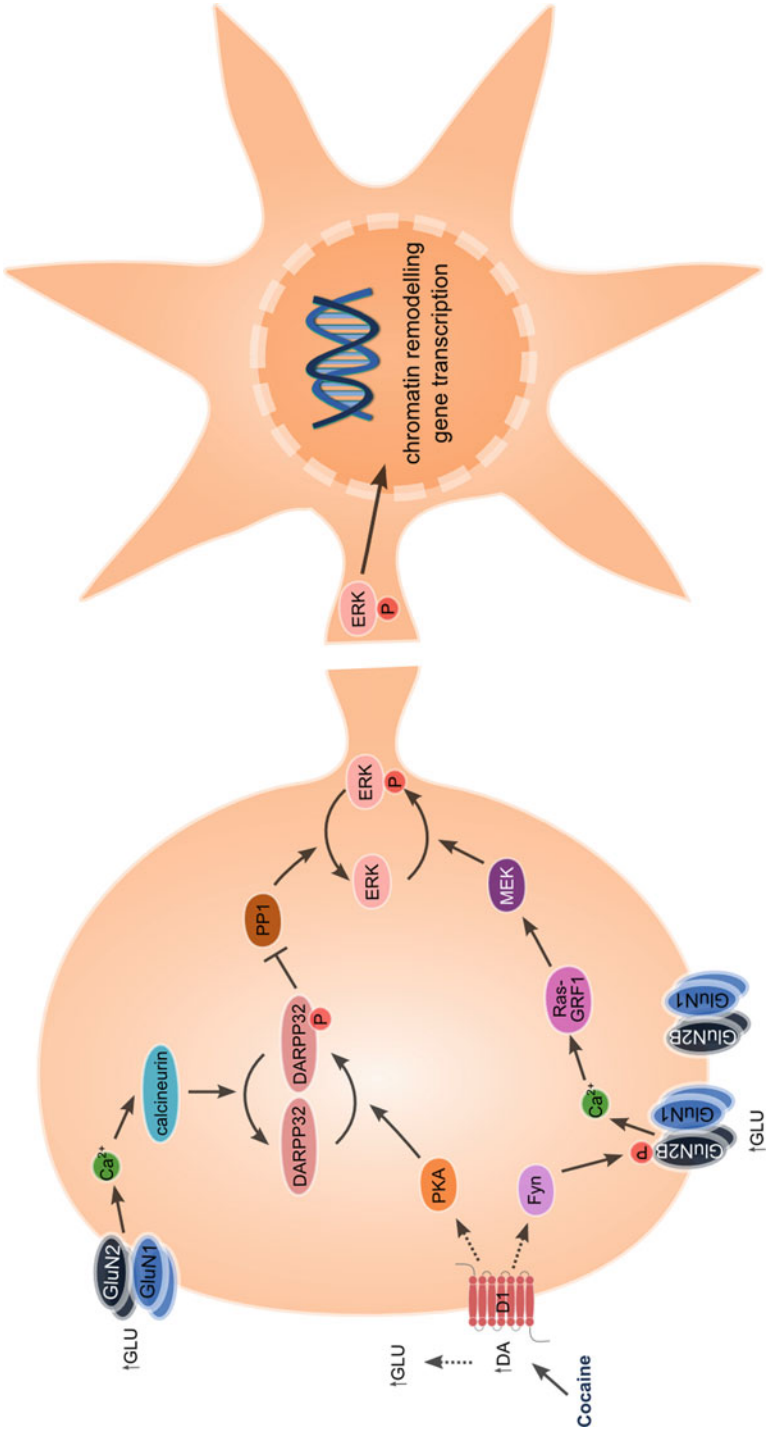

Fig. 3 (continued)

to the activation of calcineurin and dephosphorylation of DARPP32 (Nakano et al. 2010). Interestingly, interaction between glutamate and dopamine signaling in striatal medium spiny neurons seems to be critical for long-term plasticity in the striatum and may play a role in the behavioral alterations induced by cocaine (Pascoli et al. 2011). These adaptations seem to depend on activation of the ERK pathway in the striatum (Ren et al. 2010), which has been pointed out as an essential event for chromatin remodeling and gene expression associated with drug abuse (Brami-Cherrier et al. 2009). Interestingly, ERK activation upon cocaine exposure occurs selectively in dopamine D1 receptor-expressing medium spiny neurons and requires the coincident stimulation of D1 and NMDA receptors (Pascoli et al. 2011). D1 and NMDA receptor interplay upon cocaine exposure may result in ERK activation in medium spiny neurons and may be mediated by two pathways (Fig. 3). Stimulation of D1 receptors leads to PKA activation and DARPP-32 phosphorylation, inhibiting PP1 and preventing ERK dephosphorylation. Alternatively, D1 receptor activation also mediates the phosphorylation and activation of the Src-family kinase Fyn, which phosphorylates GluN2B subunits of NMDA receptors, increasing the entry of Ca^{2+} through these receptors, which leads to the activation of Ras protein-specific guanine nucleotide-releasing factor 1 (Ras-GRF1), mitogen-activated protein kinase kinase (MAPKK)/MEK, and ERK, representing a powerful mechanism of NMDA receptor potentiation (Pascoli et al. 2011).

5 Mechanisms of Neurotoxicity

Cocaine neuropathology shares some common aspects with neurodegenerative disorders, such as Parkinson's disease (PD), which also involves a dopaminergic dysfunction. As described in Sect. 3, chronic cocaine use leads to movement disorders, with similarities to parkinsonism (Karch 2009), although cocaine use does not seem to be a risk factor for the development of idiopathic PD (San Luciano and Saunders-Pullman 2009; Callaghan et al. 2012).

Fig. 3 Postsynaptic signaling in glutamate/dopamine-responsive striatal medium spiny neurons, upon chronic cocaine exposure. By increasing synaptic dopamine (*DA*) and glutamate (*GLU*), cocaine induces changes in the expression of NMDA receptor subunits and alterations in postsynaptic signaling through DARPP-32 and ERK. Stimulation of the D1 receptor leads to PKA activation, which phosphorylates DARPP-32, with subsequent inhibition of PP1, thus preventing ERK dephosphorylation. NMDA receptors also modulate DARPP-32 phosphorylation, since intracellular calcium entering through the receptor activates the phosphatase calcineurin, which dephosphorylates DARPP-32, preventing PP1 inhibition and enabling subsequent ERK dephosphorylation. Cocaine-induced ERK activation occurs selectively in D1 receptor-expressing MSNs and requires the coincident stimulation of D1 and NMDA receptors. This may be explained by the activation of Fyn following D1 receptor stimulation, which leads to phosphorylation of GluN2B subunits, resulting in increased calcium permeability, leading to the activation of Ras-GRF-1, with subsequent MEK activation and phosphorylation of ERK. Thus, activation of D1 receptors may lead to ERK activation by two independent pathways. Active ERK mediates chromatin remodeling and gene transcription that may be involved in behavioral manifestations of cocaine addiction

Cocaine exposure affects the expression of alpha-synuclein, the first protein found to be mutated in genetic cases of PD, whose accumulation in intracellular filamentous aggregates constitutes a pathological feature of PD and other neurodegenerative disorders (Perfeito et al. 2012). An overexpression of alpha-synuclein was found in dopamine neurons of cocaine abusers (Mash et al. 2003) and in the nucleus accumbens of rats exposed to high-dose cocaine (Brenz Verca et al. 2003). Increased alpha-synuclein protein levels were also found in serum from recently abstinent cocaine abusers (Mash et al. 2008). Interestingly, alpha-synuclein was reported to interact with the DAT, a cocaine classic target, modulating dopamine transmission and neuronal function (Swant et al. 2011; Bellucci et al. 2011). The presence of elevated levels of alpha-synuclein and DAT in the striatum of human cocaine abusers (Qin et al. 2005) suggested that cocaine may concomitantly regulate the binding and function of these two proteins (Fig. 3). Thus, overexpression of alpha-synuclein induced by cocaine may play a role in cocaine-induced plasticity and regulation of dopamine synaptic tone (Qin et al. 2005). Accordingly, alpha-synuclein overexpression in rat nucleus accumbens was suggested to modulate cocaine-induced locomotion and self-administration (Boyer and Dreyer 2007).

Other common characteristics observed in cocaine abuse and in several neurodegenerative diseases include oxidative stress, mitochondrial dysfunction, and apoptosis, as described in the next sections.

5.1 Cocaine Toxicity and Oxidative Stress

The occurrence of oxidative stress in neurons upon cocaine exposure has been suggested to occur due to the presence of oxidized metabolites of cocaine (Kovacic 2005), due to an accumulation of dopamine and its metabolites in the brain, or due to the deregulation of antioxidant defenses (Cunha-Oliveira et al. 2008, for review), all of which may ultimately result in increased levels of reactive oxygen species.

As described in Sect. 4.1, cocaine blocks dopamine reuptake by the DAT, resulting in the increase in extracellular dopamine levels in specific brain areas. Dopamine has been shown to be neurotoxic in vitro (Graham et al. 1978; McLaughlin et al. 1998) and in vivo (Hastings et al. 1996). Dopamine has a propensity to form reactive metabolites by enzymatic and nonenzymatic mechanisms and may induce oxidative stress in dopaminergic and neighboring cells, which may contribute to the neurotoxicity of many drugs of abuse, particularly cocaine due to its direct effect in increasing synaptic dopamine concentration. Dopamine may be metabolized intracellularly by monoamine oxidase A (MAO_A) (Fornai et al. 2000) and in a lower extent by MAO_B (Youdim et al. 2006), two isoforms of a mitochondrial enzyme that is present in the cytoplasmic side of the outer mitochondrial membrane in neurons and astrocytes. This enzyme generates 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is highly toxic and rapidly metabolized by aldehyde dehydrogenase, producing 3,4-dihydroxyphenylacetic acid (DOPAC) and hydrogen peroxide (H_2O_2) (Marchitti et al. 2007). In contrast

with enzymatic dopamine oxidation, dopamine auto-oxidation may also occur extracellularly, which is more relevant for explaining cocaine-induced oxidative stress. Auto-oxidation of the catechol ring of dopamine generates dopamine quinones, together with superoxide anion ($O_2^{\bullet -}$) and H_2O_2 (Fig. 2), which may react with transition metal ions, such as iron, via the Haber-Weiss/Fenton reactions, creating the highly toxic hydroxyl radical ($^{\bullet}OH$) (Hastings 2009). $O_2^{\bullet -}$ may also lead to the formation of the highly toxic peroxynitrite, by reaction with nitric oxide. Dopamine quinones are electron-deficient molecules and, thus, readily react with cellular nucleophiles, such as reduced sulfhydryl groups on protein cysteinyl residues, covalently modifying protein structure. Since cysteinyl residues are often localized at the active site of proteins, covalent modification by dopamine quinones often leads to inactivation of protein function, affecting cell survival (Hastings 2009).

Cocaine also affects antioxidant defenses, which protect the cells against free radicals and other reactive species, such as $O_2^{\bullet -}$ and H_2O_2. Superoxide dismutase (SOD) is an antioxidant enzyme that detoxifies $O_2^{\bullet -}$, but may contribute to increase H_2O_2 levels (Cunha-Oliveira et al. 2008, for review). The main antioxidant enzymes involved in H_2O_2 inactivation are glutathione peroxidase (GPx) and catalase, which is present in peroxisomes. These enzymes are regulated by the cells, through the control of protein expression or activity, which allows the maintenance of cellular homeostasis and leads to a contrast in the cellular effects of chronic and acute oxidant exposures. For example, whereas acute exposure to H_2O_2 induces apoptotic cell death (Benedi et al. 2004; Jang and Surh 2004), chronic exposure to low H_2O_2 concentrations induces cellular resistance to the acute toxicity of this compound (Cunha-Oliveira et al. 2006b).

Acute cocaine exposure increased H_2O_2 in the prefrontal cortex and in the striatum of rats (Dietrich et al. 2005) and decreased catalase activity in the same structures in mice (Macedo et al. 2005). The levels of the antioxidants vitamin E and reduced glutathione (GSH) were also shown to be decreased in human neuronal progenitor cells upon acute cocaine exposure (Poon et al. 2007) and in fetal rat brain upon in utero cocaine exposure (Lipton et al. 2003). Biochemical markers of oxidative injury were also found in brain cells, after cocaine exposure. The hippocampus of rats exposed prenatally to cocaine showed an increase in lipid peroxidation (Bashkatova et al. 2006), and protein oxidation was found in human neuronal progenitor cells acutely exposed to cocaine (Poon et al. 2007). Elevated malondialdehyde (MDA) and nitrite levels were also found in rat brain slices from the prefrontal cortex and nucleus accumbens, after acute cocaine exposure. This increase in oxidative damage markers was accompanied by a decrease in total antioxidant content, and both were prevented by tempol, a SOD mimetic antioxidant (Numa et al. 2008).

Repeated cocaine exposure decreased GSH concentration and GPx activity in the hippocampus of rats, which also presented learning and memory impairments (Muriach et al. 2010). Interestingly, chronic exposure of catecholaminergic cells to cocaine seems to induce adaptation to oxidative stress, because PC12 cells chronically exposed to cocaine exhibited a partial resistance against H_2O_2 toxicity (Fig. 4) (Cunha-Oliveira et al. 2006b), which suggests the occurrence of oxidative stress

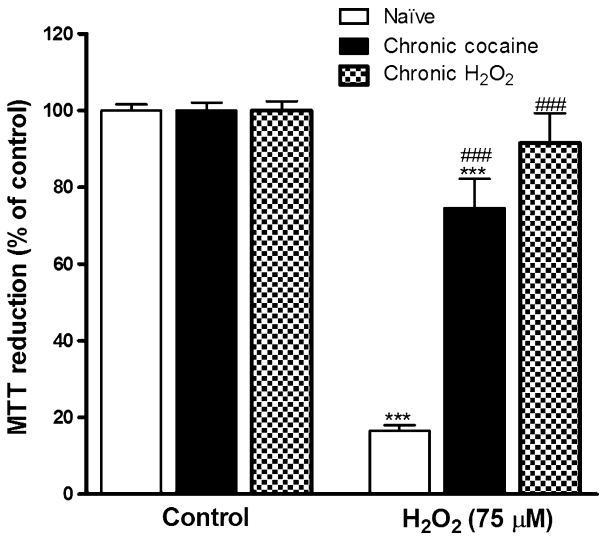

Fig. 4 Involvement of oxidative stress in chronic cocaine effects. PC12 cells were chronically exposed to subtoxic concentrations of cocaine (30 μM) or H_2O_2 (10 μM) for 7–12 months. The toxicity of an acute exposure to H_2O_2 was then assessed by the MTT assay. Cells chronically exposed to H_2O_2 were completely resistant to H_2O_2 acute toxicity, due to cellular adaptation to oxidative stress. Cells chronically exposed to cocaine were partially resistant to H_2O_2 toxicity, suggesting that chronic cocaine exposure also induces cellular adaptation to oxidative stress. Statistical analysis was performed by one-way ANOVA with Bonferroni posttest $^{***}P > 0.001$ compared to respective control; $^{\#\#\#}P > 0.001$ compared to treated naïve cells (Adapted from Toxicology, 217, T. Cunha-Oliveira, A.C. Rego, M.T. Morgadinho, T. Macedo and C.R. Oliveira, Differential cytotoxic responses of PC12 cells chronically exposed to psychostimulants or hydrogen peroxide, 54–62 (Copyright 2006. Cunha-Oliveira et al. (2006b), with permission from Elsevier)

during chronic cocaine exposure. In dopaminergic rat brain structures, repeated cocaine administration induced an increase in antioxidant enzyme activity (Dietrich et al. 2005), and repeated self-administration of cocaine in rats induced an increase in SOD activity and a reduction in MDA levels in the hippocampus, frontal cortex and dorsal striatum (Pomierny-Chamiolo et al. 2012). Induction of antioxidant defenses in models of repeated cocaine exposure may explain the resistance to oxidative agents in these models, and cocaine-induced adaptations in cellular redox balance were suggested to contribute to enduring behavioral plasticity (Uys et al. 2011).

5.2 Cocaine Toxicity and Mitochondrial Dysfunction

Mitochondrial function and energy metabolism were shown to be affected in brains of human cocaine abusers (Lehrmann et al. 2003), and cocaine was demonstrated to influence mitochondrial function in different cell types. Interestingly, acute cocaine

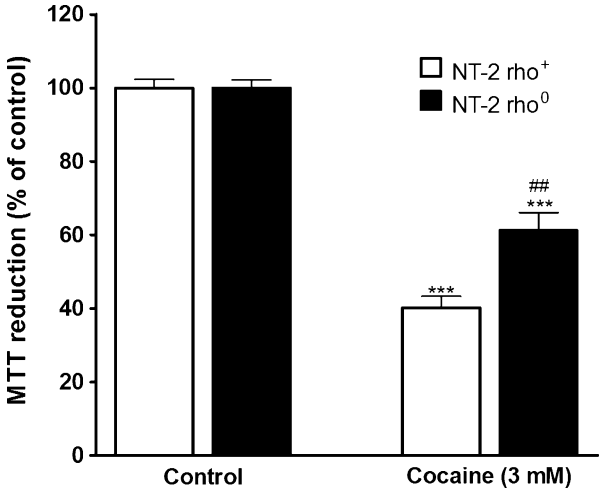

Fig. 5 Partial involvement of functional mitochondria in cocaine toxicity. Mitochondrial involvement in acute cocaine toxicity was analyzed in NT-2 rho-zero cells, depleted from mitochondrial DNA, and thus devoid of a functional mitochondrial respiratory chain. Loss of cell viability induced by cocaine was attenuated in NT-2 rho-zero cells, compared with NT-2 rho-plus cells. Statistical analysis was performed by one-way ANOVA with Bonferroni posttest $***P > 0.001$ compared to respective control; $\#\#P > 0.01$ compared to treated NT-2 rho-plus cells (Adapted from Brain Research, 1089, T. Cunha-Oliveira, A.C. Rego, S.M. Cardoso, F. Borges, R.H. Swerdlow, T. Macedo and C.R. Oliveira, Mitochondrial dysfunction and caspase activation in rat cortical neurons treated with cocaine or amphetamine, 44–54. Copyright 2006. Cunha-Oliveira et al. (2006a), with permission from Elsevier)

toxicity partially required the presence of a functional respiratory chain, because rho-zero cells, which lack functional mitochondria, were more resistant to cocaine toxicity, in comparison with rho-plus cells (Fig. 5) (Cunha-Oliveira et al. 2006a). In addition, exposure to cocaine downregulated mitochondrial gene expression in rat cingulate cortex (Dietrich et al. 2004). Accordingly, mitochondrial complex I activity and subunit expression were reduced after cocaine exposure (Yuan and Acosta, Jr. 2000; Dietrich et al. 2004; Devi and Chan 1997), a defect that is also observed in PD. Cocaine may interact directly with mitochondria and other intracellular targets (Heard et al. 2008), entering the cell due to the weak base effect, because cocaine has a positive charge at physiological pH. Studies in hepatic mitochondria showed that in vivo cocaine administration decreased state 3 respiration, the respiratory control ratio, and the activity of complexes I, II/III, and IV (Devi and Chan 1997). In isolated liver and brain mitochondria, cocaine inhibited complex-I-driven respiration, through a direct effect on this complex (Cunha-Oliveira et al. 2013).

Cocaine neurotoxicity has been associated with apoptotic cell death, and cocaine has been shown to induce biochemical features of apoptosis, including activation of caspases, loss of mitochondrial potential, and cytochrome c release (Cunha-Oliveira et al. 2008, for review). In brains of cocaine addicts, the occurrence of

aberrant cell death was suggested by the enhanced degradation of nuclear poly (ADP-ribose) polymerase (PARP)-1, an apoptotic hallmark, which appeared to be the consequence of oxidative stress and activation of nuclear AIF (Alvaro-Bartolome et al. 2011). Changes in Bcl-2 and Bax expression (Lepsch et al. 2009; Dey et al. 2007), decrease of mitochondrial cytochrome c levels, and caspase-9 activation (Cunha-Oliveira et al. 2006a) were found after in vitro cocaine exposure, suggesting the involvement of the mitochondrial apoptotic pathway. However, controversial results have been reported regarding structural apoptotic hallmarks induced by cocaine. Whereas many reports did not find structural features of neuronal apoptosis upon in vivo (Dietrich et al. 2005) or in vitro (Oliveira et al. 2002; Cunha-Oliveira et al. 2006a) cocaine exposure, others have shown that cultured fetal mouse cortical neurons exposed to cocaine exhibited apoptotic morphology (Nassogne et al. 1997) and apoptotic neurons were also observed in the fetal rat brain, upon in utero cocaine exposure, whereas the maternal brain was spared (Xiao and Zhang 2008).

Interestingly, oxidative stress seemed to precede cell death induced by cocaine, in human neuronal progenitor cells (Poon et al. 2007). Oxidative stress also seems to be involved in cocaine's mitochondrial effects, since cocaine-induced mitochondrial dysfunction could be prevented by the mitochondrial-targeted antioxidant MitoQ, in isolated cardiac mitochondria (Vergeade et al. 2010).

6 Combinations of Cocaine and Other Substances

6.1 Effects of Speedball

Cocaine abusers may also consume other drugs that may interact with cocaine and modify its effects. Speedball is a relatively popular drug combination (European Monitoring Center for Drugs and Drug Addiction 2009), which consists in simultaneous injection of cocaine and heroin, and has been reported to cause more rewarding effects in rats than cocaine or heroin alone (Ranaldi and Munn 1998). The popularity of this drug combination may derive from the reduction of the unwanted side effects of one drug by the other due to compensatory mechanisms of action and/or from enhanced effects of the combination (Leri et al. 2003).

Speedball has serious health consequences. The use of cocaine in combination with heroin is generally associated with the presence of a mental illness and may aggravate underlying psychological problems, such as bipolar disorder (European Monitoring Center for Drugs and Drug Addiction 2008). Moreover, speedball abusers seem to exhibit more severe psychopathology in comparison with other cocaine addicts and more likely fail in drug abuse treatment (Bandettini Di Poggio et al. 2006).

Self-administration of cocaine in rhesus monkeys was shown to be enhanced by otherwise inactive doses of heroin (Rowlett and Woolverton 1997). The pharmacological mechanisms underlying speedball abuse seem to involve an increase in the reward obtained from the drug combination (Rowlett and Woolverton 1997).

In accordance, self-administration of both cocaine and heroin produces synergistic elevations in extracellular dopamine concentration in the reward pathway (Hemby et al. 1999; Smith et al. 2006) that are thought to be mediated by dopamine and μ-opioid receptors (Cornish et al. 2005). This suggests that this drug combination alters the dynamics of dopamine and other neurotransmitters affected in drug addiction.

Interestingly, a chemical interaction between cocaine and morphine was found in cocaine-heroin mixtures, involving the formation of a cocaine-morphine adduct (Garrido et al. 2007), which may play a role in the effects of cocaine-heroin combinations, especially when the co-abuse occurs simultaneously. Thus, besides pharmacodynamic interactions, chemical interactions between drugs could also play a role in speedball neurotoxicity. In previous studies, we exposed primary cultures of rat cortical neurons to heroin and/or cocaine, either sequentially or simultaneously (Cunha-Oliveira et al. 2010) to investigate this possibility. The effects of cocaine seemed to predominate over heroin's effects when the cells were exposed to a mixture of the two drugs (where the probability of chemical interactions between the drugs is enhanced), but not upon sequential exposure (Cunha-Oliveira et al. 2010). Since both drugs were present at the same amounts in both situations, this result suggested that the interaction of the mixture of drugs with opioid receptors may be lower, as compared to a sequential administration of the two drugs, probably due to the presence of cocaine-morphine adducts. In addition, cocaine-morphine combinations induced apoptosis and mitochondrial dysfunction in rat cortical neurons (Cunha-Oliveira et al. 2010) and impaired the bioenergetics of isolated brain mitochondria (Cunha-Oliveira et al. 2013). These data suggest that neurotoxic pathways induced by co-administration of cocaine and heroin differ from those evoked by a sequential administration of the same drugs, possibly due to chemical interactions between the two drugs (or their metabolites), which may interfere with cell death mechanisms and may affect the capacity of cocaine to interact with its cellular targets, such as monoamine transporters or mitochondria. Thus, co-use of cocaine and heroin may lead to different effects depending on the mode of co-exposure, and polydrug abusers may be more prone to neurotoxic damage than single drug abusers.

6.2 Cocaine and Ethanol

As referred in Sect. 2, cocaine and ethanol represent another toxic drug combination. Ethanol use by cocaine abusers leads to the potentiation of cocaine's euphoric effects. However, toxic effects of both drugs are also enhanced (Bolla et al. 2000), since the drugs are combined in the liver to form a very toxic metabolite – cocaethylene (see Fig. 1). Cocaethylene also affects the brain because it is highly lipophilic and, thus, crosses the blood–brain barrier. The behavioral effects of cocaethylene are similar to those of cocaine, but more prolonged due to its longer half-life (Henry 2007).

7 Conclusion

Cocaine is a widely abused psychostimulant drug, popularized by its intense euphoric effects. Cocaine addiction may be described as a neurological disorder because cocaine abusers present several neurological impairments. Cocaine neurotoxicity may be due to vasoconstriction and subsequent hypoxia or to the impairment of neurotransmission, namely through dopamine and glutamate, and may be mediated by oxidative stress and mitochondrial dysfunction. The interaction with the dopaminergic system is the main cause of cocaine's addictive effects, leading to excess synaptic dopamine levels that may induce oxidative stress through dopamine auto-oxidation and reactive oxygen species generation. Neurotoxic cocaine metabolites may also induce oxidative stress. Cocaine also affects glutamate levels and the expression of glutamate receptors, which may mediate excitotoxic cell damage and long-term neuroadaptive effects.

Cocaine abuse shares some common aspects with neurological disorders such as PD, which also involves a dysfunction in the dopaminergic system. Cocaine exposure has been shown to regulate both alpha-synuclein and DAT binding and function, possibly affecting cocaine-induced plasticity, locomotion, and self-administration. Moreover, cocaine has been shown to impair mitochondrial function in several models, namely through the inhibition of mitochondrial respiratory chain complex I, as occurs in PD; furthermore, cocaine toxicity was demonstrated to require a functional respiratory chain. Cocaine may also induce the activation of mitochondrial apoptotic pathways in several models.

Cocaine is frequently co-abused with heroin, in a combination known as speedball, and heroin may modulate or modify cocaine's effects. A chemical interaction between cocaine and morphine was found in drug mixtures similar to those used by speedball abusers and may produce a specific neurotoxic profile and alter cocaine effects. A concomitant use of cocaine and alcohol may have additive negative effects in the brain, due to the formation of a metabolite, cocaethylene, which is more toxic than cocaine itself.

In summary, cocaine-induced neurotoxicity may underlie brain dysfunction in cocaine and polydrug abusers and may predispose the brain to neurodegeneration.

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References

Alvaro-Bartolome, M., La, H. R., Callado, L. F., Meana, J. J., & Garcia-Sevilla, J. A. (2011). Molecular adaptations of apoptotic pathways and signaling partners in the cerebral cortex of human cocaine addicts and cocaine-treated rats. *Neuroscience, 196*, 1–15.

Bandettini Di Poggio, A., Fornai, F., Paparelli, A., Pacini, M., Perugi, G., & Maremmani, I. (2006). Comparison between heroin and heroin-cocaine polyabusers: A psychopathological study. *Annals of the New York Academy of Sciences, 1074*, 438–445.

Bartzokis, G., Beckson, M., & Ling, W. (1996). Clinical and MRI evaluation of psychostimulant neurotoxicity. *NIDA Research Monograph, 163*, 300–317.

Bartzokis, G., Beckson, M., Wirshing, D. A., Lu, P. H., Foster, J. A., & Mintz, J. (1999). Choreoathetoid movements in cocaine dependence. *Biological Psychiatry, 45*, 1630–1635.

Bashkatova, V., Meunier, J., Vanin, A., & Maurice, T. (2006). Nitric oxide and oxidative stress in the brain of rats exposed in utero to cocaine. *Annals of the New York Academy of Sciences, 1074*, 632–642.

Bellucci, A., Navarria, L., Falarti, E., Zaltieri, M., Bono, F., Collo, G., Spillantini, M. G., Missale, C., & Spano, P. (2011). Redistribution of DAT/alpha-synuclein complexes visualized by "in situ" proximity ligation assay in transgenic mice modelling early Parkinson's disease. *PLoS One, 6*, e27959.

Benedi, J., Arroyo, R., Romero, C., Martin-Aragon, S., & Villar, A. M. (2004). Antioxidant properties and protective effects of a standardized extract of *Hypericum perforatum* on hydrogen peroxide-induced oxidative damage in PC12 cells. *Life Sciences, 75*, 1263–1276.

Bolla, K. I., Cadet, J. L., & London, E. D. (1998). The neuropsychiatry of chronic cocaine abuse. *The Journal of Neuropsychiatry and Clinical Neurosciences, 10*, 280–289.

Bolla, K. I., Funderburk, F. R., & Cadet, J. L. (2000). Differential effects of cocaine and cocaine alcohol on neurocognitive performance. *Neurology, 54*, 2285–2292.

Boyer, F., & Dreyer, J. L. (2007). Alpha-synuclein in the nucleus accumbens induces changes in cocaine behaviour in rats. *European Journal of Neuroscience, 26*, 2764–2776.

Brami-Cherrier, K., Roze, E., Girault, J. A., Betuing, S., & Caboche, J. (2009). Role of the ERK/MSK1 signalling pathway in chromatin remodelling and brain responses to drugs of abuse. *Journal of Neurochemistry, 108*, 1323–1335.

Brenz Verca, M. S., Bahi, A., Boyer, F., Wagner, G. C., & Dreyer, J. L. (2003). Distribution of alpha- and gamma-synucleins in the adult rat brain and their modification by high-dose cocaine treatment. *European Journal of Neuroscience, 18*, 1923–1938.

Brown, J. M., Hanson, G. R., & Fleckenstein, A. E. (2001). Regulation of the vesicular monoamine transporter-2: A novel mechanism for cocaine and other psychostimulants. *Journal of Pharmacology and Experimental Therapeutics, 296*, 762–767.

Buttner, A., Mall, G., Penning, R., Sachs, H., & Weis, S. (2003). The neuropathology of cocaine abuse. *Legal Medicine, 5*(Suppl 1), S240–S242. Tokyo.

Callaghan, R. C., Cunningham, J. K., Sykes, J., & Kish, S. J. (2012). Increased risk of Parkinson's disease in individuals hospitalized with conditions related to the use of methamphetamine or other amphetamine-type drugs. *Drug and Alcohol Dependence, 120*, 35–40.

Cornish, J. L., Lontos, J. M., Clemens, K. J., & McGregor, I. S. (2005). Cocaine and heroin ('speedball') self-administration: The involvement of nucleus accumbens dopamine and mu-opiate, but not delta-opiate receptors. *Psychopharmacology, 180*, 21–32.

Couper, F., & Logan, B. (2004). *Drugs and human performance fact sheets*. Washington, DC: National Highway Traffic Safety Administration.

Cunha-Oliveira, T., Rego, A. C., Cardoso, S. M., Borges, F., Swerdlow, R. H., Macedo, T., & de Oliveira, C. R. (2006a). Mitochondrial dysfunction and caspase activation in rat cortical neurons treated with cocaine or amphetamine. *Brain Research, 1089*, 44–54.

Cunha-Oliveira, T., Rego, A. C., Morgadinho, M. T., Macedo, T., & Oliveira, C. R. (2006b). Differential cytotoxic responses of PC12 cells chronically exposed to psychostimulants or to hydrogen peroxide. *Toxicology, 217*, 54–62.

Cunha-Oliveira, T., Rego, A. C., Garrido, J., Borges, F., Macedo, T., & Oliveira, C. R. (2010). Neurotoxicity of heroin-cocaine combinations in rat cortical neurons. *Toxicology, 276*, 11–17.

Cunha-Oliveira, T., Rego, A. C., & Oliveira, C. R. (2008). Cellular and molecular mechanisms involved in the neurotoxicity of opioid and psychostimulant drugs. *Brain Research Reviews, 58*, 192–208.

Cunha-Oliveira, T., Silva, L., Silva, A. M., Moreno, A. J., Oliveira, C. R., & Santos M. S. (2013). Mitochondrial complex I dysfunction induced by cocaine and cocaine plus morphine in brain and liver mitochondria. *Toxicology Letters, 219*, 298–306.

Devi, B. G., & Chan, A. W. (1997). Impairment of mitochondrial respiration and electron transport chain enzymes during cocaine-induced hepatic injury. *Life Sciences, 60*, 849–855.

Dey, S., Mactutus, C. F., Booze, R. M., & Snow, D. M. (2007). Cocaine exposure in vitro induces apoptosis in fetal locus coeruleus neurons by altering the Bax/Bcl-2 ratio and through caspase-3 apoptotic signaling. *Neuroscience, 144*, 509–521.

Dietrich, J. B., Mangeol, A., Revel, M. O., Burgun, C., Aunis, D., & Zwiller, J. (2005). Acute or repeated cocaine administration generates reactive oxygen species and induces antioxidant enzyme activity in dopaminergic rat brain structures. *Neuropharmacology, 48*, 965–974.

Dietrich, J. B., Poirier, R., Aunis, D., & Zwiller, J. (2004). Cocaine downregulates the expression of the mitochondrial genome in rat brain. *Annals of the New York Academy of Sciences, 1025*, 345–350.

Domingues, A., Cunha, O. T., Laco, M. L., Macedo, T. R., Oliveira, C. R., & Rego, A. C. (2006). Expression of NR1/NR2B N-methyl-D-aspartate receptors enhances heroin toxicity in HEK293 cells. *Annals of the New York Academy of Sciences, 1074*, 458–465.

European Monitoring Center for Drugs and Drug Addiction (2008) Annual Report 2008. The state of the drugs problem in Europe.

European Monitoring Center for Drugs and Drug Addiction (2009) Polydrug use: Patterns and responses.

Fornai, F., Giorgi, F. S., Bassi, L., Ferrucci, M., Alessandri, M. G., & Corsini, G. U. (2000). Modulation of dihydroxyphenylacetaldehyde extracellular levels in vivo in the rat striatum after different kinds of pharmacological treatment. *Brain Research, 861*, 126–134.

Garcia, R. C., Dati, L. M., Fukuda, S., Torres, L. H., Moura, S., de Carvalho, N. D., Carrettiero, D. C., Camarini, R., Levada-Pires, A. C., Yonamine, M., Negrini-Neto, O., Abdalla, F. M., Sandoval, M. R., Afeche, S. C., & Marcourakis, T. (2012). Neurotoxicity of anhydroecgonine methyl ester, a crack cocaine pyrolysis product. *Toxicological Sciences, 128*, 223–234.

Garrido, J. M., Marques, M. P., Silva, A. M., Macedo, T. R., Oliveira-Brett, A. M., & Borges, F. (2007). Spectroscopic and electrochemical studies of cocaine-opioid interactions. *Analytical and Bioanalytical Chemistry, 388*, 1799–1808.

Graham, D. G., Tiffany, S. M., Bell, W. R., Jr., & Gutknecht, W. F. (1978). Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Molecular Pharmacology, 14*, 644–653.

Han, D. D., & Gu, H. H. (2006). Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacology, 6*, 6.

Hastings, T. G. (2009). The role of dopamine oxidation in mitochondrial dysfunction: Implications for Parkinson's disease. *Journal of Bioenergetics and Biomembranes, 41*, 469–472.

Hastings, T. G., Lewis, D. A., & Zigmond, M. J. (1996). Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections. *Proceedings of the National Academy of Science United States of America, 93*, 1956–1961.

Heard, K., Palmer, R., & Zahniser, N. R. (2008). Mechanisms of acute cocaine toxicity. *Open Pharmacology Journal, 2*, 70–78.

Hemby, S. E., Co, C., Dworkin, S. I., & Smith, J. E. (1999). Synergistic elevations in nucleus accumbens extracellular dopamine concentrations during self-administration of cocaine/heroin combinations (Speedball) in rats. *Journal of Pharmacology and Experimental Therapeutics, 288*, 274–280.

Henry, J. (2007). Cocaine powder trail. *The Biochemist, 29*, 16–19.

Huber, J. D., Darling, S. F., Park, K. K., & Soliman, K. F. (2001). The role of NMDA receptors in neonatal cocaine-induced neurotoxicity. *Pharmacology Biochemistry and Behavior, 69*, 451–459.

Hyman, S. E., Malenka, R. C., & Nestler, E. J. (2006). Neural mechanisms of addiction: The role of reward-related learning and memory. *Annual Review of Neuroscience, 29*, 565–598.

International Programme on Chemical Safety. (1999). *Poisons information* [Monograph]: Cocaine (PIM 139).

Jang, J. H., & Surh, Y. J. (2004). Possible role of NF-kappaB in Bcl-X(L) protection against hydrogen peroxide-induced PC12 cell death. *Redox Report, 9*, 343–348.

Karch, S. B. (2009). *Karch's pathology of drug abuse*. Boca Raton: CRC Press.

Koppel, B. S., Samkoff, L., & Daras, M. (1996). Relation of cocaine use to seizures and epilepsy. *Epilepsia, 37*, 875–878.

Kovacic, P. (2005). Role of oxidative metabolites of cocaine in toxicity and addiction: Oxidative stress and electron transfer. *Medical Hypotheses, 64*, 350–356.

Langendorf, F. G., Anderson, D. C., Tupper, D. E., Rottenberg, D. A., & Weisman, I. D. (1996). Brain atrophy and chronic cocaine abuse: Background and work in progress. *NIDA Research Monograph, 163*, 27–42.

Lehrmann, E., Oyler, J., Vawter, M. P., Hyde, T. M., Kolachana, B., Kleinman, J. E., Huestis, M. A., Becker, K. G., & Freed, W. J. (2003). Transcriptional profiling in the human prefrontal cortex: Evidence for two activational states associated with cocaine abuse. *The Pharmacogenomics Journal, 3*, 27–40.

Lepsch, L. B., Munhoz, C. D., Kawamoto, E. M., Yshii, L. M., Lima, L. S., Curi-Boaventura, M. F., Salgado, T. M., Curi, R., Planeta, C. S., & Scavone, C. (2009). Cocaine induces cell death and activates the transcription nuclear factor kappa-b in pc12 cells. *Molecular Brain, 2*, 3.

Leri, F., Bruneau, J., & Stewart, J. (2003). Understanding polydrug use: Review of heroin and cocaine co-use. *Addiction, 98*, 7–22.

Lipton, J. W., Gyawali, S., Borys, E. D., Koprich, J. B., Ptaszny, M., & McGuire, S. O. (2003). Prenatal cocaine administration increases glutathione and alpha-tocopherol oxidation in fetal rat brain. *Brain Research. Developmental Brain Research, 147*, 77–84.

Liu, X. Y., Chu, X. P., Mao, L. M., Wang, M., Lan, H. X., Li, M. H., Zhang, G. C., Parelkar, N. K., Fibuch, E. E., Haines, M., Neve, K. A., Liu, F., Xiong, Z. G., & Wang, J. Q. (2006). Modulation of D2R-NR2B interactions in response to cocaine. *Neuron, 52*, 897–909.

Macedo, D. S., de Vasconcelos, S. M., dos Santos, R. S., Aguiar, L. M., Lima, V. T., Viana, G. S., & de Sousa, F. C. (2005). Cocaine alters catalase activity in prefrontal cortex and striatum of mice. *Neuroscience Letters, 387*, 53–56.

Majewska, M. D. (1996). Cocaine addiction as a neurological disorder: Implications for treatment. *NIDA Research Monograph, 163*, 1–26.

Marchitti, S. A., Deitrich, R. A., & Vasiliou, V. (2007). Neurotoxicity and metabolism of the catecholamine-derived 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylglycolaldehyde: The role of aldehyde dehydrogenase. *Pharmacological Reviews, 59*, 125–150.

Mash, D. C., Adi, N., Duque, L., Pablo, J., Kumar, M., & Ervin, F. R. (2008). Alpha synuclein protein levels are increased in serum from recently abstinent cocaine abusers. *Drug and Alcohol Dependence, 94*, 246–250.

Mash, D. C., Ouyang, Q., Pablo, J., Basile, M., Izenwasser, S., Lieberman, A., & Perrin, R. J. (2003). Cocaine abusers have an overexpression of alpha-synuclein in dopamine neurons. *Journal of Neuroscience, 23*, 2564–2571.

McLaughlin, B. A., Nelson, D., Erecinska, M., & Chesselet, M. F. (1998). Toxicity of dopamine to striatal neurons in vitro and potentiation of cell death by a mitochondrial inhibitor. *Journal of Neurochemistry, 70*, 2406–2415.

Missale, C., Fiorentini, C., Busi, C., Collo, G., & Spano, P. F. (2006). The NMDA/D1 receptor complex as a new target in drug development. *Current Topics in Medicinal Chemistry, 6*, 801–808.

Muriach, M., Lopez-Pedrajas, R., Barcia, J. M., Sanchez-Villarejo, M. V., Almansa, I., & Romero, F. J. (2010). Cocaine causes memory and learning impairments in rats: Involvement of nuclear factor kappa B and oxidative stress, and prevention by topiramate. *Journal of Neurochemistry, 114*, 675–684.

Nakano, T., Doi, T., Yoshimoto, J., & Doya, K. (2010). A kinetic model of dopamine- and calcium-dependent striatal synaptic plasticity. *PLoS Computational Biology, 6*, e1000670.

Nassogne, M. C., Louahed, J., Evrard, P., & Courtoy, P. J. (1997). Cocaine induces apoptosis in cortical neurons of fetal mice. *Journal of Neurochemistry, 68*, 2442–2450.

Numa, R., Kohen, R., Poltyrev, T., & Yaka, R. (2008). Tempol diminishes cocaine-induced oxidative damage and attenuates the development and expression of behavioral sensitization. *Neuroscience, 155*, 649–658.

Oliveira, M. T., Rego, A. C., Morgadinho, M. T., Macedo, T. R., & Oliveira, C. R. (2002). Toxic effects of opioid and stimulant drugs on undifferentiated PC12 cells. *Annals of the New York Academy of Sciences, 965*, 487–496.

Olsen, G. D. (1995). Potential mechanisms of cocaine-induced developmental neurotoxicity: A minireview. *Neurotoxicology, 16*, 159–167.

Pascoli, V., Besnard, A., Herve, D., Pages, C., Heck, N., Girault, J. A., Caboche, J., & Vanhoutte, P. (2011). Cyclic adenosine monophosphate-independent tyrosine phosphorylation of NR2B mediates cocaine-induced extracellular signal-regulated kinase activation. *Biological Psychiatry, 69*, 218–227.

Perfeito, R., Cunha-Oliveira, T., & Rego, A. C. (2012). Revisiting oxidative stress and mitochondrial dysfunction in the pathogenesis of Parkinson's disease – resemblance to the effect of amphetamine drugs of abuse. *Free Radical Biology Medicine, 53*, 1791–806.

Pomierny-Chamiolo, L., Moniczewski, A., Wydra, K., Suder, A., & Filip M. (2012). Oxidative stress biomarkers in some rat brain structures and peripheral organs underwent cocaine. *Neurotoxicity Research, 23*, 92–102.

Poon, H. F., Abdullah, L., Mullan, M. A., Mullan, M. J., & Crawford, F. C. (2007). Cocaine-induced oxidative stress precedes cell death in human neuronal progenitor cells. *Neurochemistry International, 50*, 69–73.

Qin, Y., Ouyang, Q., Pablo, J., & Mash, D. C. (2005). Cocaine abuse elevates alpha-synuclein and dopamine transporter levels in the human striatum. *Neuroreport, 16*, 1489–1493.

Ranaldi, R., & Munn, E. (1998). Polydrug self-administration in rats: Cocaine-heroin is more rewarding than cocaine-alone. *Neuroreport, 9*, 2463–2466.

Rego, A. C., & Oliveira, C. R. (2003). Mitochondrial dysfunction and reactive oxygen species in excitotoxicity and apoptosis: Implications for the pathogenesis of neurodegenerative diseases. *Neurochemical Research, 28*, 1563–1574.

Ren, Z., Sun, W. L., Jiao, H., Zhang, D., Kong, H., Wang, X., & Xu, M. (2010). Dopamine D1 and N-methyl-D-aspartate receptors and extracellular signal-regulated kinase mediate neuronal morphological changes induced by repeated cocaine administration. *Neuroscience, 168*, 48–60.

Roussotte, F., Soderberg, L., & Sowell, E. (2010). Structural, metabolic, and functional brain abnormalities as a result of prenatal exposure to drugs of abuse: Evidence from neuroimaging. *Neuropsychology Review, 20*, 376–397.

Rowlett, J. K., & Woolverton, W. L. (1997). Self-administration of cocaine and heroin combinations by rhesus monkeys responding under a progressive-ratio schedule. *Psychopharmacology, 133*, 363–371.

San, L. M., & Saunders-Pullman, R. (2009). Substance abuse and movement disorders. *Current Drug Abuse Reviews, 2*, 273–278.

Scheggi, S., Mangiavacchi, S., Masi, F., Gambarana, C., Tagliamonte, A., & De Montis, M. G. (2002). Dizocilpine infusion has a different effect in the development of morphine and cocaine sensitization: Behavioral and neurochemical aspects. *Neuroscience, 109*, 267–274.

Schilstrom, B., Yaka, R., Argilli, E., Suvarna, N., Schumann, J., Chen, B. T., Carman, M., Singh, V., Mailliard, W. S., Ron, D., & Bonci, A. (2006). Cocaine enhances NMDA receptor-mediated currents in ventral tegmental area cells via dopamine D5 receptor-dependent redistribution of NMDA receptors. *Journal of Neuroscience, 26*, 8549–8558.

Smith, J. E., Co, C., Coller, M. D., Hemby, S. E., & Martin, T. J. (2006). Self-administered heroin and cocaine combinations in the rat: Additive reinforcing effects-supra-additive effects on nucleus accumbens extracellular dopamine. *Neuropsychopharmacology, 31*, 139–150.

Sun, W. L., Zhou, L., Hazim, R., Quinones-Jenab, V., & Jenab, S. (2008). Effects of dopamine and NMDA receptors on cocaine-induced Fos expression in the striatum of Fischer rats. *Brain Research, 1243*, 1–9.

Swant, J., Goodwin, J. S., North, A., Ali, A. A., Gamble-George, J., Chirwa, S., & Khoshbouei, H. (2011). Alpha-Synuclein stimulates a dopamine transporter-dependent chloride current and modulates the activity of the transporter. *Journal of Biological Chemistry, 286*, 43933–43943.

Tzschentke, T. M., & Schmidt, W. J. (2003). Glutamatergic mechanisms in addiction. *Molecular Psychiatry, 8*, 373–382.

United Nations Office on Drugs and Crime. (2012). World drug report 2011.

Uys, J. D., Knackstedt, L., Hurt, P., Tew, K. D., Manevich, Y., Hutchens, S., Townsend, D. M., & Kalivas, P. W. (2011). Cocaine-induced adaptations in cellular redox balance contributes to enduring behavioral plasticity. *Neuropsychopharmacology, 36*, 2551–2560.

Uys, J. D., & Reissner, K. J. (2011). Glutamatergic neuroplasticity in cocaine addiction. *Progress in Molecular Biology and Translational Science, 98*, 367–400.

Vergeade, A., Mulder, P., Vendeville-Dehaudt, C., Estour, F., Fortin, D., Ventura-Clapier, R., Thuillez, C., & Monteil, C. (2010). Mitochondrial impairment contributes to cocaine-induced cardiac dysfunction: Prevention by the targeted antioxidant MitoQ. *Free Radical Biology & Medicine, 49*, 748–756.

Volkow, N. D., Fowler, J. S., & Wang, G. J. (2003). The addicted human brain: Insights from imaging studies. *The Journal of Clinical Investigation, 111*, 1444–1451.

Wickens, J. R., Horvitz, J. C., Costa, R. M., & Killcross, S. (2007). Dopaminergic mechanisms in actions and habits. *Journal of Neuroscience, 27*, 8181–8183.

Williams, J. M., & Steketee, J. D. (2004). Cocaine increases medial prefrontal cortical glutamate overflow in cocaine-sensitized rats: A time course study. *European Journal of Neuroscience, 20*, 1639–1646.

Wolf, M. E. (2010). The Bermuda Triangle of cocaine-induced neuroadaptations. *Trends in Neurosciences, 33*, 391–398.

Xiao, D., & Zhang, L. (2008). Upregulation of Bax and Bcl-2 following prenatal cocaine exposure induces apoptosis in fetal rat brain. *International Journal of Medical Sciences, 5*, 295–302.

Yamaguchi, M., Suzuki, T., Abe, S., Hori, T., Kurita, H., Asada, T., Okado, N., & Arai, H. (2002). Repeated cocaine administration differentially affects NMDA receptor subunit (NR1, NR2A-C) mRNAs in rat brain. *Synapse, 46*, 157–169.

Youdim, M. B., Edmondson, D., & Tipton, K. F. (2006). The therapeutic potential of monoamine oxidase inhibitors. *Nature Reviews Neuroscience, 7*, 295–309.

Yuan, C., & Acosta, D., Jr. (2000). Effect of cocaine on mitochondrial electron transport chain evaluated in primary cultures of neonatal rat myocardial cells and in isolated mitochondrial preparations. *Drug and Chemical Toxicology, 23*, 339–348.

5,6- and 5,7-Dihydroxytryptamines as Serotoninergic Neurotoxins

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Abstract

Dihydroxytryptamines are able to selectively destroy central serotonin neurons when catecholaminergic neurons are protected. Compared to serotonin, 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT) are indole derivatives that acquire their neurotoxicity due to additional hydroxyl groups on the indole ring. Factors influencing the action of dihydroxytryptamines include the site of injection, the speed and volume of injection, the amount and type of antioxidant used, the type of anesthesia, and the dose and type of catecholamine uptake blocker. Two major hypotheses have been proposed to explain the molecular mechanism that underlies the toxicity of 5,6- and 5,7-DHT. The first suggests that highly reactive (electrophilic) quinonoid intermediates, created as a result of 5,6- and 5,7-DHT auto-oxidation, bind covalently to molecules indispensable for neuronal function. The second hypothesis states that during the auto-oxidation of dihydroxylated tryptamines, reactive oxygen species such as superoxide radical anion (O_2^{-}), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO^{\cdot}$) are generated as toxic by-products. The use of 5,6- and 5,7-DHT in both adult and neonatal animals enables the recognition the role of 5-HT in locomotor activity (LMA), prepulse inhibition, seizure susceptibility, sleeping behavior, feeding and drinking behavior, sexual and aggressive behavior, the regulation of body temperature, the response to novel and noxious stimuli, learning and memory, the antinociceptive action of drugs, neuroendocrine regulatory mechanisms, and developmental plasticity.

Keywords

5,6-dihydroxytryptamine (5,6-DHT) • 5,7-dihydroxytryptamine (5,7-DHT) • 5-HT neurotoxins

List of Abbreviations

5-HT	5-hydroxytryptamine, serotonin
5-HTP	5-hydroxytryptophan
5,6-DHT	5,6-dihydroxytryptamine
5,7-DHT	5,7-dihydroxytryptamine
6-OHDA	6-hydroxydopamine
CNS	Central nervous system
DA	Dopamine
DRN	Dorsal raphe nuclei
DTPA	Diethylene triamine pentaacetic acid
FSH	Follicle-stimulating hormone
GH	Growth hormone
GSH	Glutathione
H_2O_2	Hydrogen peroxide
HO	Hydroxyl radical

L-5-HTP	5-hydroxy-L-tryptophan
LMA	Locomotor activity
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MRN	Median raphe nuclei
NA	Noradrenaline
NGF	Nerve growth factor
O_2^-	Superoxide radical anion
PPI	Prepulse inhibition
PTZ	Pentylenetetrazol
REM	Rapid eye movements
ROS	Reactive oxygen species
SOD	Superoxide dismutase

1 Introduction

1.1 The Significance of Serotonin and Serotonergic Neurons

Serotonergic neurons are one of the first to appear during brain and spinal cord development. They are formed very early in gestation in vertebrates (Lidov and Molliver 1982). Serotonin (5-hydroxytryptamine or 5-HT) is a neurotransmitter with developmental functions (Ahmad and Zamenhof 1978) and is involved in more behaviors, physiological processes, and diseases than any other transmitter. It affects developmental events such as cell division, neuronal migration, cell differentiation, and synaptogenesis (Lipton and Kater 1989; Azmitia 2001; Vitalis and Parnavelas 2003). Studies in vertebrates show that even a brief 5-HT deprivation in the brain leads to a loss of spines, dendritic profiles, and synapses (Yan et al. 1997).

1.2 From the First Neurotoxins to the Discovery of Dihydroxytryptamines

The roles of different neuronal types and pathways can be determined by disrupting their function. In the first half of the twentieth century, using the methods of axonal transaction and chemical or electrolytic ablation of nuclei, our knowledge of nerve function was enhanced. However, these techniques have some inherent limitations due to the lack of selectivity. A real breakthrough came in the 1950s with the discovery the first neurotoxin, an antibody to nerve growth factor (NGF), which could destroy sympathetic nerves in

whole animals. The next neurotoxin introduced in the 1960s by Thoenen and Tranzer (1968) was 6-hydroxydopamine (6-OHDA). This hydroxylated analog of dopamine (DA) promoted selective degeneration of peripheral and central noradrenergic neurons. This discovery initiated a novel period in experimental biology. Selective chemical axotomy replaced early mechanical and electrolytic lesioning techniques. After the discovery of 6-OHDA, Baumgarten and collaborators (1971) concentrated on dihydroxytryptamine derivatives. They found that these compounds are able to cause selective destruction of central serotonergic neurons when noradrenergic neurons are protected through pharmacological inhibition of the noradrenaline reuptake carrier (Baumgarten and Lachenmayer 1972; Baumgarten et al. 1973). In 1978, Jacoby and Lytle first used the term "serotonin neurotoxins" in the title of their symposium proceedings volume to describe these compounds.

The mechanism of action of dihydroxytryptamines is analogous to the effects triggered by 6-OHDA in catecholaminergic terminals. Initially, 5,6-dihydroxytryptamine (5,6-DHT) was introduced to neuroscience as a serotoninergic neurotoxin. However, consecutive studies regarding the mechanism of its action revealed the limitations of 5,6-DHT use (Baumgarten and Schlossberger 1973), which motivated scientists to utilize 5,7-dihydroxytryptamine (5,7-DHT), an isomer of 5,6-DHT.

2 Dihydroxytryptamines

2.1 Chemical Structure

Dihydroxytryptamines are indole derivatives that acquire their neurotoxicity due to additional hydroxyl groups (in comparison with 5-HT) on the indole ring (Schlossberger 1978).

2.2 The Mode of Administration

5,6-DHT as well as 5,7-DHT can be administered intracerebroventricularly, intracerebrally, intracisternally, and intrathecally (Baumgarten et al. 1978b).

2.3 Cytochrome-c Oxidase and the Neurotoxicity of Dihydroxytryptamines

Cytochrome-c oxidase is a common feature in the mechanisms of toxicity of both 5,6-DHT and 5,7-DHT (Baumgarten et al. 1982b).

3 Dose-Dependent Action of Dihydroxytryptamines

3.1 5,6-Dihydroxytryptamine

5,6-DHT was the first dihydroxytryptamine introduced to neurobiology. Its injection into the cerebrospinal fluid of rats at doses of 25–75 μg leads to degeneration of indolamine-containing axons and axon terminals in distinct regions of the brain and spinal cord (Baumgarten et al. 1971). At this range of doses, 5,6-DHT causes no considerable changes in DA and noradrenaline (NA) levels in any region of the rat brain (Baumgarten et al. 1972). The same doses injected intracerebroventricularly trigger long-lasting depletion of 5-HT in the spinal cord and smaller effects in the brain (Baumgarten et al. 1971; Daly et al. 1973). Doses higher than 75 μg do not enhance the neurotoxic effects of 5,6-DHT on serotonergic pathways and are extremely toxic to the animals, leading to muscle paralysis, convulsions, tremor, and death in the majority of animals. Furthermore, such doses cause damage to other types of neurons. Studies show nonselective destruction of myelinated fiber systems and glial cells and shrinkage of the striatum on the side of the injection. In doses above 75 μg administered intracerebroventricularly, 5,6-DHT induces a significant reduction of DA and NA levels in distinct areas of the brain (Baumgarten et al. 1972; Bjorklund et al. 1973). Bjorklund and collaborators (1973) demonstrated that this dose restriction can be overcome by infusion of small amounts of the neurotoxin directly into the brain parenchyma.

3.2 5,7-Dihydroxytryptamine

Limitations in the use of 5,6-DHT and need for neurotoxins with less general cytotoxicity inspired the discovery of more selective serotonergic neurotoxins. In 1972, Baumgarten and Lachenmayer described the effects of intracerebral injection of another 5-HT neurotoxin – 5,7-DHT. One of the basic distinctions between 5,6- and 5,7-DHT is the ability to use doses up to 300 μg, which are ordinarily well tolerated. Further, the injection can be made intracerebroventricularly, so the neurotoxin does not have to be injected directly into brain tissue (Baumgarten and Lachenmayer 1972). However, doses up to 100 μg administered intracerebroventricularly in rats often lead to convulsions that can be prevented by previous treatment with barbiturates (Baumgarten and Lachenmayer 1972). Baumgarten and collaborators (1973) demonstrated that even 10 μg of 5,7-DHT injected intracerebroventricularly triggers a significant reduction (approximately 25 %) in the levels of 5-HT and NA in the rat brain. A dose of 50 μg leads to a further decrease in the concentration both of neurotransmitters to approximately 50 % of control values. Successively increasing doses from 75 to 200 μg cause a further decline in the 5-HT level to about 25 % of the control value, without further reduction in the NA level. In early studies, even large doses of 5,7-DHT did not affect the brain levels of DA, a finding that was verified by subsequent

research demonstrating that 5,7-DHT also damages DA axons (Wuttke et al. 1977; Baumgarten et al. 1978b). It is important that the neurotoxic action of 5,7-DHT is not equal in all central nervous system (CNS) regions. Doses as low as 10 μg are sufficient to cause almost complete (85 %) reduction in the 5-HT level in the spinal cord (Baumgarten et al. 1973). Depletion in the 5-HT level in rats after injection of 200 μg ranged from 55 % in the medulla and pons to approximately 85 % in the forebrain and septum (Baumgarten et al. 1973). The most considerable decrease in 5-HT level was noted in the regions of the CNS rich in serotonergic axons and terminals (i.e., the septum, striatum, and hypothalamus), whereas much lower depletion was observed in areas containing serotonergic cell bodies (i.e., mesencephalon, medulla/pons) (Baumgarten et al. 1973).

4 Dihydroxytryptamines: Differences

4.1 Neurotoxicity

A comparison of mono-, di-, and trihydroxylated tryptamines reveals that the principal factor affecting the neurotoxicity of these compounds is the velocity of their oxidation at biological pH. Moderate reaction velocity provides the best potential for toxicity. In the case of 5,6,7-trihydroxytryptamine, a very high oxidation rate causes its immediate transformation into a quinone derivative that does not serve as a substrate for monoaminergic transport mechanisms. A similar mechanism is responsible for the lower in vivo neurotoxicity of 5,6-DHT in comparison to 5,7-DHT, even though 5,6-DHT shows a higher affinity to the amine carrier of serotoninergic neurons (Baumgarten 1981, 1982b). 5,6-DHT undergoes rapid oxidation to o-quinone, which forms melanin-like polymers by aggregation and/or binds covalently and irreversibly to brain proteins (Baumgarten et al. 1975a). These features ensure that 5,6-DHT never penetrates into deeply located 5-HT uptake sites. Moreover, they reduce 5,6-DHT toxicity to 5-HT neurons and increase the risk of nonselective toxic side effects. Conversely, 5,7-DHT is not oxidized as easily, does not create insoluble polymers, and rapidly penetrates into the brain, thus giving it high intrinsic neurotoxicity despite a lower affinity for the 5-HT uptake mechanism (Baumgarten et al. 1975b).

The key feature that determines dihydroxytryptamines activity is the localization of the hydroxyl groups. 5,7-DHT is a meta-substituted dihydroxytryptamine, while 5,6-DHT is ortho-substituted. The meta-substitution enables 5,7-DHT to exist in solution as an equilibrium of distinct tautomeric keto-enol forms, which provide affinity to the 5-HT carrier despite the oxidation of some fraction of 5,7-DHT in vivo. Oxidation does not cause the total loss of 5,7-DHT affinity to the 5-HT carrier (unlike to 5,6-DHT) because one of the hydroxyl groups in the 5,7-DHT structure remains functional (Baumgarten et al. 1982b).

4.2 IC_{50} for 5-HT Uptake Inhibition

IC_{50} values for the inhibition of 5-HT uptake are 4 μM for 5,7-DHT and 0.6 μM for 5,6-DHT, making 5,7-DHT almost seven times less potent than 5,6-DHT (Bjorklund et al. 1975c). However, 5,7-DHT is a better long-term depletor of brain and spinal cord 5-HT than 5,6-DHT, possibly due to differences in metabolism and reactivity of 5,7-DHT (Baumgarten et al. 1975b).

4.3 Selectivity for Catecholamine Uptake

Early studies suggested that 5,7-DHT was a superior serotonergic neurotoxin to 5,6-DHT due to lower nonspecific toxicity and higher specific neurotoxicity (Baumgarten et al. 1973), but further studies by Wuttke et al. (1977) and then by Baumgarten and collaborators (1978b) revealed that 5,7-DHT affects both central NA fibers and DA axons. The results by Bjorklund et al. (1975c) showed that 5,6-DHT is a more specific inhibitor of 5-HT uptake than catecholamine uptake, despite the small differences between the affinity of 5,6-DHT to the 5-HT, NA, and DA transporters (% inhibition of uptake is 59.0 ± 2.3; 43.3 ± 2.7; 44.7 ± 0.7, respectively). 5,7-DHT has a different selectivity pattern. It inhibits the uptake of catecholamines, particularly NA, more effectively than 5-HT (% inhibition of uptake is 25.7 ± 1.2; 32.5 ± 1.3; 15.4 ± 1.7 for 5-HT, NA, and DA, respectively (Bjorklund et al. 1975c)). These results are different from those presented by Baumgarten and collaborators (1978b). On the basis of K_m values for NA uptake (10.0×10^{-6} M) and 5-HT uptake (6.0×10^{-7} M), it appears that 5,7-DHT has a 16-fold higher affinity for the 5-HT transport mechanism. However, there are also studies that show that 5,7-DHT exhibits dose-dependent differences in its selectivity. In doses up to 50 μg, it depletes NA and 5-HT uptake to a similar degree, whereas in doses higher than 50 μg, it affects CNS 5-HT levels substantially more (Baumgarten et al. 1971, 1973, 1975b).

The toxicity of dihydroxytryptamines to non-5-HT neurons can be counteracted by the use of nomifensine, which is a NA/DA uptake blocker (Baumgarten et al. 1979). The effects of 5,7-DHT on NA neurons can be abolished by desmethylimipramine, a blocker of the amine pump of NA neurons. In numerous studies, a combination of desmethylimipramine and 5,7-DHT has been used to achieve selective chemical ablation of 5-HT axons and terminals in the rat brain (Bjorklund et al. 1975a; Sachs and Jonsson 1975). Other methods to block the effect of 5,7-DHT on NA neurons include the use of monoamine oxidase inhibitors (MAOI) such as pargyline, iproniazid, and pheniprazine (Breese and Cooper 1975) and pretreatment with desipramine (Wuttke et al. 1977; Bjorklund et al. 1975b).

4.4 Half-Life and Stability

There are also differences between the half-life and stability of 5,6- and 5,7-DHT. 5,7-DHT is more stable because it lacks autocatalytic promotion of its oxidation

and does not create macromolecular polymers. In contrast, the auto-oxidation of 5,6-DHT is promoted by H_2O_2, and the product of its oxidation, o-quinone, reacts with proteins and peptides to generate high molecular weight polymers through interaction with non-oxidized 5,6-DHT (Baumgarten et al. 1982b).

4.5 The Rate of Oxygen Consumption

5,6- and 5,7-DHT differ in their rates of oxygen consumption. The initial O_2 consumption is 2.7 nmol O_2/min for 5,6-DHT and 33.4 nmol O_2/min for 5,7-DHT. This difference reflects the fact that oxidation of 5,7-DHT is a second-order reaction. However, the rate of oxidation of 5,6-DHT increases after an initial period of slow oxygen consumption (Baumgarten et al. 1982b).

4.6 Diffusion

5,6-DHT has limited diffusion into the brain parenchyma of the adult rat due to its capacity to create macromolecular polymers (Baumgarten et al. 1977). 5,7-DHT infiltrates much deeper into the brain. Notably, 5,7-DHT has the ability to freely diffuse across the brain and to cross the blood-brain barrier during the first week of life. These features enable denervation of the entire 5-HT neuronal system following the intraperitoneal administration of 5,7-DHT to rat pups (Sachs and Jonsson 1975; Kostrzewa et al. 1994).

5 Factors That Affect the Action of 5-HT Neurotoxins

Factors influencing the action of dihydroxytryptamines include the site of injection, the speed and volume of injection, the amount and type of antioxidant used, the type of anesthesia, and the dose and type of catecholamine uptake blocker. These factors influence the specificity of the treatment, the extent of amine depletion, the time course of degeneration and regeneration, the development and recovery of a postsynaptic supersensitivity, and the symmetry of neurotoxic action (Baumgarten et al. 1982b).

5.1 The Site of Injection

The site of injection affects the degree and selectivity of the serotonin neurotoxin action, as demonstrated by comparing the effects of 5,7-DHT intracerebral injection into the lateral and fourth ventricle. Injection of 100 μg of 5,7-DHT into the left lateral ventricle causes rapid loss of 5-HT from the forebrain with no considerable recovery up to 55 days following injection. Administration of 5,7-DHT into the fourth ventricle (termed intracisternal injection) evokes a less extensive and more delayed 5-HT depletion, with mild but significant recovery occurring

within 2 months of injection (Baumgarten et al. 1978a, b). The route of administration also influences the amount of nonspecific toxic damage to the CNS, which is higher when the neurotoxin is injected into the lateral ventricle than into fourth ventricle (Hedreen 1975).

5.2 The Speed and the Volume of Injection

The speed of 5,7-DHT injection affects the extent and symmetry of its action in periventricular structures when the neurotoxin is administered into one of the lateral ventricles. Comparison of two distinct velocities of injection revealed that slow infusion (1 μl/min) causes a more extensive reduction of amine uptake and leads to a more symmetric loss of 5-HT uptake (75 % and 50 % in the left and right striatum, respectively), while fast injection (3 μl/min) leads to asymmetric decreases in 5-HT uptake (Gershanik et al. 1979). Furthermore, large interindividual discrepancies can result from different times elapsing between the neurotoxin administration and sacrifice (2–7 days). Time of sacrifice is an important factor because the damage of 5-HT axons is a time-dependent process (Baumgarten et al. 1973, 1977). Slow versus fast 5-HT neurotoxin administration also produces entirely different results when observed at different time points. One hour after infusion, rapid injection causes symmetric, whereas slow injection causes asymmetric reduction in the 5-HT content of the brain. However, when assessment of 5-HT levels is performed 24 h after neurotoxin administration, initial differences become equalized (Baumgarten et al. 1982b).

5.3 The Influence of Anesthesia

There are inconsistent results regarding the influence of anesthesia on the efficiency of 5,7-DHT neurotoxicity. Baumgarten and collaborators (1982a) suggest that administration of pentobarbitone with ketamine does not exert a major effect on 5,7-DHT action, whereas in an earlier study, the application of pentobarbitone attenuated the toxicity of 5,6-DHT (Baumgarten et al. 1972). Furthermore, the anesthetic can affect not only the neurotoxin activity but also the activity of the catecholamine uptake blocker that is used to gain the high selectivity of the neurotoxin action. Pentobarbitone was shown to interfere with nomifensine in animals given a slow injection of 5,7-DHT (Baumgarten et al. 1982a).

6 Mechanism of 5,6- and 5,7-Dihydroxytryptamine Neurotoxicity

The mechanism of action of dihydroxylated tryptamines is connected with their uptake into neuronal terminals by the active transport systems that are normally responsible for the reuptake of 5-HT (Baumgarten et al. 1978c). The neurotoxins accumulate

intraneuronally to achieve a critical concentration that causes axonal damage. The specificity and potency of each neurotoxin is determined by its affinity for a given monoamine uptake mechanism, its rate of clearance via cellular metabolism, and the ability of its breakdown products to bind to proteins (Bjorklund et al. 1975c). Two major hypotheses have been proposed to explain the molecular mechanism that underlies the toxicity of 5,6- and 5,7-DHT. The first suggests that highly reactive (electrophilic) quinonoid intermediates, created as a result of 5,6- and 5,7-DHT auto-oxidation, bind covalently to molecules indispensable for neuronal function (Rotman et al. 1976). The second hypothesis states that during the auto-oxidation of dihydroxylated tryptamines, reactive oxygen species (ROS) such as superoxide radical anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO^{\cdot}$) are generated as toxic by-products (Baumgarten et al. 1978b, 1982a, b). Studies show that free radical scavengers can provide some protection to peripheral nerves against the degeneration caused by 5,7-DHT (Cohen and Heikkila 1978). Moreover, it has been speculated that quinine imine intermediates of 5,7-DHT can also trigger toxicity via an interaction with the electron transport chain (Klemm et al. 1980).

6.1 Mechanism of 5,7-DHT Action

The auto-oxidation of 5,7-DHT in a buffered aqueous solution at pH 7.4 causes the solution to turn pink (Schlossberger 1978; Cohen and Heikkila 1978; Tabatabaie et al. 1990). The auto-oxidation reaction is second order (Klemm et al. 1980) and at physiological pH leads to the formation of two major products – 5-hydroxytryptamine-4,7-dione and 6,6-bi-5-hydroxytryptamine-4,7-dione (Tabatabaie et al. 1990). Auto-oxidation of 5,7-DHT produces molecular oxygen that catalyzes the oxidation of brain mitochondria (Cohen and Heikkila 1978). This result was confirmed in 1980 by Klemm and collaborators. Moreover, they ruled out any pivotal participation of MAO in the catalytic action of mitochondria. Mitochondria play an important role in catalyzing the auto-oxidation of 5,7-DHT and may be sites of accumulation of damage caused by 5,7-DHT (Tabatabaie and Dryhurst 1998). In 1992, Tabatabaie and Dryhurst revealed that in the presence of superoxide dismutase (SOD), catalase, and diethylene triamine pentaacetic acid (DTPA), which eliminate the catalytic action of O_2^-, trace transition metal ions, and organic peroxy intermediates, the auto-oxidation of 5,7-DHT is much slower than normal. Among the transition metal ions, Fe^{3+}, Fe^{2+}, Cu^{2+}, and Mn^{2+} (Tabatabaie and Dryhurst 1992) are strong candidates to catalyze 5,7-DHT auto-oxidation. The rate of auto-oxidation decreases after catalase and SOD application but increases after the addition of O_2^-. The lack of a stimulatory effect after H_2O_2 administration is striking, especially considering inhibitory catalase action. It has been suggested that the ability of catalase to decompose organic peroxides plays pivotal role in the auto-oxidation of 5,7-DHT (Kadlubar et al. 1973). O_2^- also plays a role in auto-oxidation of 5,7-DHT. It is not only a by-product, but it also plays significant role in potentiating the rate of the reaction. Studies suggest that the catalytic action of O_2^- is not directly connected with oxidation of 5,7-DHT because O_2^- is a weak

oxidizing agent (Sawyer et al. 1978). However, it may react with organic hydroperoxides and contribute to a free radical chain reaction (Tabatabaie and Dryhurst 1998). Moreover, it has been proposed that O_2^- stimulates the deprotonation of 5,7-DHT, which is the initial step in auto-oxidation (Tabatabaie and Dryhurst 1992).

Due to its structure similarity to 5-HT, 5,7-DHT is taken up and concentrated in serotonergic nerve terminals (Baumgarten et al. 1978c). There, the result of 5,7-DHT oxidation is the generation of 5-hydroxytryptamine-4,7-dione and 6,6-bi-5-hydroxytryptamine-4,7-dione as major products. Both of these compounds are more toxic than 5,7-DHT. Their LD_{50} values, measured by ventricular administration in mice, are 30 and 25 μg, respectively, whereas the LD_{50} for 5,7-DHT is 52 μg (Tabatabaie et al. 1993). However, they do not have the ability to cause profound and long-lasting depletion of 5-HT as occurs after 5,7-DHT administration (Tabatabaie and Dryhurst 1998). The result of 5,7-DHT oxidation is the formation of O_2^- and H_2O_2, which, in the presence of trace levels of transition metal ions, cause the generation of cytotoxic $HO^{\cdot}$ (Halliwell 1992). The other effect of this auto-oxidation is increased consumption of molecular oxygen, leading to hypoxia, changes in metabolism, and cellular damage (Kappus 1986). The auto-oxidation product 5-hydroxytryptamine-4,7-dione also reacts with glutathione (GSH) to cause depletion of GSH storage, exacerbating damage caused by ROS (Tabatabaie and Dryhurst 1998).

6.2 Mechanism of 5,6-DHT Action

The ultimate product of 5,6-DHT auto-oxidation at physiological pH is a black, insoluble, melanin-like polymer (Klemm et al. 1980). The by-product of this reaction is a high amount of H_2O_2 (Klemm et al. 1980; Singh and Dryhurst 1990). Studies show that H_2O_2, O_2^-, and trace levels of transition metal ions play an important role in 5,6-DHT auto-oxidation because the application of catalase, SOD, and iron-complexing agents inhibit this reaction. However, the removal of agents such as transition metal ions and molecular oxygen from the reaction site causes 5,6-DHT not to be oxidized by H_2O_2 (Singh and Dryhurst 1990). In turn, the application of even very low amounts of Fe^{3+}, Cu^{2+}, and O_2^- augments the oxidation of 5,6-DHT. These results indicate that H_2O_2 does not participate directly in 5,6-DHT auto-oxidation, but it undergoes a transformation (via the transition metal ion-catalyzed Fenton reaction) leading to $HO^{\cdot}$ generation, which directly affects 5,6-DHT. In vitro as well as in vivo studies show that products of 5,6-DHT auto-oxidation undergo covalent binding with protein nucleophiles (Baumgarten et al. 1978b). However, auto-oxidation of 5,6-DHT ultimately leads to melanin-like polymer formation. An assessment of the role of covalent binding products of 5,6-DHT auto-oxidation revealed that they are not essential for the cytotoxic effects of 5,6-DHT (Sinhababu et al. 1985). The other mechanism that triggers the neurodegenerative action of 5,6-DHT is its ability to deplete intraneuronal antioxidants (e.g., GSH) and generate ROS that induce oxidative damage of lipids, proteins, and

DNA (Tabatabaie and Dryhurst 1998). The result of intraneuronal auto-oxidation of 5,6-DHT is the alkylation of proteins and, in favorable conditions, the cross-linking of proteins with GSH. Both reactions can lead to lethal consequences (Tabatabaie and Dryhurst 1998).

7 Behavioral Effects

At the beginning of this subsection, we would like to emphasize that there are age-related differences in the response of 5-HT neurons to dihydroxytryptamines. Young neurons display increased sensitivity to dihydroxytryptamines due to underdeveloped detoxifying mechanisms such as degrading enzymes, 5,7-DHT storage capacity, and the ability to scavenge radicals and neutralize oxidation intermediates through nucleophilic compounds such as GSH (Breese and Mueller 1978). Moreover, the accumulation of 5,7-DHT in the relatively small 5-HT perikarya of neonatal rats is more efficient than in adult rats where the surface/volume relationship is less favorable. Brain weight has to be taken into account because the brain weight of adult rats is about tenfold higher than newborn rats (Baumgarten et al. 1982b). On the other hand, infant brains have a greater potential for functional recovery than adult brains because they have greater potential for neuronal regeneration (Jonsson et al. 1978).

7.1 Adult Animal Studies

7.1.1 Changes in Locomotor Activity

Intraventricular administration of one of the dihydroxytryptamines (5,6- or 5,7-DHT) to rats causes dramatic behavioral changes (Massotti et al. 1974). Behavioral depression and sedation during the first 2 h after the infusion is typical (Baumgarten and Schlossberger 1973; Massotti et al. 1974). Following this initial period, increased irritability, explosive running, disorganized locomotion, and hyperreactivity to any type of sensory stimulation are observed. These responses are accompanied by bizarre social behavior (fighting in a stereotyped position and manner) and increased copulatory activity between the 1st and 3rd day after 5,6-DHT administration (Baumgarten and Schlossberger 1973). The greatest locomotor hyperactivity occurs during the first week after neurotoxin (5,7-DHT) administration (Pranzatelli and Snodgrass 1986b), and increased locomotor activity (LMA) persists 1–3 weeks following the intracisternal (Stewart et al. 1979) and intracerebroventricular (Lyness and Moore 1981) injection of 5,7-DHT. The stimulation of LMA has been observed after stereotactic injection of 5,7-DHT into the nucleus accumbens septi, substantia nigra, and striatum (Lyness and Moore 1981; Carter and Pycock 1981). The destruction of the medial raphe nuclei (MRN) also leads to increased LMA that manifests mainly as nocturnal hyperactivity and is correlated with hippocampal 5-HT depletion and a limbic mechanism (Geyer et al. 1976). The forebrain may be involved in the changes to LMA following the serotonergic manipulations (Geyer et al. 1976; Carter and Pycock 1981).

After this period of increased LMA, behavior returns to normal, and it is difficult to distinguish DHT-treated animals from controls. This normalization is termed "recovery" and is related to functional reorganization and supersensitivity (Glick 1974; Finger and Almli 1985). Despite the recovery of LMA, the supersensitivity to 5-hydroxy-L-tryptophan (L-5-HTP) may remain for at least 12 months after 5,7-DHT administration (Pranzatelli and Snodgrass 1986a). In 1978, Mackenzie and collaborators revealed that serotonin depletion by 5,7-DHT in rats causes tilt-cage hyperactivity but decreases activity in the open-field test. Kusljic et al. (2003) showed differential involvement of serotonergic projections deriving from medial raphe nuclei and dorsal raphe nuclei (DRN) in the regulation of locomotor hyperactivity. 5,7-DHT injected into the MRN causes a marked increase of phencyclidine-induced locomotor hyperactivity, whereas administration of DHT into the DRN does not.

7.1.2 Prepulse Inhibition

Prepulse inhibition (PPI) is a normal reduction of a startle response taking place when a weak stimulus occurs within a very short time period before a startle-evoking stimulus (Graham 1975). PPI measures sensorimotor gating and reflects the ability to filter out irrelevant stimuli. Fletcher et al. (2001) found that severe depletion of 5-HT (by 5,7-DHT injection into the DRN and MRN) disrupts PPI of the acoustic startle reflex without influencing basal startle activity. Kusljic and coworkers (2003) demonstrated that, similar to LMA, the MRN and DRN regulate PPI in different ways. 5,7-DHT injected into the MRN or the DRN leads to disruption of PPI, but the PPI effect is more significant in MRN-lesioned rats, while the startle amplitude is markedly increased in DRN-lesioned animals.

7.1.3 Seizure Susceptibility

The contribution of serotonin to seizure susceptibility was first suggested in 1954 by Chen and collaborators (Chen et al. 1954). They revealed that the seizure-facilitating effects of reserpine derive from its depletion of 5-HT as well as catecholamines. In adult rats pretreated with protriptyline and 5,7-DHT, the severity of pentylenetetrazol (PTZ)-induced seizures is augmented. These animals also display an earlier seizure onset and a greater severity of seizures than control rats that received vehicle pretreatment. Thus, 5,7-DHT-treated rats seem to be more seizure prone than controls (Browning et al. 1978). Similar results have been obtained by Trindade-Filho and coworkers (2008), who assessed the effects of 5,7-DHT administration into the MRN in a rat pilocarpine model of epilepsy. They showed that the frequency of seizures in treated animals was significantly increased compared to control rats.

7.1.4 Serotonin Syndrome

The classical 5-HT syndrome manifests as lateral head "weaving" (side-to-side movement), reciprocal forepaw "treading," "resting tremor," hindlimb abduction, rigidly arched (Straub) tail, hyperreactivity to auditory stimuli, limb and axial rigidity or hypertonicity, and autonomic responses (salivation, erection, and

ejaculation) (Jacobs 1976). Dihydroxytryptamines evoke supersensitivity to 5-HT precursors and agonists. Studies in 5,7-DHT-treated adult rats revealed that application of L-5-HTP (precursor of 5-HT) leads to 5-HT syndrome (Browning et al. 1978). Low doses of 5-hydroxytryptophan (5-HTP) cause a self-limiting syndrome characterized by the appearance of myoclonus, other dyskinesias, stereotypies, and abnormalities of posture and tone (Stewart et al. 1976, 1979; Pranzatelli and Snodgrass 1986b). It has been suggested that the brainstem and spinal cord are the brain areas involved in mediating this serotonergic behavioral syndrome (Jacobs and Klemfuss 1975).

7.1.5 Feeding and Drinking Behavior

Intraventricular injection of dihydroxytryptamines leads to immediate and transient anorexia, hypodipsia, and weight loss. However, treated animals quickly return to normal body weight and show normal patterns of food and water intake; permanent alteration of drinking preference is rare (Myers 1975; Melchior and Myers 1976). Saller and Stricker (1976) suggested that the inability to observe consistent changes in digestive behavior derives from the effect of dihydroxytryptamines on neurons containing NA. They showed that animals treated with desmethylimipramine and subsequently intraventricularly with 5,7-DHT become hyperphagic and obese, but animals injected with only 5,7-DHT show no change or minor reductions in eating, drinking, and body weight (Saller and Stricker 1976). Opposite results were reported by Myers (1978) in monkeys and rats treated with 5,6-DHT infused directly into hypothalamic structures. A significant reduction of food consumption was observed in monkeys following neurotoxin infusion in the anterior hypothalamic, preoptic region. Water consumption was decreased but not significantly different from the baseline intake. In most cases, the decline of food and water intake occurred not only on the day of 5,6-DHT injection but also on the following day. In rats, hypophagia lasts 4–5 days after the infusion. Water consumption in rats was changed in a similar manner as was food intake. Further, rats lost between 30 g and 50 g of body weight at the higher doses of 5,6-DHT (Myers 1978). The comparison of food intake and body weight studies involving administration of 5,6-DHT, 5,7-DHT, and 6-OHDA to different strains of rats reveals that 5,7-DHT injected intraventricularly produces the greatest loss of body weight (Melchior and Myers 1976).

7.1.6 Body Temperature

In 1963, it was first suggested that 5-HT within the anterior hypothalamus participates in the control of heat production (Feldberg and Myers 1963). Currently, we know that neurons containing 5-HT take part in the regulation of body temperature, but we still do not know the exact role of this transmitter in the mechanism due to inconsistent results between different studies. The effects of dihydroxytryptamines depend on the species and the interval between drug injection and examination. In rats, intraventricular injection of 5,6-DHT causes immediate, transient hypothermia (Breese et al. 1974a). On the other hand, studies in rats and monkeys suggest that infusion of 5,6-DHT into the anterior hypothalamus causes not hypo- but

hyperthermia (Waller and Myers 1974; Myers 1975). Myers (1978) emphasizes that the thermic response to 5,6-DHT is dose dependent and if too large a dose of neurotoxin is injected, transient hypothermia always precedes the hyperthermia.

7.1.7 Sexual Behavior

Neurons containing 5-HT mediate the inhibition of copulatory behavior (Meyerson 1964, 1966). Studies using serotonergic neurotoxins confirm that serotonin nerves inhibit sexual behavior and depletion of 5-HT increases sexual activity in rats (Da Prada et al. 1972). Intracerebroventricular injection of 5,6-DHT induces hyperexcitability and fighting in rats. The main characteristic effect of such treatment is mounting activity (Da Prada et al. 1972; Baumgarten and Schlossberger 1973). If 5-HT levels are normalized by 5-HTP administration, mounting behavior is completely abolished. Similar effects were observed in 5,7-DHT-lesioned rats treated with 5-HTP. Grouped female rats injected with 5,7-DHT also exhibit sexual activity analogous to the mounting displayed by male rats. A similar effect can be triggered by 5,6-DHT treatment. In addition, protection of 5-HT neurons with the 5-HT inhibitor Lu 10-171 before 5,7-DHT administration prevents fighting and mounting behaviors (Da Prada et al. 1978).

7.1.8 Aggressive Behavior

Serotonergic neurons may play a pivotal role in predatory and irritable aggression, defined as aggressive behavior against an animal of another species and against animals of any species triggered by an aversive stimulus, respectively (Eichelman and Thoa 1973). Depletion of 5-HT increases predatory aggression toward mice (also termed muricidal behavior). Intracisternal injection of 5,6-DHT evokes a marked increase in muricidal behavior, even 1 week after infusion. Pretreatment with pargyline significantly augments this predatory aggression. Similar results were found in rats injected with 5,6-DHT in the midbrain raphe nuclei. However, pargyline pretreatment did not significantly increase muricidal behavior (Breese et al. 1974a). Intracisternally injected 5,7-DHT also increases muricidal behavior, observed 19 days after neurotoxin administration. Treatment with 5,7-DHT, pargyline + 5,7-DHT, or desipramine + 5,7-DHT causes killing in 71 %, 89 % and 40 % of rats, respectively (Breese and Cooper 1975).

7.1.9 Response to Novel and Noxious Stimuli

The presentation of a novel stimulus triggers exploratory activity and behavioral vigilance. Subsequent exposures to the same stimulus cause a graded attenuation of these behaviors until they completely disappear. Neural processes termed habituation and sensitization are responsible for this behavior (Davis and Sheard 1974). 5-HT is involved in both the sensitization process (Carlton and Advokat 1973) and the response to a noxious stimulus (Messing and Lytle 1977). However, investigations with 5,6-DHT or 5,7-DHT injected intraventricularly (Blasig et al. 1973) or directly into the midbrain (Hole and Lorens 1975) did not reveal any changes in the behavioral response to noxious stimuli. Lieben and collaborators (2006) found that 5,7-DHT lesion of the dorsal raphe nuclei impairs the ability to identify a novel object.

7.1.10 Learning and Memory Functions

It has been suggested 5-HT participates in both short-term and long-term memory (Bailey et al. 1992; Barbas et al. 2003). There is some literature showing that 5,7-DHT injection does not change learning and memory capabilities even when severe 5-HT depletion is induced electrolytically (Asin and Fibiger 1984). However, there are also data providing quite distinct results that show that 5,7-DHT administration affects short-term memory. Hritcu and collaborators (2007) found that disruption of short-term memory does not accompany alterations in long-term memory. Ciobica et al. (2010) found a significant defect in short-term memory, demonstrated by working memory errors in the radial arm maze. Ricaurte and coworkers (1993) noted an impairment of choice accuracy following 5,7-DHT treatment, which also suggests alterations in short-term memory. Moreover, Cassaday et al. (2003) found that rats treated with 5,7-DHT had difficulty in learning new tasks but no impairment in performing previously learned working memory tasks. However, data demonstrating a lack of short-term memory deficits after neurotoxin injection also exist (Wirth et al. 2000).

7.1.11 Effect of Dihydroxytryptamines on the Antinociceptive Actions of Drugs

5-HT is involved in determining the pain threshold and in regulating the perceptual and behavioral responses to painful stimuli (Lorens 1978). Decreasing 5-HT levels (by different methods) reduces the analgesic potency of morphine (Tenen 1968), whereas increasing 5-HT concentrations augments morphine analgesia (Sewell and Spencer 1974). However, not all results confirm this hypothesis. Some studies indicate that mesencephalic raphe lesions and intra-midbrain infusion of 5,7-DHT (10 μg) do not change pain sensitivity (Harvey et al. 1974; Hole and Lorens 1975). Blasig and collaborators (1973) noticed that intraventricular injection of 5,6-DHT did not significantly change the antinociceptive effect of morphine. Moreover, they showed that neurotoxin did not affect the stimulation threshold before morphine administration. These results suggest that a decreased 5-HT level is not sufficient to trigger the development of hyperalgesia (Lorens 1978). On the other hand, the majority of studies show different results. Genovese and collaborators (1973) found that, following intraventricular injection of 5,6-DHT in rats, the pain threshold was modestly but not significantly reduced. Following morphine infusion, the pain threshold was clearly increased in controls and sham-injected animals, but in rats that received 5,6-DHT, it was only slightly augmented. This result demonstrates that 5,6-DHT treatment reduces the antinociceptive action of morphine. Similar effects have been obtained in intrathecally 5,6- and 5,7-DHT-injected rats. Both neurotoxins evoke a decrease in the analgesic action of morphine, and no marked distinctions were noted between animals treated with 5,6- or 5,7-DHT (Rodriguez and Rodriguez 1989).

7.1.12 Sleeping Behavior

Manipulations that reduce forebrain 5-HT also decrease the duration of slow-wave sleep. This suggests a pivotal role for 5-HT in the initiation as well as maintenance

of this stage of sleep (Jouvet 1969, 1972). Studies in cats have shown that intraventricular injection of 5,6-DHT causes long-term reductions in slow-wave and REM (rapid eye movement) sleep time (Jouvet and Pujol 1974). Analogous results were not observed following intraventricular infusion of 5,7-DHT in rats. 5,7-DHT does not prominently affect the circadian pattern of sleep. The interesting difference between 5,7-DHT-treated and control rats was the amount of paradoxical sleep, which was significantly higher for the 5,7-DHT group on days 6 and 8. Additionally, 5,7-DHT rats displayed a large number of long duration paradoxical sleep epochs (Ross et al. 1976). Moreover, Jouvet and Pujol (1974) showed that direct administration of 5,6-DHT into the dorsal raphe or superior centralis nuclei of cats leads to a transient decrease in slow-wave sleep and markedly increases the duration of REM sleep within the first 24 h after treatment. These parameters subsequently return to normal. In 1978, Pujol and collaborators revealed that selective and diffuse damage of 5-HT terminals in cats causes a marked increase in waking time. Such evoked insomnia might be derived from decreases of stage 2 and paradoxical sleep as well as a simultaneous increase of the waking mechanism.

7.1.13 Neuroendocrine Consequences of Dihydroxytryptamine Administration

Many studies of the role of 5-HT in neuroendocrine regulatory mechanisms have used dihydroxytryptamines. In 1974, Ladosky and Noronha (1974) revealed that 5,6-DHT prominently increases the release of gonadotropin. However, other studies have provided different results. Significant changes in plasma and pituitary follicle-stimulating hormone (FSH) levels were not observed in rats treated at 21–23 days of age with 5,6-DHT (Saller and Stricker 1976). The influence of 5,7-DHT on plasma prolactin concentration has also been studied. Gil-ad et al. (1976) demonstrated that 5,7-DHT causes a reduction in prolactin levels, whereas Wuttke et al. (1978) and Clemens (1978) found no impairments in prolactin release in animals injected with 5,7-DHT. There are also inconsistent results in studies of the influence of 5-HT neurotoxins on growth hormone (GH). Collu (1978) showed that 5,6-DHT does not affect GH levels in male prepubertal rats, whereas Baumgarten and Bjorklund (1976) found that administration of 5,7-DHT increases plasma GH concentration.

7.1.14 Effect of 5,7-DHT on the Dopaminergic System

When 5,7-DHT was administered in adulthood to rats that were neonatally lesioned with 6-hydroxydopamine, attenuated dopamine D_1 receptor supersensitivity and intense spontaneous hyperlocomotor activity were observed (Kostrzewa et al. 1994). Amphetamine and m-chlorophenylpiperazine attenuation of the profound hyperlocomotion in rats lesioned with both 6-hydroxydopamine and 5,7-DHT reinforces the suggestion that such rats are a useful animal model of attention deficit hyperactivity disorder (Kostrzewa et al. 1994; Brus et al. 2004; Nowak et al. 2007).

7.2 Neonatal Animal Studies

7.2.1 Locomotor Activity

Studies suggest that serotonergic pathways play an inhibitory role in LMA (Breese et al. 1974b; Hollister et al. 1976). Neonatal rats injected with 5,7-DHT also display hyperactivity that persists for 1 month after the injection of neurotoxin. Breese and Mueller (1978) showed that the depletion of brain amines was greater in juveniles than in adults despite the lower doses of 5,7-DHT used. Moreover, they reported a significant fivefold elevation in LMA at 14 days in animals treated with 50 μg of 5,7-DHT at 3 days of age. The LMA of animals treated with pargyline prior to 5,7-DHT was comparable to rats treated 5,7-DHT alone. This result suggests that rats pup hyperactivity does not require a reduction of NA fibers that increased LMA in adult rats (Smith et al. 1973). However, when the rats are tested at 28 days of age, the neurotoxin-treated groups demonstrate considerably less LMA than the control group (Breese and Mueller 1978).

7.2.2 Seizure Susceptibility

Pranzatelli and collaborators (Pranzatelli et al. 1989) demonstrated that intracisternal application of 5,7-DHT causes transient postinjection convulsions in rat pups. The first infusion leads to continuous paddling and writhing for at least 30 min without forward locomotion or vocalization. Moreover, 11 of 13 rats that received 5,7-DHT were unable to stand upright and fell over on their backs or sides. Subsequent 5,7-DHT injections caused more severe convulsions, whereas intraperitoneal injection of 5,7-DHT did not induce seizures during the 2-hour period of observation (Pranzatelli et al. 1989).

7.2.3 Serotonin Syndrome

Intracisternal and intraperitoneal injection of 5,7-DHT into rat pups, followed by the administration of 5-HTP 2 and 14 weeks later, leads to behavioral supersensitivity in adult rats. Both routes of 5,7-DHT administration provide supersensitivity to 5-HTP, but shaking behavior is greater following intraperitoneal infusion, whereas forepaw myoclonus and head weaving are greater following intracisternal injection (Pranzatelli et al. 1989).

7.2.4 Body Weights

A comparison of body weights between 5,7-DHT-treated rats and control animals at 14 days of age revealed that the former had markedly lower body weights. Pretreatment with pargyline prevents this weight loss. One possible explanation of this difference is that neurotoxin-treated rat pups bite the mother during suckling, which causes them to receive less nutrition (Breese and Mueller 1978).

7.2.5 Learning and Memory Function

Volpe et al. (1992), in their research with intracisternally administered 5,7-DHT to three-day-old rat pups pretreated with pargyline, revealed that 5,7-DHT destroys 5-HT neurons but does not influence spatial memory, tested by the Morris water

maze and the 12-arm radial maze. We also demonstrated that despite a severe and permanent decrease in hippocampal, prefrontal, and striatal 5-HT levels, neonatal treatment with 5,7-DHT caused no spatial learning and memory impairment in the Morris water maze (Piechal et al. 2012).

7.2.6 Emotional and Exploratory Behaviors

In our study, neonatal 5,7-DHT depletion reduced LMA in the open-field test and attenuated social interaction in non-aversive conditions and exploration of a novel objects in adult rats. Ultravocalization, but not freezing, was increased in the contextual fear-conditioning paradigm in 5-HT-depleted rats. There was no difference in the pain threshold between groups (Rok-Bujko et al. 2012).

7.2.7 Prepulse Inhibition

Our results clearly demonstrated that neonatal 5,7-DHT depletion does not interrupt PPI of the acoustic startle reflex. In contrast to the effect on PPI, neonatal treatment with 5,7-DHT increased basal startle activity (Kołomańska et al. 2011).

7.2.8 Developmental Plasticity

Studies using several doses and schedules of 5,6-DHT administration in rat pups revealed that this neurotoxin is not useful for research in developmental studies because most pups died soon after treatment. Conversely, 5,7-DHT is well tolerated by young animals, even when injected repeatedly in doses up to 200 mg/kg. Systemic injection of 5,7-DHT in neonatal rats caused changes in 5-HT neurons that did not occur in adult animals. These changes include significant denervations of most distally located 5-HT nerve terminals, but cell bodies often remain intact. Moreover, in regions adjacent to cell bodies, compensatory outgrowth occurs that causes an increased number of 5-HT nerve terminals. This mechanism leads to redistribution of the terminals and bifurcations of 5-HT neurons and is termed "pruning" and "sprouting," respectively (Jonsson et al. 1978).

7.2.9 Effect of 5,7-DHT on Dopaminergic, Noradrenergic, and Histaminergic Systems

Neonatal 6-hydroxydopamine treatment is associated with supersensitization of dopamine D_1 receptors, and this effect was attenuated by neonatal 5,7-DHT depletion (Brus et al. 1994). In contrast, the neonatal destruction of 5-HT fibers by 5,7-DHT produces supersensitization of the dopamine D_2 receptor complex (Brus et al. 1995). Furthermore, the ontogenic destruction of serotonergic and noradrenergic neurons in rat brain is associated with prominent D_2 and D_3 receptor supersensitivity, as reflected by enhanced dopamine D_2 and D_3 agonist-induced stereotyped activities (Nowak et al. 2009). These findings indicate that both serotonergic and noradrenergic fibers have important influence on the process of dopamine D_1 and D_2 receptor sensitivity.

Finally, there is some evidence that ontogenetic 5-HT depletion alters histaminergic activity in adult rats. Neonatal 5,7-DHT treatment of rats was associated with enhanced oral and locomotor activity induced by the histamine H_3 receptor antagonist thioperamide (Jośko et al. 2011).

8 Conclusion

5,6-dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT) are serotonin neurotoxins able to selectively destroy central serotonin neurons when catecholaminergic neurons are protected. Both compounds are indole derivatives, and their neurotoxicity derives from the additional hydroxyl group compared to serotonin (5-HT). The administration of 5,6- or 5,7-DHT to animals is a useful method to study the role of serotonin neurons and pathways in the central nervous system (CNS). Despite high structural similarity, 5,6-DHT differs from 5,7-DHT. The basic differences are connected to the ability of 5,6-DHT to rapidly oxidize and form consecutive melanin-like polymers. These features lower the neurotoxicity of 5,6-DHT to 5-HT neurons and make it more generally cytotoxic. Therefore, 5,7-DHT is used more frequently. The action of serotonin neurotoxins depends on the proper selection of injection sites, the speed and volume of administration, the type and amount of antioxidant used, and the type and dose of catecholamine uptake blocker used to prevent nonspecific toxicity. The mechanism of dihydroxytryptamine action assumes that, because of their structurally similarity to 5-HT, they accumulate in 5-HT neurons where the products of their oxidation bind covalently to molecules indispensable for neuronal function. Moreover, reactive oxygen species (ROS) generated as by-products of oxidation also contribute to the destruction of 5-HT neurons. The use of 5,6- and 5,7-DHT in both adult and neonatal animals enables the recognition the role of 5-HT in locomotor activity (LMA), prepulse inhibition, seizure susceptibility, sleeping behavior, feeding and drinking behavior, sexual and aggressive behavior, the regulation of body temperature, the response to novel and noxious stimuli, learning and memory, the antinociceptive action of drugs, neuroendocrine regulatory mechanisms, and developmental plasticity.

References

Ahmad, G., & Zamenhof, S. (1978). Serotonin as a growth factor for chick embryo brain. *Life Sciences, 22*(11), 963–970.

Asin, K. E., & Fibiger, H. C. (1984). Spontaneous and delayed spatial alternation following damage to specific neuronal elements within the nucleus medianus raphe. *Behavioural Brain Research, 13*(3), 241–250.

Azmitia, E. C. (2001). Modern views on an ancient chemical: Serotonin effects on cell proliferation, maturation, and apoptosis. *Brain Research Bulletin, 56*(5), 413–424.

Bailey, C. H., Montarolo, P., Chen, M., Kandel, E. R., & Schacher, S. (1992). Inhibitors of protein and RNA synthesis block structural changes that accompany long-term heterosynaptic plasticity in Aplysia. *Neuron, 9*(4), 749–758.

Barbas, D., DesGroseillers, L., Castellucci, V. F., Carew, T. J., & Marinesco, S. (2003). Multiple serotonergic mechanisms contributing to sensitization in aplysia: Evidence of diverse serotonin receptor subtypes. *Learning and Memory, 10*(5), 373–386.

Baumgarten, H. G., & Bjorklund, A. (1976). Neurotoxic indoleamines and monoamine neurons. *Annual Review of Pharmacology and Toxicology, 16*, 101–111.

Baumgarten, H. G., & Lachenmayer, L. (1972). 5,7-dihydroxytryptamine: Improvement in chemical lesioning of indoleamine neurons in the mammalian brain. *Zeitschrift für Zellforschung und mikroskopische Anatomie, 135*(3), 399–414.

Baumgarten, H. G., & Schlossberger, H. G. (1973). Effects of 5,6-dihydroxytryptamine on brain monoamine neurons in the rat. In J. D. Barchas (Ed.), *Serotonin and behavior* (pp. 209–224). New York: Academic.

Baumgarten, H. G., Bjorklund, A., Lachenmayer, L., Nobin, A., & Stenevi, U. (1971). Long-lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine. *Acta Physiologica Scandinavica. Supplementum, 373*, 1–15.

Baumgarten, H. G., Evetts, K. D., Holman, R. B., Iversen, L. L., Vogt, M., & Wilson, G. (1972). Effects of 5,6-dihydroxytryptamine on monoaminergic neurones in the central nervous system of the rat. *Journal of Neurochemistry, 19*(6), 1587–1597.

Baumgarten, H. G., Bjorklund, A., Lachenmayer, L., & Nobin, A. (1973). Evaluation of the effects of 5,7-dihydroxytryptamine on serotonin and catecholamine neurons in the rat CNS. *Acta Physiologica Scandinavica. Supplementum, 391*, 1–19.

Baumgarten, H. G., Bjorklund, A., & Bogdanski, D. F. (1975a). Similarities and differences in the mode of action of 6-hydroxy-dopamine and neurotoxic indoleamines. In G. Jonnson, T. Malmfors, & C. H. Sachs (Eds.), *Chemical tools in catecholamine research* (pp. 59–66). Amsterdam/North-Holland/New York: American Elsevier.

Baumgarten, H. G., Bjorklund, A., Nobin, A., Rosengren, E., & Schlossberger, H. G. (1975b). Neurotoxicity of hydroxylated tryptamines: Structure-activity relationships. 1. Long-term effects on monoamine content and fluorescence morphology of central monoamine neurons. *Acta Physiologica Scandinavica. Supplementum, 429*, 5–27.

Baumgarten, H. G., Lachenmayer, L., & Bjorklund, A. (1977). Chemical lesioning of indoleamine pathways. In R. D. Myers (Ed.), *Methods in psychobiology* (pp. 47–98). New York: Academic.

Baumgarten, H. G., Bjorklund, A., & Wuttke, W. (1978a). Neural control of pituitary LH, FSH and prolactin secretion: The role of serotonin. In D. E. Scott, G. P. Kozlowski, & A. Weindl (Eds.), *Brain-endocrine interaction* (pp. 327–343). Basel/New York: Karger.

Baumgarten, H. G., Klemm, H. P., Lachenmayer, L., Bjorklund, A., Lovenberg, W., & Schlossberger, H. G. (1978b). Mode and mechanism of action of neurotoxic indoleamines: A review and a progress report. *Annals of the New York Academy of Sciences, 305*, 3–24.

Baumgarten, H. G., Klemm, H. P., Lachenmayer, L., & Schlossberger, H. G. (1978c). Effect of drugs on the distribution of [14C]-5,6-dihydroxytryptamine and [14C]-5,7-dihydroxytryptamine in rat brain. *Annals of the New York Academy of Sciences, 305*, 107–118.

Baumgarten, H. G., Jenner, S., & Schlossberger, H. G. (1979). Serotonin neurotoxins: Effects of drugs on the destruction of brain serotonergic, noradrenergic and dopaminergic axons in the adult rat by intraventricularly, intracisternally or intracerebrally administered 5,7-dihydroxytryptamine and related compounds. In I. W. Chubb & L. B. Geffen (Eds.), *Neurotoxins, fundamental and clinical advances* (pp. 221–226). Adelaide: Adelaide University Union Press.

Baumgarten, H. G., Jenner, S., & Klemm, H. P. (1981). Serotonin neurotoxins: Recent advances in the mode of administration and molecular mechanism of action. *Journal of Physiology, Paris, 77*(2–3), 309–314.

Baumgarten, H. G., Jenner, S., Bjorklund, A., Klemm, H. P., & Schlossberger, H. G. (1982a). Serotonin neurotoxin. In N. N. Osborne (Ed.), *Biology of serotonergic transmission* (pp. 249–277). New York: Wiley.

Baumgarten, H. G., Klemm, H. P., Sievers, J., & Schlossberger, H. G. (1982b). Dihydroxytryptamines as tools to study the neurobiology of serotonin. *Brain Research Bulletin, 9*(1–6), 131–150.

Bjorklund, A., Nobin, A., & Stenevi, U. (1973). The use of neurotoxic dihydroxytryptamines as tools for morphological studies and localized lesioning of central indolamine neurons. *Zeitschrift für Zellforschung und mikroskopische Anatomie, 145*(4), 479–501.

Bjorklund, A., Baumgarten, H. G., Lachenmayer, L., & Rosengren, E. (1975a). Recovery of brain noradrenaline after 5,7-dihydrixytryptamine-induced axonal lesions in rat. *Cell and Tissue Research, 161*(2), 145–155.

Bjorklund, A., Baumgarten, H. G., & Rensch, A. (1975b). 5,7-Dihydroxytryptamine: Improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. *Journal of Neurochemistry, 24*(4), 833–835.

Bjorklund, A., Horn, A. S., Baumgarten, H. G., Nobin, A., & Schlossberger, H. G. (1975c). Neurotoxicity of hydroxylated tryptamines: Structure-activity relationships. 2. In vitro studies on monoamine uptake inhibition and uptake impairment. *Acta Physiologica Scandinavica. Supplementum, 429*, 29–60.

Blasig, J., Reinhold, K., & Herz, A. (1973). Effect of 6-hydroxydopamine, 5,6-dihydroxytryptamine and raphe lesions on the antinociceptive actions of morphine in rats. *Psychopharmacologia, 31*(2), 111–119.

Breese, G. R., & Cooper, B. R. (1975). Behavioral and biochemical interactions of 5,7-dihydroxytryptamine with various drugs when administered intracisternally to adult and developing rats. *Brain Research, 98*(3), 517–527.

Breese, G. R., & Mueller, R. A. (1978). Alterations in the neurocytotoxicity of 5,6-dihydroxytryptamine by pharmacologic agents in adult and developing rats. *Annals of the New York Academy Sciences, 305*, 160–170.

Breese, G. R., Cooper, B. R., Grant, L. D., & Smith, R. D. (1974a). Biochemical and behavioural alterations following 5,6-dihydroxytryptamine administration into brain. *Neuropharmacology, 13*(3), 177–187.

Breese, G. R., Cooper, B. R., & Mueller, R. A. (1974b). Evidence for involvement of 5-hydroxytryptamine in the actions of amphetamine. *British Journal of Pharmacology, 52*(2), 307–314.

Browning, R. A., Hoffman, W. E., & Simonton, R. L. (1978). Changes in seizure susceptibility after intracerebral treatment with 5,7-dihydroxytryptamine: role of serotonergic neurons. *Annals of the New York Academy Sciences, 305*, 437–456.

Brus, R., Kostrzewa, R. M., Perry, K. W., & Fuller, R. W. (1994). Supersensitization of the oral response to SKF 38393 in neonatal 6-hydroxydopamine-lesioned rats is eliminated by neonatal 5,7-dihydroxytryptamine treatment. *Journal of Pharmacol and Experimental Therapeutics, 268*, 231–237.

Brus, R., Plech, A., & Kostrzewa, R. M. (1995). Enhanced quinpirole response in rats lesioned neonatally with 5,7-dihydroxytryptamine. *Pharmacology Biochemistry and Behavior, 50*, 649–653.

Brus, R., Nowak, P., Szkilnik, R., Mikolajun, U., & Kostrzewa, R. M. (2004). Serotoninergics attenuate hyperlocomotor activity in rats. Potential new therapeutic strategy for hyperactivity. *Neurotoxicity Research, 6*, 317–325.

Carlton, P. L., & Advokat, C. (1973). Attenuated habituation due to parachlorophenylalanine. *Pharmacology Biochemistry and Behavior, 1*(6), 657–663.

Carter, C. J., & Pycock, C. J. (1981). The role of 5-hydroxytryptamine in dopamine-dependent stereotyped behaviour. *Neuropharmacology, 20*(3), 261–265.

Cassaday, H. J., Norman, C., Shilliam, C. S., Vincent, C., & Marsden, C. A. (2003). Intraventricular 5,7-dihydroxytryptamine lesions disrupt acquisition of working memory task rules but not performance once learned. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27*(1), 147–156.

Chen, G., Ensor, C. R., & Bohner, B. (1954). A facilitation action of reserpine on the central nervous system. *Proceedings of the Society for Experimental Biology and Medicine, 86*(3), 507–510.

Ciobica, A., Hritcu, L., Padurariu, M., Dobrin, R., & Bild, V. (2010). Effects of serotonin depletion on behavior and neuronal oxidative stress status in rat: Relevance for anxiety and affective disorders. *Advances in Medical Sciences, 55*(2), 289–296.

Clemens, J. A. (1978). Effects of serotonin neurotoxins on pituitary hormone release. *Annals of the New York Academy of Sciences, 305*, 399–410.

Cohen, G., & Heikkila, R. E. (1978). Mechanisms of action of hydroxylated phenylethylamine and indoleamine neurotoxins. *Annals of the New York Academy of Sciences, 305*, 74–84.

Collu, R. (1978). Endocrine effects of brain serotonin depletion by 5,6-dihydroxytryptamine in prepubertal male rats. *Annals of the New York Academy of Sciences, 305*, 411–422.

Da Prada, M., Carruba, M., O'Brien, R. A., Saner, A., & Pletscher, A. (1972). The effect of 5,6-dihydroxytryptamine on sexual behaviour of male rats. *European Journal of Pharmacology, 19*(2), 288–290.

Da Prada, M., Pieri, L., Keller, H., Pieri, M., & Bonetti, E. P. (1978). Effects of 5,6-dihydroxytryptamine and 5,7-dihydroxytryptamine on the rat central nervous system after intraventricular or intracerebral application and on blood platelets in vitro. *Annals of the New York Academy of Sciences, 305*, 595–620.

Daly, J., Fuxe, K., & Jonsson, G. (1973). Effects of intracerebral injections of 5,6-dihydroxytryptamine on central monoamine neurons: Evidence for selective degeneration of central 5-hydroxytryptamine neurons. *Brain Research, 49*(2), 476–482.

Davis, M., & Sheard, M. H. (1974). Habituation and sensitization of the rat startle response: Effects of raphe lesions. *Physiology and Behavior, 12*(3), 425–431.

Eichelman, B. S., Jr., & Thoa, N. B. (1973). The aggressive monoamines. *Biological Psychiatry, 6*(2), 143–164.

Feldberg, W., & Myers, R. D. (1963). A new concept of temperature regulation by amines in the hypothalamus. *Nature, 200*, 1325.

Finger, S., & Almli, C. R. (1985). Brain damage and neuroplasticity: Mechanisms of recovery or development? *Brain Research, 357*(3), 177–186.

Fletcher, P. J., Selhi, Z. F., Azampanah, A., & Sills, T. L. (2001). Reduced brain serotonin activity disrupts prepulse inhibition of the acoustic startle reflex: Effects of 5,7-dihydroxytryptamine and p-chlorophenylalanine. *Neuropsychopharmacology, 24*(4), 399–409.

Genovese, E., Zonta, N., & Mantegazza, P. (1973). Decreased antinociceptive activity of morphine in rats pretreated intraventricularly with 5,6-dihydroxytryptamine, a long-lasting selective depletor of brain serotonin. *Psychopharmacologia, 32*(4), 359–364.

Gershanik, O. S., Heikkila, R. E., & Duvoisin, R. C. (1979). Asymmetric action of intraventricular monoamine neurotoxins. *Brain Research, 174*(2), 345–350.

Geyer, M. A., Puerto, A., Menkes, D. B., Segal, D. S., & Mandell, A. J. (1976). Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. *Brain Research, 106*(2), 257–269.

Gil-ad, I., Zambotti, F., Carruba, M. O., Vicentini, L., & Muller, E. E. (1976). Stimulatory role for brain serotoninergic system on prolactin secretion in the male rat. *Proceedings of the Society for Experimental Biology and Medicine, 151*(3), 512–518.

Glick, S. D. (1974). Changes in drug sensitivity of mechanism of functional recovery following brain damage. In D. G. Stein, J. J. Rosen, & N. Butters (Eds.), *Plasticity and recovery of function in the central nervous system* (pp. 339–372). New York: Academic.

Graham, F. K. (1975). Presidential Address, 1974. The more or less startling effects of weak prestimulation. *Psychophysiology, 12*(3), 238–248.

Halliwell, B. (1992). Reactive oxygen species and the central nervous system. *Journal of Neurochemistry, 59*(5), 1609–1623.

Harvey, J. A., Schlosberg, A. J., & Yunger, L. M. (1974). Effect of p-chlorophenylalanine and brain lesions on pain sensitivity and morphine analgesia in the rat. *Advances in Biochemical Psychopharmacology, 10*, 233–245.

Hedreen, J. (1975). Increased nonspecific damage after lateral ventricle injection of 6-OHDA compared with fourth ventricle injection in rat brain. In G. Jonnson, T. Malmfors, & C. H. Sachs (Eds.), *Chemical tools in catecholamine research* (pp. 91–100). Amsterdam/North-Holland/New York: American Elsevier.

Hole, K., & Lorens, S. A. (1975). Response to electric shock in rats: Effects of selective midbrain raphe lesions. *Pharmacology Biochemistry and Behavior, 3*(1), 95–102.

Hollister, A. S., Breese, G. R., Kuhn, C. M., Cooper, B. R., & Schanberg, S. M. (1976). An inhibitory role for brain serotonin-containing systems in the locomotor effects of d-amphetamine. *Journal of Pharmacol and Experimental Therapeutics, 198*(1), 12–22.

Hritcu, L., Clicinschi, M., & Nabeshima, T. (2007). Brain serotonin depletion impairs short-term memory, but not long-term memory in rats. *Physiology and Behavior, 91*(5), 652–657.

Jacobs, B. L. (1976). An animal behavior model for studying central serotonergic synapses. *Life Sciences, 19*(6), 777–785.

Jacobs, B. L., & Klemfuss, H. (1975). Brain stem and spinal cord mediation of a serotonergic behavioral syndrome. *Brain Research, 100*(2), 450–457.

Jacoby, J. A., & Lytle, L. D. (1978). Serotonin neurotoxins. *Annals of the New York Academy of Sciences, 305*, 1–665.

Jonsson, G., Pollare, H., Hallman, H., & Sachs, C. (1978). Developmental plasticity of central serotonin neurons after 5,7-dihydrotryptamine treatment. *Annals of the New York Academy of Sciences, 305*, 328–345.

Jośko, J., Drab, J., Jochem, J., Nowak, P., Szkilnik, R., Korossy-Mruk, E., Boroń, D., Kostrzewa, R. M., Brus, H., & Brus, R. (2011). Ontogenetic serotoninergic lesioning alters histaminergic activity in rats in adulthood. *Neurotoxicity Research, 20*, 103–108.

Jouvet, M. (1969). Biogenic amines and the states of sleep. *Science, 163*(3862), 32–41.

Jouvet, M. (1972). The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergeb Physiology, 64*, 166–307.

Jouvet, M., & Pujol, J. F. (1974). Effects of central alterations of serotoninergic neurons upon the sleep-waking cycle. *Advances in Biochemical Psychopharmacology, 11*, 199–209.

Kadlubar, F. F., Morton, K. C., & Ziegler, D. M. (1973). Microsomal-catalyzed hydroperoxide-dependent C-oxidation of amines. *Biochemical and Biophysical Research Communications, 54*(4), 1255–1261.

Kappus, H. (1986). Overview of enzyme systems involved in bio-reduction of drugs and in redox cycling. *Biochemical Pharmacology, 35*(1), 1–6.

Klemm, H. P., Baumgarten, H. G., & Schlossberger, H. G. (1980). Polarographic measurements of spontaneous and mitochondria-promoted oxidation of 5,6- and 5,7-dihydroxytryptamine. *Journal of Neurochemistry, 35*(6), 1400–1408.

Kołomańska, P., Wyszogrodzka, E., Rok-Bujko, P., Krząścik, P., Kostowski, W., Zaniewska, M., et al. (2011). Neonatal serotonin (5-HT) depletion does not disrupt prepulse inhibition of the startle response in rats. *Pharmacological Reports, 63*(5), 1077–1084.

Kostrzewa, R. M., Brus, R., Kalbfleisch, J. H., Perry, K. W., & Fuller, R. W. (1994). Proposed animal model of attention deficit hyperactivity disorder. *Brain Research Bulletin, 34*, 161–167.

Kusljic, S., Copolov, D. L., & van den Buuse, M. (2003). Differential role of serotonergic projections arising from the dorsal and median raphe nuclei in locomotor hyperactivity and prepulse inhibition. *Neuropsychopharmacology, 28*(12), 2138–2147.

Ladosky, W., & Noronha, J. G. (1974). Further evidence for an inhibitory role of serotonin in the control of ovulation. *Journal of Endocrinology, 62*(3), 677–678.

Lidov, H. G., & Molliver, M. E. (1982). An immunohistochemical study of serotonin neuron development in the rat: Ascending pathways and terminal fields. *Brain Research Bulletin, 8*(4), 389–430.

Lieben, C. K., Steinbusch, H. W., & Blokland, A. (2006). 5,7-DHT lesion of the dorsal raphe nuclei impairs object recognition but not affective behavior and corticosterone response to stressor in the rat. *Behavioural Brain Research, 168*(2), 197–207.

Lipton, S. A., & Kater, S. B. (1989). Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. *Trends in Neurosciences, 12*(7), 265–270.

Lorens, S. A. (1978). Some behavioral effects of serotonin depletion depend on method: A comparison of 5,7-dihydroxytryptamine, p-chlorophenylalanine, p-chloroamphetamine, and electrolytic raphe lesions. *Annals of the New York Academy of Sciences, 305*, 532–555.

Lyness, W. H., & Moore, K. E. (1981). Destruction of 5-hydroxytryptaminergic neurons and the dynamics of dopamine in nucleus accumbens septi and other forebrain regions of the rat. *Neuropharmacology, 20*(4), 327–334.

Mackenzie, R. G., Hoebel, B. G., Norelli, H. C., & Trulson, M. E. (1978). Increased tilt-cage activity after serotonin depletion by 5,7-dihydroxytryptamine. *Neuropharmacology, 17*(11), 957–963.

Massotti, M., Scotti de Carolis, A., & Longo, V. G. (1974). Effects of three dihydroxylated derivatives of tryptamine on the behavior and on brain amine content in mice. *Pharmacology Biochemistry and Behavior, 2*(6), 769–776.

Melchior, C. L., & Myers, R. D. (1976). Genetic differences in ethanol drinking of the rat following injection of 6-OHDA, 5,6-DHT or 5,7-DHT into the cerebral ventricles. *Pharmacology Biochemistry and Behavior, 5*(1), 63–72.

Messing, R. B., & Lytle, L. D. (1977). Serotonin-containing neurons: Their possible role in pain and analgesia. *Pain, 4*(1), 1–21.

Meyerson, B. J. (1964). The effect of neuropharmacological agents on hormone-activated estrus behaviour in ovariectomised rats. *Archives Internationales de Pharmacodynamie et de Therapie, 150*, 4–33.

Meyerson, B. J. (1966). Oestrous behaviour in oestrogen treated ovariectomized rats after chlorpromazine alone or in combination with progesterone, tetrabenazine or reserpine. *Acta Pharmacologica et Toxicologica (Copenh), 24*(4), 363–376.

Myers, R. D. (1975). Impairment of thermoregulation, food and water intakes in the rat after hypothalamic injections of 5,6-dihydroxytryptamine. *Brain Research, 94*(3), 491–506.

Myers, R. D. (1978). Hypothalamic actions of 5-hydroxytryptamine neurotoxins: Feeding, drinking and body temperature. *Annals of New York Academy of Sciences, 305*, 556–575.

Nowak, P., Bortel, A., Dabrowska, J., Oswiecimska, J., Drosik, M., Kwiecinski, A., Opara, J., Kostrzewa, R. M., & Brus, R. (2007). Amphetamine and mCPP effects on dopamine and serotonin striatal in vivo microdialysates in an animal model of hyperactivity. *Neurotoxicity Research, 11*, 131–144.

Nowak, P., Nitka, D., Kwieciński, A., Jośko, J., Drab, J., Pojda-Wilczek, D., Kasperski, J., Kostrzewa, R. M., & Brus, R. (2009). Neonatal co-lesion by DSP-4 and 5,7-DHT produces adulthood behavioral sensitization to dopamine D(2) receptor agonists. *Pharmacological Reports, 61*, 311–318.

Piechal, A., Blecharz-Klin, K., Wyszogrodzka, E., Kolomanska, P., Rok-Bujko, P., Krzascik, P., et al. (2012). Neonatal serotonin (5-HT) depletion does not affect spatial learning and memory in rats. *Pharmacological Reports, 64*(2), 266–274.

Pranzatelli, M. R., & Snodgrass, S. R. (1986a). Motor habituation in the DHT model: Bin analysis of daytime and nocturnal locomotor activity. *Pharmacology Biochemistry and Behavior, 24*(6), 1679–1686.

Pranzatelli, M. R., & Snodgrass, S. R. (1986b). Serotonin-lesion myoclonic syndromes. II. Analysis of individual syndrome elements, locomotor activity and behavioral correlations. *Brain Research, 364*(1), 67–76.

Pranzatelli, M. R., Huang, Y. Y., Dollison, A. M., & Stanley, M. (1989). Brainstem serotonergic hyperinnervation modifies behavioral supersensitivity to 5-hydroxytryptophan in the rat. *Brain Research. Developmental Brain Research, 50*(1), 89–99.

Pujol, J. F., Keane, P., Bobillier, P., Renaud, B., & Jouvet, M. (1978). 5,6-Dihyhydroxytryptamine as a tool for studying sleep mechanisms and interactions between monoaminergic systems. *Annals of New York Academy of Sciences, 305*, 576–589.

Ricaurte, G. A., Markowska, A. L., Wenk, G. L., Hatzidimitriou, G., Wlos, J., & Olton, D. S. (1993). 3,4-Methylenedioxymethamphetamine, serotonin and memory. *Journal of Pharmacol and Experimental Therapeutics, 266*(2), 1097–1105.

Rodriguez, F. D., & Rodriguez, R. E. (1989). Intrathecal administration of 5,6-DHT or 5,7-DHT reduces morphine and substance P-antinociceptive activity in the rat. *Neuropeptides, 13*(2), 139–146.

Rok-Bujko, P., Krzascik, P., Szyndler, J., Kostowski, W., & Stefanski, R. (2012). The influence of neonatal serotonin depletion on emotional and exploratory behaviours in rats. *Behavioural Brain Research, 226*(1), 87–95.

Ross, C. A., Trulson, M. E., & Jacobs, B. L. (1976). Depletion of brain serotonin following intraventricular 5,7-dihydroxytryptamine fails to disrupt sleep in the rat. *Brain Research, 114*(3), 517–523.

Rotman, A., Daly, J. W., & Creveling, C. R. (1976). Oxygen-dependent reaction of 6-hydroxydopamine, 5,6-dihydroxytryptamine, and related compounds with proteins in vitro: A model for cytotoxicity. *Molecular Pharmacology, 12*(6), 887–899.

Sachs, C., & Jonsson, G. (1975). 5,7-Dihydroxytryptamine induced changes in the postnatal development of central 5-hydroxytryptamine neurons. *Medical Biology, 53*(3), 156–164.

Saller, C. F., & Stricker, E. M. (1976). Hyperphagia and increased growth in rats after intraventricular injection of 5,7-dihydroxytryptamine. *Science, 192*(4237), 385–387.

Sawyer, D. T., Gibian, M. J., Morrison, M. M., & Seo, E. T. (1978). Chemical reactivity of superoxide ion. *Journal of the American Chemical Society, 100*(2), 627–628.

Schlossberger, H. G. (1978). Synthesis and chemical properties of some indole derivatives. *Annals of New York Academy of Sciences, 305*, 25–35.

Sewell, R. D., & Spencer, P. S. (1974). Proceedings: Modification of the antinociceptive activity of narcotic agonists and antagonists by intraventricular injection of biogenic amines in mice. *British Journal of Pharmacology, 51*(1), 140–141.

Singh, S., & Dryhurst, G. (1990). Further insights into the oxidation chemistry and biochemistry of the serotonergic neurotoxin 5,6-dihydroxytryptamine. *Journal of Medicinal Chemistry, 33*(11), 3035–3044.

Sinhababu, A. K., Ghosh, A. K., & Borchardt, R. T. (1985). Molecular mechanism of action of 5,6-dihydroxytryptamine. Synthesis and biological evaluation of 4-methyl-, 7-methyl-, and 4,7-dimethyl-5,6-dihydroxytryptamines. *Journal of Medicinal Chemistry, 28*(9), 1273–1279.

Smith, R. D., Cooper, B. R., & Breese, G. R. (1973). Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine: Evidence for involvement of brain dopamine. *Journal of Pharmacol and Experimental Therapeutics, 185*(3), 609–619.

Stewart, R. M., Growdon, J. H., Cancian, D., & Baldessarini, R. J. (1976). Myoclonus after 5-hydroxytryptophan in rats with lesions of indoleamine neurons in the central nervous system. *Neurology, 26*(7), 690–692.

Stewart, R. M., Campbell, A., Sperk, G., & Baldessarini, R. J. (1979). Receptor mechanisms in increased sensitivity to serotonin agonists after dihydroxytryptamine shown by electronic monitoring of muscle twitches in the rat. *Psychopharmacology, 60*(3), 281–289.

Tabatabaie, T., & Dryhurst, G. (1992). Chemical and enzyme-mediated oxidation of the serotonergic neurotoxin 5,7-dihydroxytryptamine: Mechanistic insights. *Journal of Medicinal Chemistry, 35*(12), 2261–2274.

Tabatabaie, T., & Dryhurst, G. (1998). Molecular mechanisms of action of 5,6- and 5,7-dihydroxytryptamine. In R. M. Kostrzewa (Ed.), *Highly selective neurotoxins: Basic and clinical applications* (pp. 269–291). Totowa: Humana Press.

Tabatabaie, T., Wrona, M. Z., & Dryhurst, G. (1990). Autoxidation of the serotonergic neurotoxin 5,7-dihydroxytryptamine. *Journal of Medicinal Chemistry, 33*(2), 667–672.

Tabatabaie, T., Goyal, R. N., Blank, C. L., & Dryhurst, G. (1993). Further insights into the molecular mechanisms of action of the serotonergic neurotoxin 5,7-dihydroxytryptamine. *Journal of Medicinal Chemistry, 36*(2), 229–236.

Tenen, S. S. (1968). Antagonism of the analgesic effect of morphine and other drugs by p-chlorophenylalanine, a serotonin depletor. *Psychopharmacologia, 12*(4), 278–285.

Thoenen, H., & Tranzer, J. P. (1968). Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. *Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie, 261*(3), 271–288.

Trindade-Filho EM, de Castro-Neto EF, de A Carvalho R, Lima E, Scorza FA, Amado D, et al. (2008). Serotonin depletion effects on the pilocarpine model of epilepsy. *Epilepsy Research, 82*(2–3), 194–199.

Vitalis, T., & Parnavelas, J. G. (2003). The role of serotonin in early cortical development. *Developmental Neuroscience, 25*(2–4), 245–256.

Volpe, B. T., Hendrix, C. S., Park, D. H., Towle, A. C., & Davis, H. P. (1992). Early post-natal administration of 5,7-dihydroxytryptamine destroys 5-HT neurons but does not affect spatial memory. *Brain Research, 589*(2), 262–267.

Waller, M. B., & Myers, R. D. (1974). Similar actions of intra-hypothalamic 5,6-DHT and 5-HT in evoking hyperthermia in the monkey. *Research Communications in Chemical Pathology and Pharmacology, 9*(3), 421–429.

Wirth, S., Lehmann, O., Bertrand, F., Lazarus, C., Jeltsch, H., & Cassel, J. C. (2000). Preserved olfactory short-term memory after combined cholinergic and serotonergic lesions using 192 IgG-saporin and 5,7-dihydroxytryptamine in rats. *NeuroReport, 11*(2), 347–350.

Wuttke, W., Bjorklund, A., Baumgarten, H. G., Lachenmayer, L., Fenske, M., & Klemm, H. P. (1977). De- and regeneration of brain serotonin neurons following 5,7-dihydroxytryptamine treatment: Effects on serum LH, FSH and prolactin levels in male rats. *Brain Research, 134*(2), 317–331.

Wuttke, W., Hancke, J. L., Höhn, K. G. & Baumgarten, H. G. (1978). Effect of intraventricular injection of 5,7-dihydroxytryptamine on serum gonadotropins and prolactin. *Annals of the New York Academy of Sciences,* 305: 423–436. doi: 10.1111/j.1749-6632.1978.tb31539.x.

Yan, W., Wilson, C. C., & Haring, J. H. (1997). Effects of neonatal serotonin depletion on the development of rat dentate granule cells. *Brain Research. Developmental Brain Research, 98*(2), 177–184.

2′-NH_2-MPTP: A Serotonin and Norepinephrine Neurotoxin

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Contents

Abstract

Altered serotonin and norepinephrine neurotransmission has been implicated in a variety of psychiatric and neurodegenerative diseases. Selective ablation of these systems represents one avenue for studying their roles in normal behavior, disease processes, and therapeutic interventions. 2′-NH_2-MPTP, an amine-substituted analog of the dopaminergic neurotoxin MPTP, can be used to degenerate serotonin and/or norepinephrine forebrain innervation to investigate

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pathophysiological and behavioral consequences in several strains of mice, rats, and nonhuman primates. 2′-NH_2-MPTP is converted to a toxic metabolite by monoamine oxidase type-A and type-B. This metabolite, a presumed pyridinium, is a substrate for serotonin and norepinephrine transporters. Acute effects immediately following 2′-NH_2-MPTP administration are characteristic of serotonin syndrome. Genetic, biochemical, and immunocytochemical evidence indicates that neurodegeneration caused by 2′-NH_2-MPTP is selective, long lasting, and occurs by an oxyradical mechanism.

Keywords

Mice • Monkeys • MPTP analog • Neurodegeneration • Norepinephrine • Rats • Serotonin

List of Abbreviations

2′-NH_2-MPP^+	1-Methyl-4-(2′-aminophenyl)-phenylpyridinium
2′-NH_2-MPTP	1-Methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine serotonin
5,6-DHT	5,6-Dihydroxytryptamine
5,7-DHT	5,7-Dihydroxytryptamine
BDNF	Brain-derived neurotrophic factor
CSF	Cerebrospinal fluid
DA	Dopamine
DAT	Dopamine transporter
DOPAC	3,4Dihydroxyphenylacetic acid
DSP-4 N	(2-Chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride
GFAP	Glial fibrillary acidic protein
HVA	Homovanillic acid
Ip	Intraperitoneal
Iv	Intravenous
LD_{50}	Median lethal dose
MAO-A	Monoamine oxidase type-A
MAO-B	Monoamine oxidase type-B
MHPG	3-Methoxy-4-hydroxyphenylglycol
MPP^+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NE	Norepinephrine
NET	Norepinephrine transporter
PET	Positron emission tomography
Ro 4–1284	2-Hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,5,6,7-hexahydrobenzo[a]chinolizine
SERT	Serotonin transporter
VMAT2	Vesicular monoamine transporter type-2

1 Introduction

Changes in brain monoamine neurotransmitters, i.e., serotonin, norepinephrine, and dopamine, are involved in many neuropsychiatric illnesses and their treatment, including major depressive disorder, anxiety disorders, and schizophrenia (Nestler et al. 2008; López-Muñoz and Álamo 2012). Moreover, degeneration of monoamine neurotransmitter systems is linked with normal aging and late-onset neurological diseases such as Parkinson's disease and Alzheimer's disease. However, studying brain disorders is inherently complicated by difficulties associated with the long time frames over which pathologies develop, the relative inaccessibility of investigating neurotransmission in humans, modeling and relating complex pathophysiology to specific endophenotypes, and a poor understanding of underlying etiologies. As such, chemical neurotoxins that cause controlled and selective damage to monoamine neurotransmitter systems in animals provide avenues to investigate aspects of specific neurotransmitter loss and neurodegeneration as they pertain to brain disorders.

2 Discovery of 2′-NH_2-MPTP

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces degeneration of dopamine (DA) nigrostriatal neurons, which is the hallmark of idiopathic Parkinson's disease. MPTP came to the forefront in the early 1980s when a group of northern California drug users developed irreversible parkinsonian-like symptoms (Langston and Palfreman 1995). MPTP, a by-product of the illicit synthesis of a derivative of the opioid meperidine, was discovered as the causative agent.

Many analogs of MPTP have been synthesized for structure-activity studies (Bradbury et al. 1985; Youngster et al. 1987, 1989; Heikkila et al. 1988; Booth et al. 1989; Johnson et al. 1989; Maret et al. 1990; Saporito et al. 1992). MPTP-related compounds with a substituent at the 2′ position, i.e., 2′-R-MPTP, R = CH_3, CH_2CH_3, Cl, F, and CF_3, have been shown to be potent dopamine neurotoxins. Amine-functionalized MPTP analogs were of interest because of the ability to covalently cross-link primary amines to brain tissue via aldehyde fixation. When coupled with immunohistochemical detection, amine-substituted MPTP compounds could be useful in mapping distribution at the ultrastructural level (Johannessen et al. 1987). However, adding an amine substituent to various positions on the phenyl ring of MPTP led to analogs that were relatively weak dopamine neurotoxins in mice.

Interestingly, the 2′-amino-substituted MPTP derivative, 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH_2-MPTP; Fig. 1a), produced reductions in striatal serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), while having no effect on dopamine in striatum (Johannessen et al. 1987). Because the goals of Johannessen et al. were to use amino-substituted analogs of MPTP to study toxicity related to Parkinsonism, no further work with

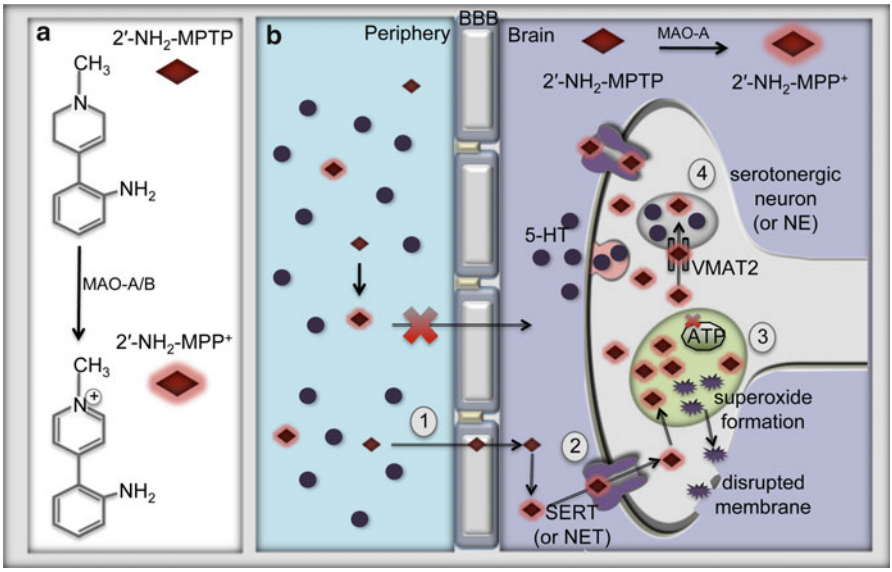

Fig. 1 *Mechanisms of serotonin and norepinephrine neurotoxicity after exposure to 2′-NH$_2$-MPTP*. (**a**) Shown is the chemical structure of 2′-NH$_2$-MPTP, a structural analog of the dopamine neurotoxin MPTP. Toxicity due to 2′-NH$_2$-MPTP is inhibited by pretreating mice with monoamine oxidase type-A (MAO-A) and/or MAO-B inhibitors. Similar to MPTP, which is converted to MPP$^+$ by MAO-B, 2′-NH$_2$-MPTP is hypothesized to be converted to a toxic pyridinium, 2′-NH$_2$-MPP$^+$. (**b**) (1) While 2′-NH$_2$-MPTP appears to cross the blood–brain barrier, 2′-NH$_2$-MPP$^+$ is not expected to do so based on findings with MPP$^+$. 2′-NH$_2$-MPTP toxicity is prevented by inhibiting plasma membrane serotonin transporters (SERT) or norepinephrine transporters (NET), which is hypothesized to block uptake of 2′-NH$_2$-MPP$^+$ into serotonin and norepinephrine (NE) neurons, respectively. (3) Superoxide radicals are thought to be formed at mitochondria leading to membrane instability, DNA damage, and ultimately, axonal degeneration and to a lesser extent, cell death. Increasing superoxide dismutase activity prevents 2′-NH$_2$-MPTP-induced toxicity. (4) Unlike MPP$^+$, which is readily accumulated in synaptic vesicles via vesicular monoamine transporters type-2 (VMAT2), 2′-NH$_2$-MPP$^+$ appears to be a weaker substrate for VMAT2 and only low dose 2′-NH$_2$-MPTP toxicity is potentiated by pharmacologic or genetic inhibition of VMAT2

2′-NH$_2$-MPTP appeared warranted. However, the effects of 2′-NH$_2$-MPTP on serotonin did not escape notice, and several years later, additional research established the unique neurotoxicity of 2′-NH$_2$-MPTP (Andrews and Murphy 1993a, b, c).

3 2′-NH$_2$-MPTP Is a Serotonin and Norepinephrine Neurotoxin in Mice

Initial investigation confirmed striatal serotonin loss in response to 2′-NH$_2$-MPTP administration (Andrews and Murphy 1993a). Furthermore, 2′-NH$_2$-MPTP was

shown to decrease norepinephrine (NE), in addition to serotonin in the forebrain, in contrast to the dopamine-selective toxicity produced by MPTP and its most potent analog, 2′-CH_3-MPTP. When administered to C57Bl/6 mice, 2′-NH_2-MPTP reduced serotonin, 5-HIAA, and norepinephrine levels by 70 % in frontal cortex and 60 % in hippocampus, with depletions occurring to a lesser extent in striatum and brain stem. Dopamine and its major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were relatively unaffected 1 week after 2′-NH_2-MPTP (Andrews and Murphy 1993a). Notably, no other MPTP analogs, including all amino-substituted and 2′-substituted analogs, have been reported to produce similar serotonin and norepinephrine neurotoxicity in mice.

4 Acute Effects of 2′-NH_2-MPTP

The median lethal dose (LD_{50}) is the dose required to kill half of a treated population and is an indicator of the acute lethality of substances. The LD_{50} for 2′-NH_2-MPTP in C57Bl/6 mice is 20–40 mg/kg, ip (Andrews and Murphy 1993a). It is likely to be similar in Swiss Webster mice (Andrews and Murphy 1993b) and somewhat lower in CD-1 mice (Andrews et al. 1996). When treated with high doses of 2′-NH_2-MPTP, i.e., $\geq$40 mg/kg, mice had tonic-clonic seizures and died shortly afterwards (Andrews and Murphy 1993a). Tonic-clonic seizures followed by death in some cases also occurred in rats at 20 mg/kg 2′-NH_2-MPTP (Unger et al. 2002). Acute and massive release of monoamine neurotransmitters has been shown to cause precipitous elevations in blood pressure and heart rate (Fuller et al. 1988). Death subsequent to high-dose 2′-NH_2-MPTP administration is likely to be due to cardiovascular consequences and not neurodegeneration.

Similar to MPTP, the dose regimen most commonly used for 2′-NH_2-MPTP in rodents consists of four intraperitoneal (ip) injections (15–20 mg/kg) at 2-h intervals. Behavioral changes in mice and rats immediately following each injection include piloerection, salivation, Straub (straight) tail, flattened body posture, intermittent walking, forepaw treading (piano-playing), and wet-dog shakes. Behavioral responses generally subside prior to each subsequent injection of 2′-NH_2-MPTP and become less pronounced after repeated injections. Acute behavioral changes in rodents are characteristic of serotonin syndrome, which results from large increases in central serotonin release (Zhang et al. 2009). Serotonin syndrome arising from single drug overdose or multiple serotonergic agents acting via different but converging mechanisms is potentially life threatening in humans (Boyer and Shannon 2005).

Serotonin is also known to be involved in thermoregulation (Murphy et al. 1991). Acutely, 2′-NH_2-MPTP causes a decrease in core body temperature in mice (Numis et al. 2004). This hypothermic effect is similar to that seen after MPTP (Freyaldenhoven et al. 1995; Moy et al. 1998) but opposite to substituted amphetamines, which cause hyperthermia (Miller and O'Callaghan 1995). It is not known why 2′-NH_2-MPTP and substituted amphetamines, both of which are thought to cause acute serotonin release, have differing effects on core body temperature.

Loss of serotonin and norepinephrine in frontal cortex and hippocampus has been detected as early as 30 min after the last injection of 2′-NH_2-MPTP in mice (Luellen et al. 2003). In contrast, decreases in 5-HIAA developed slowly over the first week after treatment. This probably reflects high levels of metabolism of serotonin from degenerating axons. In striatum, hypothalamus, and brain stem, where long-term depletions are more modest, serotonin and norepinephrine levels showed substantial recovery over the 72 h period following treatment. Transient decreases (30–40 %) in striatal dopamine were also reported during the initial 2 h after 2′-NH_2-MPTP, but dopamine levels recovered by 24 h posttreatment. Thus, forebrain serotonergic and noradrenergic axons that project far from their origins in the brain stem show the most pronounced early evidence of toxicity. In addition to acute behavioral, physiological, and neurochemical changes, mice show a 1–2 g decrease in body weight during the first 48 h after 2′-NH_2-MPTP. Throughout the first week after exposure, mice exhibit noise sensitivity and agitation.

5 Mechanism of Action of 2′-NH_2-MPTP

While the mechanisms underlying 2′-NH_2-MPTP-induced neurotoxicity are not fully understood, they have many similarities to those of MPTP. Both are small, lipophilic molecules that cross the blood–brain barrier (Fig. 1b). MPTP is a prodrug that requires oxidation by monoamine oxidase type-B (MAO-B) to exert toxicity as 1-methyl-4-phenylpyridinium (MPP^+) (Markey et al. 1984; Heikkila et al. 1985). 2′-NH_2-MPTP is oxidized by both MAO-B and monoamine oxidase type-A (MAO-A) (Fig. 1b) (Andrews and Murphy 1993b; Andrews 2000). Therefore, like MPTP, 2′-NH_2-MPTP probably exerts its toxic effects through the oxidized pyridinium, 2′-amino-1-methyl-4-phenylpyridinium (2′-NH_2-MPP^+) (Fig. 1a). Preliminary findings show monoamine oxidase-dependent production of 2′-NH_2-MPP^+ in brain tissue incubated with 2′-NH_2-MPTP. 2′-NH_2-MPP^+ was identified by tandem mass spectrometry and comparison with authentic 2′-NH_2-MPP^+ via high-performance liquid chromatography.

The MAO-catalyzed metabolite of 2′-NH_2-MPTP gains access to serotonergic and noradrenergic neurons via serotonin transporters (SERT) and norepinephrine transporters (NET), respectively (Andrews and Murphy 1993b, c; Numis et al. 2004) (Fig. 1b), just as MPTP targets dopaminergic neurons via uptake of MPP^+ by dopamine transporters (DAT) (Melamed et al. 1985; Gainetdinov et al. 1997). Studies have shown that pretreating mice with the serotonin-selective reuptake inhibiting antidepressants fluoxetine or paroxetine prevent 2′-NH_2-MPTP-induced serotonergic neurotoxicity (Andrews and Murphy 1993b, c). Pretreatment with the norepinephrine-selective reuptake inhibitor desipramine prevents norepinephrine depletion. 2′-NH_2-MPTP neurotoxicity can also be limited by genetic inactivation of SERT to produce selective loss of norepinephrine (Numis et al. 2004).

Administration of 2′-NH_2-MPTP to mice genetically deficient in NET (Xu et al. 2000) has not been investigated.

Similar to MPTP, 2′-NH_2-MPTP appears to cause neurotoxicity via generation of oxygen free radicals. MPP^+ has been shown to block the mitochondrial respiratory chain by inhibiting complex I (Cleeter et al. 1992) with two major consequences. First, conversion of NAD^+ to NADH is decreased, which interferes with mitochondrial proton gradients and subsequent production of ATP, depleting neurons of vital chemical energy. Second, metabolic disruption is purported to cause oxidative stress associated with an increase in oxyradicals and damage to DNA, proteins, and the cytoskeleton (Halliwell 1992). Mice genetically engineered to overexpress Cu-Zn superoxide dismutase, the soluble enzyme isoform that converts highly toxic superoxide radicals to hydrogen peroxide, were protected from 2′-NH_2-MPTP toxicity, implicating superoxide mechanistically (Andrews et al. 1996). Together, findings on 2′-NH_2-MPTP and MPTP suggest that inhibition of mitochondrial complex I by pyridiniums to generate oxyradicals is a primary mechanism of toxicity, not release and auto-oxidation of dopamine, which is specific to MPTP.

In addition to differential transporter specificity, there is another key difference between the molecular mechanisms of 2′-NH_2-MPTP and MPTP. Previous studies have shown that MPP^+ is a substrate for the vesicular monoamine transporter type-2 (VMAT2), and sequestration of MPP^+ into synaptic vesicles reduces neurotoxicity (Gainetdinov et al. 1998; Staal and Sonsalla 2000b). Thus, MPP^+ mimics dopamine via high-affinity transport by DAT and VMAT2. In general, vesicular sequestration of exogenous toxins by VMAT2 has been implicated as one of several lines of neuronal defense (Miller et al. 1999). However, 2′-NH_2-MPP^+ appears to be a poor substrate for VMAT2 (Numis et al. 2004). Mice with partial genetic reductions in VMAT2 expression, as well as mice treated with the VMAT2 inhibitor, Ro 4–1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydrobenzo[alpha]chinolizin hydrochloride), showed only slight potentiation of toxicity when treated with low doses of 2′-NH_2-MPTP.

With regard to sex, female mice appear to be less susceptible to the neurotoxic effects of 2′-NH_2-MPTP. Compared to male mice with 60–80 % loss of serotonin in frontal cortex and hippocampus 6 weeks after treatment, female CD-1 mice only showed 30–40 % serotonin depletions in the same brain regions (Fig. 2). Studies have shown that female mice are resistant to dopamine depletion after MPTP treatment (Miller et al. 1998; Dluzen 2000; Morissette et al. 2007). Castrated male mice pretreated with estrogen are also protected from MPTP toxicity (Dluzen et al. 1996). Estrogen may exert neuroprotective effects through direct antioxidant activity (Subbiah et al. 1993). However, these effects are only observed at low physiological concentrations of estrogen (Ramirez et al. 2003), implicating other mechanisms including gene regulation of estrogen receptors (Kenchappa et al. 2004) or altered DAT affinity for MPP^+ (Ookubo et al. 2009). Similar mechanisms may underlie sex-related differences in 2′-NH_2-MPTP neurotoxicity.

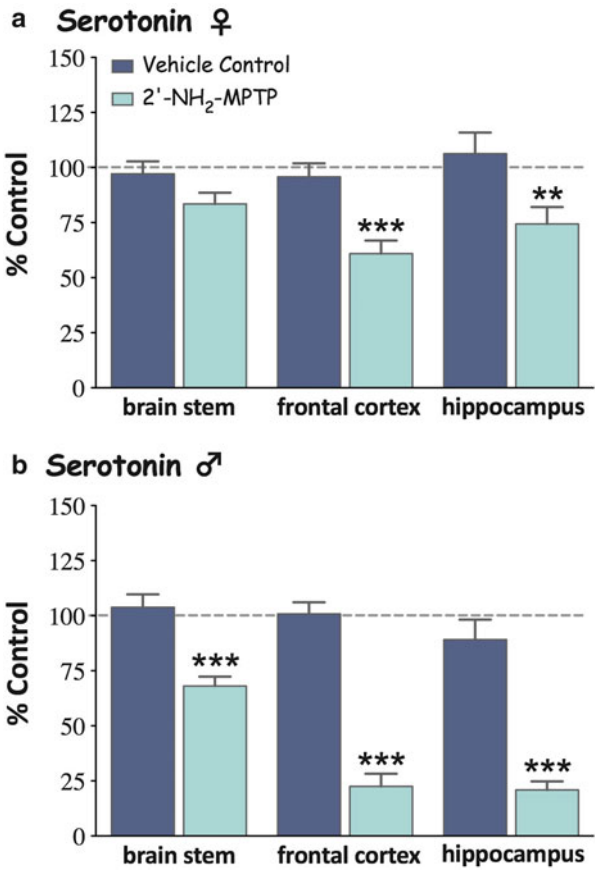

Fig. 2 *Changes in serotonin levels following 2′-NH_2-MPTP treatment*. Brain tissue neurotransmitter levels were analyzed by high-performance liquid chromatography with electrochemical detection. Data are percents of non-injected control levels in vehicle- and 2′-NH_2-MPTP-treated mice. Serotonin levels were analyzed in (**a**) female mice and (**b**) male mice. Statistical significances: $^{**}P < 0.01$ and $^{***}P < 0.001$ 2′-NH_2-MPTP-treated versus uninjected mice. $N = 10$ for control groups of both sexes; $N = 9$ for 2′-NH_2-MPTP-treated group of both sexes

Thus, 2′-NH_2-MPTP produces toxicity by cellular mechanisms with many similarities to those of MPTP, although different neuronal populations are targeted and the neuroprotective role of VMAT2 varies. Recently, α-synuclein was found to be neuroprotective in the context of MPTP but not 2′-NH_2-MPTP toxicity suggesting that α-synuclein plays a role in dopaminergic toxicity but not serotonin and norepinephrine toxicity (Thomas et al. 2011). 2′-NH_2-MPTP differs from other serotonin neurotoxins, such as 5,6- and 5,7-dihydroxytryptamine (5,6- and 5,7-DHT), which must be administered directly into the brain. 2′-NH_2-MPTP is also mechanistically dissimilar from the norepinephrine neurotoxin DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride) (Hallman and Jonsson 1984; Kostrzewa 2009) and the substituted amphetamines, e.g., 3,4-methylenedioxylmethamphetamine, fenfluramine, and *p*-chloroamphetamine (Baumgarten and Lachenmayer 2004). The toxicity profiles of substituted amphetamines differ from 2′-NH_2-MPTP, particularly in mice, where these amphetamines also affect dopamine levels (O'Callaghan and Miller 1994) (Jayanthi et al. 1999), or in the case of fenfluramine, where long-term neurotoxic effects are questionable.

6 Evidence for Neurodegeneration

Multiple lines of investigation suggest that reductions in forebrain serotonin and norepinephrine are indicative of 2′-NH_2-MPTP-induced neurodegeneration. This is compatible with findings of MPTP-induced dopaminergic neurodegeneration (Markey et al. 1986). Reductions in cortical and hippocampal serotonin and norepinephrine persist up to six months after treatment of mice with 2′-NH_2-MPTP (Luellen et al. 2003). Furthermore, a number of indicators of neurodegeneration are present after 2′-NH_2-MPTP administration.

When some types of neurons, including serotonin neurons, are damaged, i.e., during aging, disease, or injury, they increase production of brain-derived neurotrophic factor (BDNF), a protein involved in synaptic plasticity, neurogenesis, and neuronal survival (Mattson et al. 2004). Along these lines, 2′-NH_2-MPTP-treated mice show acute increases in hippocampal BDNF (Szapacs et al. 2004; Luellen et al. 2006). Further evidence for degenerating neurons is hypertrophy of astrocytes in a process known as astrogliosis. Glial fibrillary acidic protein (GFAP) is an intermediate filament protein whose expression is universally upregulated in astrocytes in response to brain injury (O'Callaghan 1991). In addition to BDNF, 2′-NH_2-MPTP administration to mice causes transient elevations in GFAP in frontal cortex and hippocampus, brain regions showing long-term serotonergic and noradrenergic depletion indicative of neuronal injury (Luellen et al. 2003). Interestingly, administration of 2′-NH_2-MPTP to mice 2 weeks prior to treatment with the dopaminergic neurotoxin 2′-CH_3-MPTP prevents striatal increases in GFAP in response to 2′-CH_3-MPTP suggesting that serotonin is a modulator of astrogliosis (Luellen et al. 2003).

Immunocytochemistry has also been used to study neurodegeneration. A few days after 2′-NH_2-MPTP treatment, serotonin-immunopositive axons exhibited a tortuous, beaded, and punctate appearance indicating ongoing neurodegeneration (Luellen et al. 2006). Three weeks later, significant serotonergic axonal losses were detected. The hippocampus and frontal cortex were the brain regions most severely affected, strongly correlating with reductions in neurotransmitter levels (Fig. 3). Silver staining has also been used for over a century as a histopathological indicator of neuronal death (Uchihara 2007). Immunocytochemical evidence of argyrophilia, the ability of degenerating neurons to take up silver, occurs in serotonin cell bodies in the dorsal raphe further indicating neurodegeneration after 2′-NH_2-MPTP (Luellen et al. 2006). From these converging lines of evidence, it can be concluded that 2′-NH_2-MPTP is a regionally selective, potent, and long-lasting serotonergic and noradrenergic neurotoxin.

7 2′-NH_2-MPTP Toxicity in Rats and Nonhuman Primates

Notwithstanding the physiological and neurochemical similarities between 2′-NH_2-MPTP and MPTP, different patterns of species sensitivity are observed. Notably, key differences between these two compounds occur regarding their effects in rats. Rats are virtually resistant to the effects of MPTP (Chiueh et al. 1984), a phenomenon that

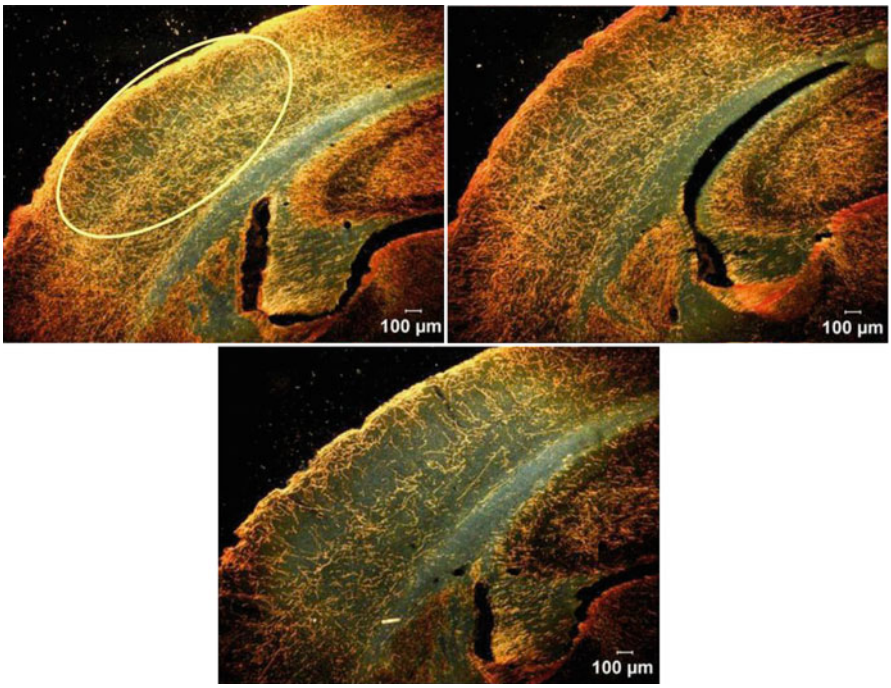

Fig. 3 *Immunocytochemistry of serotonin axons following acute and chronic administration of 2′-NH_2-MPTP*. Representative dark field photomicrographs of sections of primary somatosensory cortex from male CD-1 mice are shown. Serotonin axons are gold threadlike structures. Mice were treated with 4 × 20 mg/kg 2′-NH_2-MPTP at 2 h intervals. Compared to control mice (*upper* left panel), mice sacrificed 21 days (*lower* panel) but not 3 days (*upper* right panel) post-2′-NH_2-MPTP treatment showed significant reductions in serotonergic axon innervation in the barrel field region (*yellow* oval) (Reprinted with permission from (Luellen et al. 2006))

contributed to the difficulties of identifying MPTP as the cause of Parkinsonism in human drug users (Davis et al. 1979; Lewin 1984). However, systemic treatment of Sprague Dawley rats with 2′-NH_2-MPTP (4 × 15 mg/kg, ip, every 2 h) was highly toxic, depleting forebrain serotonin and norepinephrine levels without affecting striatal dopamine (Unger et al. 2002). Conversely, 2′-CH_3-MPTP-treated rats showed no toxic effects.

In terms of molecular mechanisms of MPTP and its analogs, there appear to be several ways in which species can differ. Some animals, rats in particular, have high MAO-B activity at the blood–brain barrier, where effective conversion of MPTP to MPP^+ can take place (Kalaria et al. 1987). Positively charged pyridinium species produced at the blood–brain barrier are then prevented from entering the brain (Fig. 1b). Rats may therefore be partly protected from MPTP toxicity by effective MPTP metabolism at the blood–brain barrier. Furthermore, rats have a greater capacity to sequester MPP^+ compared to mice (Staal and Sonsalla 2000a). However, 2′-NH_2-MPP^+ appears to be a poor substrate for VMAT2 in

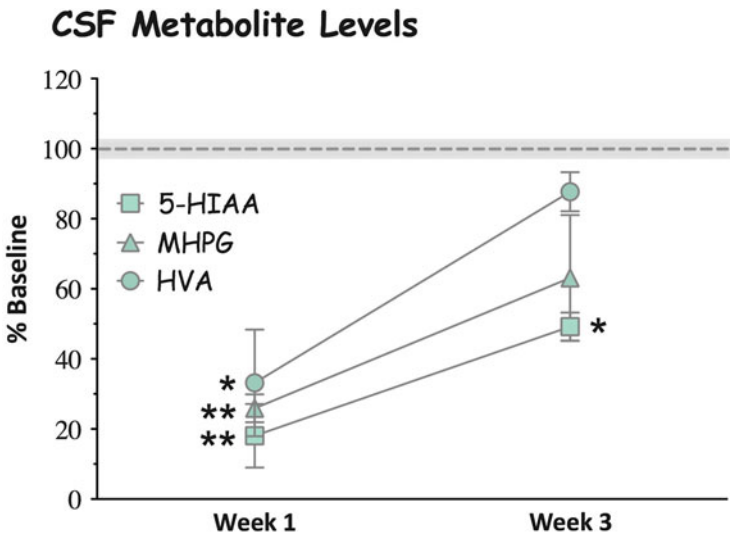

Fig. 4 *Cerebrospinal fluid metabolite levels after administration of 2′-NH_2-MPTP in African green monkeys*. Levels of 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylglycol (MHPG), and homovanillic acid (HVA) were measured in cerebrospinal fluid (CSF) 1 and 3 weeks after administration of 2′-NH_2-MPTP to African *green* monkeys (20 mg/kg total dose). These are metabolites of serotonin, norepinephrine, and dopamine, respectively. All metabolites were decreased 1 week after treatment with 2′-NH_2-MPTP and remained reduced for 5-HIAA 3 weeks after treatment. The *gray-shaded* region represents ± the standard error of the mean (SEM) for all metabolites in saline-treated monkeys. Data are means ± SEMs. *$P < 0.05$ and **$P < 0.01$ versus metabolite levels in saline-treated animals

mice (Numis et al. 2004) and rats (Unger et al. 2002). Lower blood–brain barrier metabolism of 2′-NH_2-MPTP and synaptic vesicular uptake of 2′-NH_2-MPP^+ are hypothesized to contribute to the sensitivity of rats to 2′-NH_2-MPTP toxicity.

In addition to rodents, 2-NH_2-MPTP has been administered to two nonhuman-primate species. In one study, six adult African green monkeys (*C. aethiops*) were given intravenous (iv) 2′-NH_2-MPTP with the objective of investigating serotonin and norepinephrine neurotoxicity in a species more closely related to humans. Initial doses of 2′-NH_2-MPTP (7–10 mg/kg) to one monkey produced behavior indicative of hallucinations followed by nonlethal tonic-clonic seizure at the highest dose. A subsequent dose of 5 mg/kg to the same animal was without overt behavioral effects. Two additional monkeys received 4 × 5 mg/kg 2′-NH_2-MPTP. Bolus iv doses were administered at a 6 h interval twice per day for two consecutive days. This dosing schedule of 2′-NH_2-MPTP is similar to other serotonin neurotoxin regimens in nonhuman primates (Ricaurte et al. 1988).

Cerebrospinal fluid (CSF) was analyzed after 2′-NH_2-MPTP to assess changes in neurotransmitter metabolite levels (Fig. 4). The serotonin and norepinephrine metabolites 5-HIAA and 3-methoxy-4-hydroxyphenylglycol (MHPG), respectively, were significantly reduced 1 week after 2′-NH_2-MPTP treatment compared to levels in

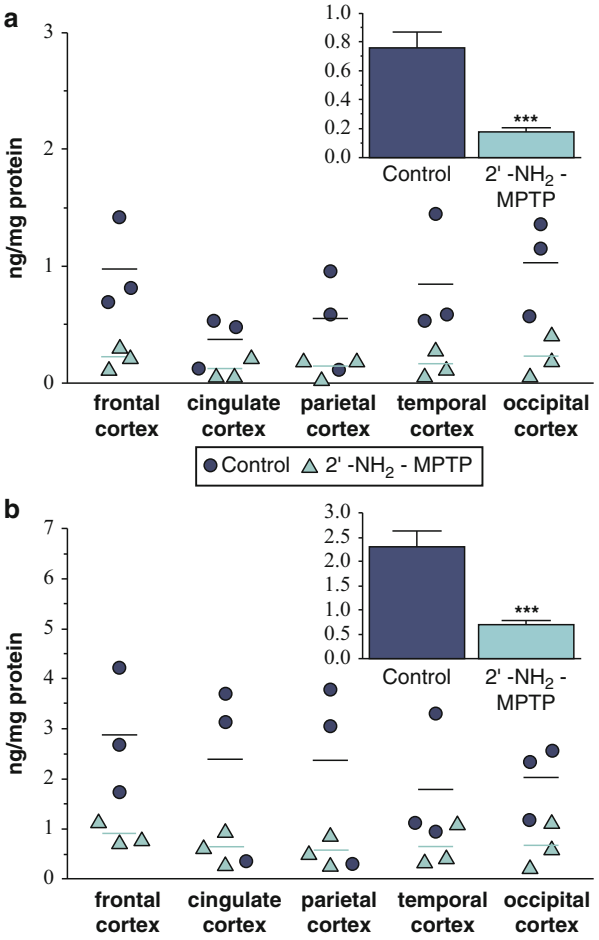

Fig. 5 *Neurotransmitter levels in cortical regions 3 weeks after 2′-NH_2-MPTP treatment in African green monkeys*. (**a**) Serotonin and (**b**) norepinephrine levels are significantly reduced across cortical regions. Data points represent neurotransmitter levels in individual animals. *Horizontal lines* depict means for 2′-NH_2-MPTP-treated versus saline-treated monkeys. Data from different subregions were combined and compared between treatment groups, as depicted in the insets ($[t_{(28)} = 5.0, P < 0.0001]$ and $[t_{(28)} = 4.7, P < 0.0001]$ for serotonin and norepinephrine, respectively)

saline-treated monkeys. Depressed CSF 5-HIAA levels continued to be evident at 3 weeks posttreatment with 2′-NH_2-MPTP. The dopamine metabolite HVA was also moderately reduced 1 week after drug treatment. Changes in CSF metabolite levels could indicate loss of brain serotonergic and noradrenergic (and possibly dopaminergic) axons/neurons. They might also reflect inhibition of MAO activity. Studies have shown that when MPTP is oxidized to form MPP^+, MAO-B is irreversibly inactivated (Singer et al. 1986). "Suicide" inhibition of MAO is expected to reduce monoamine neurotransmitter metabolites. Notably, MAO inhibition by 2′-NH_2-MPTP has not yet been investigated. In addition to CSF metabolites, serotonin and norepinephrine levels across cortical regions were globally decreased in African green monkeys treated 3 weeks earlier with 2′-NH_2-MPTP (Fig. 5) suggesting that like MPTP, nonhuman primates are susceptible to 2′-NH_2-MPTP neurotoxicity. In African green monkeys, brain dopamine levels were modestly affected in the caudate (−24 %) but not the putamen (−9 %).

2′-NH_2-MPTP has also been administered to four adult baboons (*P. anubis*). Here, the neurotoxin was infused unilaterally into the carotid artery. This route of administration has been used with MPTP resulting in dopaminergic neurodegeneration largely confined to the ipsilateral side of the brain (Bankiewicz et al. 1986; Guttman et al. 1990). Intracarotid administration of MPTP produces a hemiparkinsonian nonhuman primate model where animals can still feed and care for themselves (Ding et al. 2008). Additionally, one hemisphere is relatively unaffected and serves as an intra-animal control in morphological studies (Przedborski et al. 1991). Intracarotid administration of 2′-NH_2-MPTP to baboons was carried out to attempt to produce a model of unilateral loss of forebrain serotonergic and noradrenergic axons that could be used to evaluate positron emission tomography (PET) ligands. Like unilateral MPTP, each 2′-NH_2-MPTP-treated animal would serve as its own control in imaging studies.

A 10 mg/kg infusion of 2′-NH_2-MPTP to a single baboon resulted in nonlethal seizure. Doses of 2′-NH_2-MPTP in the remaining baboons were lowered to 6–7 mg/kg. Bilateral biopsies of cortex suggested unilateral reductions in serotonin and norepinephrine. However, the data were inconclusive due to large variations in cortical neurotransmitter levels across samples. Postmortem brain tissue analysis showed evidence for loss of dopamine in the caudate and putamen of 2′-NH_2-MPTP-treated baboons. One baboon treated unilaterally with 2′-NH_2-MPTP underwent PET scanning using [^{11}C]-McN5652 to label SERT in extant neurons (Parsey et al. 2000). SERT density was unilaterally reduced across all brain regions except in the cerebellum. While some studies find SERT (albeit at extremely low levels), and 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{3A} receptors in the cerebellum, direct evidence that 5-HT neurons innervate this region remains inconclusive (Kish et al. 2005; Arpin-Bott et al. 2006; Xie et al. 2006; Yew et al. 2009). In fact, many investigators use the cerebellum to define nonspecific binding when analyzing SERT levels.

Together, findings in nonhuman primates suggest that similar to rats and mice, 2′-NH_2-MPTP may be neurotoxic to serotonin and norepinephrine neurons in primate species. These data also suggest minor dopaminergic neurotoxicity of 2′-NH_2-MPTP in nonhuman primates. The latter might be due to the routes of administration or high doses of 2′-NH_2-MPTP used in monkeys. Small groups of animals and inherent variability across individuals also contributed to the incomplete yet highly suggestive nature of these first studies on 2′-NH_2-MPTP in primates.

8 Long-Term Behavioral Consequences of 2′-NH_2-MPTP

Compared to research on the neurotoxic mechanisms of 2′-NH_2-MPTP in different animal models, relatively little work has been done to understand the behavioral consequences of serotonergic and/or noradrenergic forebrain axonal loss. The effects of 2′-NH_2-MPTP neurotoxicity were recently studied on locomotor activity (Unger et al. 2006), anxiety-related behavior (Walf and Frye 2007), and low-stress spatial learning and memory (Bressler et al. 2010). Here, CD-1 mice were pretreated with desipramine to produce serotonin-selective

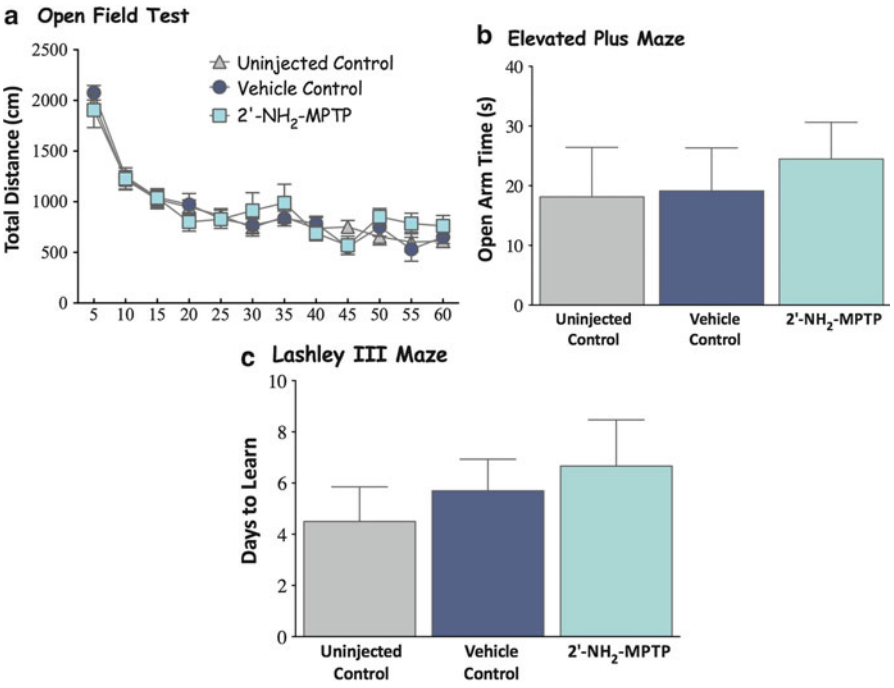

Fig. 6 *Effects of 2′-NH_2-MPTP treatment on behavior in male mice*. (**a**) Locomotor behavior was assessed in open field activity monitors (40 cm × 40 cm × 30 cm), where mice were able to explore freely for 1 h. No changes in total distance traveled were associated with 2′-NH_2-MPTP treatment. (**b**) The elevated plus maze was used to investigate changes in anxiety-related behaviors as indicated by time spent in the aversive open arms of the maze. No changes in anxiety-related behavior were observed after 2′-NH_2-MPTP. (**c**) Low-stress route learning was investigated via the Lashley III maze. A mouse was considered as having learned the maze (reached criterion) after completing two consecutive nightly trials with only 0 errors or 1 error. Mice were tested once per night for seven consecutive nights. Mice treated with 2′-NH_2-MPTP ($N = 9$) took a similar number of days to learn the maze compared with unhandled ($N = 10$) and vehicle controls ($N = 10$) indicating intact learning capabilities

2′-NH_2-MPTP-induced toxicity. It was hypothesized that serotonergic axonal degeneration would be associated with impaired cognition and increased anxiety-related behavior, similar to changes in brain function observed in humans with neurodegenerative disorders having serotonin-system involvement. Nonetheless, 60–70 % reductions in serotonin in frontal cortex and hippocampus (Fig. 2) were not associated with changes in locomotor, anxiety-related, and learning behavior in the open field, elevated plus maze, or Lashley III maze, respectively (Fig. 6).

Studies have shown that disruption of the serotonin system in response to another serotonin neurotoxin, 5,7-DHT, led to changes in learning and memory in rodents and nonhuman primates (Lieben et al. 2006; Clarke et al. 2007), although not all studies have been in agreement (Adams et al. 2008). Anxiety-like behaviors in 5,7-DHT-treated animals were not altered (Lieben et al. 2006). Here, adult mice ages 12–16 weeks were studied. Thus, critical early or late windows of vulnerability could

have been missed when degeneration of forebrain serotonin axons influences behavior. Alternately, behavioral changes might occur in 2′-NH_2-MPTP-treated mice, but the tests focused on were not directed at putative behavioral changes, e.g., depression-related behavior. Further research will also need to be carried out with 2′-NH_2-MPTP in genetically engineered disease-specific models to understand the ramifications of serotonin and norepinephrine degeneration on behavior.

9 Conclusion

A number of studies show that 2′-NH_2-MPTP induces significant serotonergic and noradrenergic neurotoxicity in mice, rats, and probably, nonhuman primates. The mechanisms of 2′-NH_2-MPTP neurotoxicity are similar to those of MPTP, yet significant differences occur with regard to the isoforms of monoamine oxidase that produce proximal pyridinium neurotoxins; 2′-NH_2-MPTP is a substrate for MAO-A and MAO-B while MPTP is metabolized almost exclusively by MAO-B. Moreover, rats are relatively insensitive to the dopamine-depleting effects of MPTP (and 2′-CH_3-MPTP). Conversely, 2′-NH_2-MPTP produces serotonin and norepinephrine toxicity in rats at doses comparable to those used in mice. The latter is likely to be associated, at least in part, to differential affinities of MPP^+ versus 2′-NH_2-MPP^+ for VMAT2 in rats and mice. The most important differences between 2′-NH_2-MPTP and MPTP arise from their affinities for plasma monoamine uptake transporters. This mechanistic aspect bestows relative dopamine-selective neurotoxicity to MPTP and 2′-CH_3-MPTP. By contrast, the MAO-catalyzed oxidative metabolite of 2′-NH_2-MPTP is a substrate for serotonin and norepinephrine transporters, conferring 2′-NH_2-MPTP with the ability to produce serotonergic and noradrenergic neurotoxicity largely in the absence of dopaminergic neurotoxicity. Further research in nonhuman primates and with regard to the behavioral consequences of serotonergic and noradrenergic neurodegeneration seems to be warranted. In turn, 2′-NH_2-MPTP animal models could be used to investigate potential treatments for serotonin-related pathologies. Moreover, a clearer understanding of links between serotonin deficiency and behavior may ultimately help to unravel the complex pathophysiology of depression and other neuropsychiatric and neurodegenerative disorders.

References

Adams, W., Kusljic, S., & van den Buuse, M. (2008). Serotonin depletion in the dorsal and ventral hippocampus: Effects on locomotor hyperactivity, prepulse inhibition and learning and memory. *Neuropharmacology, 55*, 1048–1055.

Andrews, A. M. (2000). The use of 2′-NH_2-MPTP as a tool to probe central serotonin and norepinephrine neurotransmitter systems. *Neurotransmissions, 16*, 18–21.

Andrews, A. M., & Murphy, D. L. (1993a). Sustained depletion of cortical and hippocampal serotonin and norepinephrine but not striatal dopamine by 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH_2-MPTP): A comparative study with 2′-CH_3-MPTP and MPTP. *Journal of Neurochemistry, 60*, 1167–1170.

Andrews, A. M., & Murphy, D. L. (1993b). 2′-NH_2-MPTP in Swiss Webster mice: Evidence for long-term (6-month) depletions in cortical and hippocampal serotonin and norepinephrine, differential protection by selective uptake inhibitors or clorgyline and functional changes in central serotonin neurotransmission. *The Journal of Pharmacology and Experimental Therapeutics, 267*, 1432–1439.

Andrews, A. M., & Murphy, D. L. (1993c). Fluoxetine and desipramine selectively attenuate 2′-NH_2-MPTP-induced depletions in serotonin and norepinephrine. *European Journal of Pharmacology, 250*, 215–221.

Andrews, A. M., Ladenheim, B., Epstein, C. J., Cadet, J. L., & Murphy, D. L. (1996). Transgenic mice with high levels of superoxide dismutase activity are protected from the neurotoxic effects of 2′-NH2-MPTP on serotonergic and noradrenergic nerve terminals. *Molecular Pharmacology, 50*, 1511–1519.

Arpin-Bott, M. P., Dietrich, J. B., Dirrig-Grosch, S., Aunis, D., & Zwiller, J. (2006). Induction by cocaine of the serotonergic 5-HT3 receptor in rat cerebellum. *Annals of the New York Academy of Sciences, 1074*, 382–389.

Bankiewicz, K. S., Oldfield, E. H., Chiueh, C. C., Doppman, J. L., Jacobowitz, D. M., & Kopin, I. J. (1986). Hemiparkinsonism in monkeys after unilateral internal carotid artery infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Life Sciences, 39*, 7–16.

Baumgarten, H. G., & Lachenmayer, L. (2004). Serotonin neurotoxins–past and present. *Neurotoxicity Research, 6*, 589–614.

Booth, R. G., Trevor, A., Singer, T. P., & Castagnoli, N. J. (1989). Studies on semirigid tricyclic analogues of the nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Journal of Medicinal Chemistry, 32*, 473–477.

Boyer, E. W., & Shannon, M. (2005). The serotonin syndrome. *The New England Journal of Medicine, 352*, 1112–1120.

Bradbury, A. J., Costall, B., Domeney, A. M., Testa, B., Jenner, P. G., Marsden, C. D., & Naylor, R. J. (1985). The toxic actions of MPTP and its metabolite MPP^+ are not mimicked by analogues of MPTP lacking an N-methyl moiety. *Neuroscience Letters, 61*, 121–126.

Bressler, A. J., Blizard, D. A., & Andrews, A. M. (2010). Low-stress route learning using the Lashley III maze in mice. *Journal of Visualized Experiments*, *39*. doi:10.3791/1786

Chiueh, C. C., Markey, S. P., Burns, R. S., Johannessen, J. N., Pert, A., & Kopin, I. J. (1984). Neurochemical and behavioral effects of systemic and intranigral administration of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the rat. *European Journal of Pharmacology, 100*, 189–194.

Clarke, H. F., Walker, S. C., Dalley, J. W., Robbins, T. W., & Roberts, A. C. (2007). Cognitive inflexibility after prefrontal serotonin depletion is behaviorally and neurochemically specific. *Cerebral Cortex, 17*, 18–27.

Cleeter, M. W., Cooper, J. M., & Schapira, A. H. (1992). Irreversible inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium: Evidence for free radical involvement. *Journal of Neurochemistry, 58*, 786–789.

Davis, G. C., Williams, A. C., Markey, S. P., Ebert, M. H., Caine, E. D., Reichert, C. M., & Kopin, I. J. (1979). Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Research, 1*, 249–254.

Ding, F., Luan, L., Ai, Y., Walton, A., Gerhardt, G. A., Gash, D. M., Grondin, R., & Zhang, Z. (2008). Development of a stable, early stage unilateral model of Parkinson's disease in middle-aged rhesus monkeys. *Experimental Neurology, 212*, 431–439.

Dluzen, D. E. (2000). Neuroprotective effects of estrogen upon the nigrostriatal dopaminergic system. *Journal of Neurocytology, 29*, 387–399.

Dluzen, D. E., McDermott, J. L., & Liu, B. (1996). Estrogen as a neuroprotectant against MPTP-induced neurotoxicity in C57/B1 mice. *Neurotoxicology and Teratology, 18*, 603–606.

Freyaldenhoven, T. E., Ali, S. F., & Hart, R. W. (1995). MPTP- and MPP(+)-induced effects on body temperature exhibit age- and strain-dependence in mice. *Brain Research, 688*, 161–170.

Fuller, R. W., Hemrick, L. S., Kindt, M. V., & Heikkila, R. E. (1988). Different effects of monoamine oxidase inhibition on MPTP depletion of heart and brain catecholamines in mice. *Life Sciences, 42*, 263–271.

Gainetdinov, R. R., Fumagalli, F., Jones, S. R., & Caron, M. G. (1997). Dopamine transporter is required for in vivo MPTP neurotoxicity: Evidence from mice lacking the transporter. *Journal of Neurochemistry, 69*, 1322–1325.

Gainetdinov, R. R., Fumagalli, F., Wang, Y. M., Jones, S. R., Levey, A. I., Miller, G. W., & Caron, M. G. (1998). Increased MPTP neurotoxicity in vesicular monoamine transporter 2 heterozygote knockout mice. *Journal of Neurochemistry, 70*, 1973–1978.

Guttman, M., Fibiger, H. C., Jakubovic, A., & Calne, D. B. (1990). Intracarotid 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration: Biochemical and behavioral observations in a primate model of hemiparkinsonism. *Journal of Neurochemistry, 54*, 1329–1334.

Halliwell, B. (1992). Reactive oxygen species and the central nervous system. *Journal of Neurochemistry, 59*, 1609–1623.

Hallman, H., & Jonsson, G. (1984). Pharmacological modifications of the neurotoxic action of the noradrenaline neurotoxin DSP4 on central noradrenaline neurons. *European Journal of Pharmacology, 103*, 269–278.

Heikkila, R. E., Manzino, L., Cabbat, F. S., & Duvoisin, R. C. (1985). Studies on the oxidation of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine by monoamine oxidase B. *Journal of Neurochemistry, 45*, 1049–1054.

Heikkila, R. E., Youngster, S. K., Panek, D. U., Giovanni, A., & Sonsalla, P. K. (1988). Studies with the neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and several of its analogs. *Toxicology, 49*, 493–501.

Jayanthi, S., Ladenheim, B., Andrews, A. M., & Cadet, J. L. (1999). Overexpression of human copper/zinc superoxide dismutase in transgenic mice attenuates oxidative stress caused by methylenedioxymethamphetamine (Ecstasy). *Neuroscience, 91*, 1379–1387.

Johannessen, J. N., Savitt, J. M., Markey, C. J., Bacon, J. P., Weisz, A., Hanselman, D. S., & Markey, S. P. (1987). The development of amine substituted analogues of MPTP as unique tools for the study of MPTP toxicity and Parkinson's disease. *Life Sciences, 40*, 697–704.

Johnson, E. A., Wu, E. Y., Rollema, H., Booth, R. G., Trevor, A. J., & Castagnoli, N., Jr. (1989). 1-methyl-4-phenylpyridinium (MPP+) analogs: In vivo neurotoxicity and inhibition of striatal synaptosomal dopamine uptake. *European Journal of Pharmacology, 166*, 65–74.

Kalaria, R. N., Mitchell, M. J., & Harik, S. I. (1987). Correlation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity with blood–brain barrier monoamine oxidase activity. *Proceedings of the National Academy of Sciences of the United States of America, 84*, 3521–3525.

Kenchappa, R. S., Diwakar, L., Annepu, J., & Ravindranath, V. (2004). Estrogen and neuroprotection: Higher constitutive expression of glutaredoxin in female mice offers protection against MPTP-mediated neurodegeneration. *The FASEB Journal, 18*, 1102–1104.

Kish, S. J., Furukawa, Y., Chang, L. J., Tong, J., Ginovart, N., Wilson, A., Houle, S., & Meyer, J. H. (2005). Regional distribution of serotonin transporter protein in postmortem human brain: Is the cerebellum a SERT-free brain region? *Nuclear Medicine and Biology, 32*, 123–128.

Kostrzewa, R. M. (2009). Evolution of neurotoxins: From research modalities to clinical realities. *Current Protocols in Neuroscience, 46*, 1.18.1–1.18.10.

Langston, J. W., & Palfreman, J. (1995). *The Case of the Frozen Addicts*. New York: Pantheon.

Lewin, R. (1984). Trail of ironies to Parkinson's disease [news]. *Science, 224*, 1083–1085.

Lieben, C. K., Steinbusch, H. W., & Blokland, A. (2006). 5,7-DHT lesion of the dorsal raphe nuclei impairs object recognition but not affective behavior and corticosterone response to stressor in the rat. *Behavioural Brain Research, 168*, 197–207.

López-Muñoz, F., & Álamo, C. (Eds.). (2012). *Neurobiology of Depression*. Boca Raton, FL: Taylor and Francis Group, LLC.

Luellen, B. A., Miller, D. B., Chisnell, A. C., Murphy, D. L., O'Callaghan, J. P., & Andrews, A. M. (2003). Neuronal and astroglial responses to the serotonin and norepinephrine neurotoxin:

1-Methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine. *The Journal of Pharmacology and Experimental Therapeutics, 307*, 923–931.

Luellen, B. A., Szapacs, M. E., Materese, C. K., & Andrews, A. M. (2006). The neurotoxin 2′-NH_2-MPTP degenerates serotonin axons and evokes increases in hippocampal BDNF. *Neuropharmacology, 50*, 297–308.

Maret, G., el Tayar, N., Carrupt, P. A., Testa, B., Jenner, P., & Baird, M. (1990). Toxication of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and analogs by monoamine oxidase. A structure-reactivity relationship study. *Biochemical Pharmacology, 40*, 783–792.

Markey, S. P., Johannessen, J. N., Chiueh, C. C., Burns, R. S., & Herkenham, M. A. (1984). Intraneuronal generation of a pyridinium metabolite may cause drug-induced parkinsonism. *Nature, 311*, 464–467.

Markey, S. P., Castagnoli, N., Trevor, A. J., & Kopin, I. J. (Eds.). (1986). *MPTP: A Neurotoxin Producing a Parkinsonian Syndrome*. Orlando: Academic Press, Inc.

Mattson, M. P., Maudsley, S., & Martin, B. (2004). BDNF and 5-HT: A dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends in Neurosciences, 27*, 589–594.

Melamed, E., Rosenthal, J., Cohen, O., Globus, M., & Uzzan, A. (1985). Dopamine but not norepinephrine or serotonin uptake inhibitors protect mice against neurotoxicity of MPTP. *European Journal of Pharmacology, 116*, 179–181.

Miller, D. B., & O'Callaghan, J. P. (1995). The role of temperature, stress, and other factors in the neurotoxicity of the substituted amphetamines 3,4-methylenedioxymethamphetamine and fenfluramine. *Molecular Neurobiology, 11*, 177–192.

Miller, D. B., Ali, S. F., O'Callaghan, J. P., & Laws, S. C. (1998). The impact of gender and estrogen on striatal dopaminergic neurotoxicity. *Annals of the New York Academy of Sciences, 844*, 153–165.

Miller, G. W., Gainetdinov, R. R., Levey, A. I., & Caron, M. G. (1999). Dopamine transporters and neuronal injury. *Trends in Pharmacological Sciences, 20*, 424–429.

Morissette, M., Jourdain, S., Al Sweidi, S., Menniti, F. S., Ramirez, A. D., & Di Paolo, T. (2007). Role of estrogen receptors in neuroprotection by estradiol against MPTP toxicity. *Neuropharmacology, 52*, 1509–1520.

Moy, L. Y., Albers, D. S., & Sonsalla, P. K. (1998). Lowering ambient or core body temperature elevates striatal MPP^+ levels and enhances toxicity to dopamine neurons in MPTP-treated mice. *Brain Research, 790*, 264–269.

Murphy, D. L., Lesch, K. P., Aulakh, C. S., & Pigott, T. A. (1991). Serotonin-selective arylpiperazines with neuroendocrine, behavioral, temperature, and cardiovascular effects in humans. *Pharmacological Reviews, 43*, 527–552.

Nestler, E. J., Hyman, S. E., & Malenka, R. C. (2008). *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience* (2nd ed.). New York: McGraw-Hill Professional.

Numis, A. L., Unger, E. L., Sheridan, D. L., Chisnell, A. C., & Andrews, A. M. (2004). The role of membrane and vesicular monoamine transporters in the neurotoxic and hypothermic effects of 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH(2)-MPTP). *Molecular Pharmacology, 66*, 718–727.

O'Callaghan, J. P. (1991). Assessment of neurotoxicity: Use of glial fibrillary acidic protein as a biomarker. *Biomedical and Environmental Sciences, 4*, 197–206.

O'Callaghan, J. P., & Miller, D. B. (1994). Neurotoxicity profiles of substituted amphetamines in the C57BL/6 J mouse. *The Journal of Pharmacology and Experimental Therapeutics, 270*, 741–751.

Ookubo, M., Yokoyama, H., Kato, H., & Araki, T. (2009). Gender differences on MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxicity in C57BL/6 mice. *Molecular and Cellular Endocrinology, 311*, 62–68.

Parsey, R. V., Kegeles, L. S., Hwang, D. R., Simpson, N., Abi-Dargham, A., Mawlawi, O., Slifstein, M., Van Heertum, R. L., Mann, J. J., & Laruelle, M. (2000). In vivo quantification of brain serotonin transporters in humans using [11C]McN 5652. *Journal of Nuclear Medicine, 41*, 1465–1477.

Przedborski, S., Jackson-Lewis, V., Popilskis, S., Kostic, V., Levivier, M., Fahn, S., & Cadet, J. L. (1991). Unilateral MPTP-induced parkinsonism in monkeys. A quantitative autoradiographic study of dopamine D1 and D2 receptors and re-uptake sites. *Neurochirurgie, 37*, 377–382.

Ramirez, A. D., Liu, X., & Menniti, F. S. (2003). Repeated estradiol treatment prevents MPTP-induced dopamine depletion in male mice. *Neuroendocrinology, 77*, 223–231.

Ricaurte, G. A., DeLanney, L. E., Irwin, I., & Langston, J. W. (1988). Toxic effects of MDMA on central serotonergic neurons in the primate: Importance of route and frequency of drug administration. *Brain Research, 446*, 165–168.

Saporito, M. S., Heikkila, R. E., Youngster, S. K., Nicklas, W. J., & Geller, H. M. (1992). Dopaminergic neurotoxicity of 1-methyl-4-phenylpyridinium analogs in cultured neurons: Relationship to the dopamine uptake system and inhibition of mitochondrial respiration. *The Journal of Pharmacology and Experimental Therapeutics, 260*, 1400–1409.

Singer, T. P., Salach, J. I., Castagnoli, N. J., & Trevor, A. (1986). Interactions of the neurotoxic amine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine with monoamine oxidases. *The Biochemical Journal, 235*, 785–789.

Staal, R. G., & Sonsalla, P. K. (2000a). Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata. *The Journal of Pharmacology and Experimental Therapeutics, 293*, 336–342.

Staal, R. G., & Sonsalla, P. K. (2000b). Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata. *The Journal of Pharmacology and Experimental Therapeutics, 293*, 336–342.

Subbiah, M. T., Kessel, B., Agrawal, M., Rajan, R., Abplanalp, W., & Rymaszewski, Z. (1993). Antioxidant potential of specific estrogens on lipid peroxidation. *The Journal of Clinical Endocrinology and Metabolism, 77*, 1095–1097.

Szapacs, M. E., Numis, A. L., & Andrews, A. M. (2004). Late onset loss of hippocampal 5-HT and NE is accompanied by increases in BDNF protein expression in mice co-expressing mutant APP and PS1. *Neurobiology of Disease, 16*, 572–580.

Thomas, B., Mandir, A. S., West, N., Liu, Y., Andrabi, S. A., Stirling, W., Dawson, V. L., Dawson, T. M., & Lee, M. K. (2011). Resistance to MPTP-neurotoxicity in alpha-synuclein knockout mice is complemented by human alpha-synuclein and associated with increased beta-synuclein and Akt activation. *PLoS One, 6*, e16706.

Uchihara, T. (2007). Silver diagnosis in neuropathology: Principles, practice and revised interpretation. *Acta Neuropathologica, 113*, 483–499.

Unger, E. L., Mazzola-Pomietto, P., Murphy, D. L., & Andrews, A. M. (2002). 2′-NH(2)-MPTP [1-Methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine] depletes serotonin and norepinephrine in rats: A comparison with 2′-CH(3)-MPTP [1-methyl-4-(2′-methylphenyl)-1,2,3,6-tetrahydropyridine]. *The Journal of Pharmacology and Experimental Therapeutics, 303*, 527–533.

Unger, E. L., Eve, D. J., Perez, X. A., Reichenbach, D. K., Xu, Y., Lee, M. K., & Andrews, A. M. (2006). Locomotor hyperactivity and alterations in dopamine neurotransmission are associated with overexpression of A53T mutant human alpha-synuclein in mice. *Neurobiology of Disease, 21*, 431–443.

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols, 2*, 322–328.

Xie, T., Tong, L., McLane, M. W., Hatzidimitriou, G., Yuan, J., McCann, U., & Ricaurte, G. (2006). Loss of serotonin transporter protein after MDMA and other ring-substituted amphetamines. *Neuropsychopharmacology, 31*, 2639–2651.

Xu, F., Gainetdinov, R. R., Wetsel, W. C., Jones, S. R., Bohn, L. M., Miller, G. W., Wang, Y. M., & Caron, M. G. (2000). Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. *Nature Neuroscience, 3*, 465–471.

Yew, D. T., Yeung, L. Y., Wai, M. S., & Mak, Y. T. (2009). 5-HT 1A and 2A receptor positive cells in the cerebella of mice and human and their decline during aging. *Microscopy Research and Technique, 72*, 684–689.

Youngster, S. K., Sonsalla, P. K., & Heikkila, R. E. (1987). Evaluation of the biological activity of several analogs of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Journal of Neurochemistry, 48*, 929–934.

Youngster, S. K., Sonsalla, P. K., Sieber, B. A., & Heikkila, R. E. (1989). Structure-activity study of the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity. I. Evaluation of the biological activity of MPTP analogs. *The Journal of Pharmacology and Experimental Therapeutics, 249*, 820–828.

Zhang, G., Krishnamoorthy, S., Ma, Z., Vukovich, N. P., Huang, X., & Tao, R. (2009). Assessment of 5-hydroxytryptamine efflux in rat brain during a mild, moderate and severe serotonin-toxicity syndrome. *European Journal of Pharmacology, 615*, 66–75.

Methamphetamine and MDMA Neurotoxicity: Biochemical and Molecular Mechanisms

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Contents

Abstract

Methamphetamine (METH) and its analogs, methylenedioxymethamphetamine (MDMA), are psychostimulant drugs with high abuse liability. The two drugs are also very neurotoxic. In the case of METH, the behavioral and neurotoxic effects of the drug occur because it alters dopamine terminal physiology and causes massive release of dopamine (DA) in the synaptic cleft in brain regions that receive dopaminergic projections from

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the midbrain. The increase of synaptic DA is compounded by the ability of METH to block DA reuptake into DA terminals. METH toxicity is not only accompanied by terminal dysfunction but also by causing dysfunction of complex networks that subserve cognitive and emotional processes. MDMA is a ring-substituted derivative of phenylisopropylamine which is structurally similar to METH. MDMA is a substrate of the serotonin transporter (SERT) via which it enters monoaminergic neurons and causes release of serotonin (5-HT) from storage vesicles. This is followed by 5-HT release into the synaptic cleft by reversal of normal SERT function. MDMA is selectively neurotoxic to serotonergic nerve terminals in rats, guinea pigs, and nonhuman primates. MDMA users consistently show reduced SERT radionuclide ligand binding across multiple brain regions. There is also evidence that MDMA users can suffer from cognitive deficits. However, the relation of DA and/or 5-HT depletion to cognitive impairments remains to be clarified in METH and MDMA users. Results from these studies are likely to impact the therapeutic approaches to the treatment of patients who suffer from METH and MDMA addiction.

List of Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	Serotonin
α-MT	α-methyl-p-tyrosine
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CuZnSOD	Copper–zinc superoxide dismutase
DA	Dopamine
DAT	Dopamine transporter
ER-	Endoplasmic reticulum-
HHA	3,4-Dihydroxyamphetamine
HHMA	3,4-Dihydroxymethamphetamine
HMA	4-Hydroxy-3-methoxy-amphetamine
L-DOPA	L-3,4-Dihydroxyphenylalanine
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
METH	Methamphetamine
MK-801	Dizocilpine
MTF-1	Metal-responsive transcription factor 1
MTs	Metallothioneins
NET	Norepinephrine transporter
Nrf2	NF-E2-Related factor 2
PARP	Poly(ADP-ribose) polymerase
ROS	Reactive oxygen species
SERT	Serotonin transporter
TH	Tyrosine hydroxylase
TPH	Tryptophan hydroxylase
VMAT2	Vesicular monoamine transporter 2

1 Introduction

Some psychostimulants including methamphetamine (METH) are presently prescribed for weight control, narcolepsy, and attention deficit disorder. These drugs are also used recreationally, with some individuals developing abuse followed by severe addiction. METH addiction is prevalent throughout the world, being second only to marijuana abuse among illicit drugs. During the past two decades, it has become clear that dopamine (DA) plays a critical role in the behavioral pharmacology and addictive properties of abused drugs. DA contributes to incentive salience and is necessary for identifying wanted elements from the environment (Berridge 2007). Drugs of abuse that increase dopaminergic signals alter and sensitize dynamic mesolimbic mechanisms that evolved to attribute incentive salience to rewards (Le Moal and Koob 2007). Such drugs interact with incentive salience integrating Pavlovian associative information with physiological state signals (Berridge 2007). Specific transporters in the synaptic membrane of dopaminergic terminals serve to remove DA from the synaptic cleft to end its action (Sulzer et al. 2005). METH alters dopamine terminal physiology and causes massive release of DA in the synaptic cleft in brain regions that receive dopaminergic projections from the midbrain (Krasnova and Cadet 2009). This is compounded by its ability to block DA reuptake into DA terminals. These mechanisms work together to cause degeneration of monoaminergic terminals in the striatum (O'Callaghan and Miller 1994; Krasnova and Cadet 2009). In the following paragraphs, we describe specific toxic effects and the mechanisms by which METH and its analogs, methylenedioxymethamphetamine (MDMA), induce damage of monoaminergic terminals and non-aminergic cell bodies in the brain.

2 Methamphetamine Toxicity

2.1 Dopamine and Oxidative Stress

Methamphetamine enters DA neurons through the DAT and via passive diffusion. In the cell, it accumulates in vesicles, disrupts the pH gradient required for vesicular DA sequestration, and displaces DA into the cytoplasm (Sulzer and Rayport 1990). METH causes release of monoamines from the neuronal cytosol via plasmalemmal uptake transporters, particularly the DA transporter (DAT), the norepinephrine transporter (NET), and the serotonin (5-HT) transporter (SERT) via a mechanism referred to as reverse transport (Sulzer et al. 2005). METH acts through the vesicular monoamine transporter 2 (VMAT-2) to cause excessive release of dopamine into the cytoplasm followed by DA release into the synaptic cleft through the DAT (Sulzer et al. 2005).

DA accumulates in the cytoplasm which alters the concentration gradient and likely helps to favor the reverse transport of DA via the DAT (see Sulzer et al. 2005).

DA is an important component of the mechanisms that underlie METH neurotoxicity (Sulzer and Rayport 1990).

Neurotoxic effects of METH have been demonstrated in many species including rats, mice, guinea pigs, cats, and monkeys (for detailed reviews see McCann and Ricaurte 2004 and Krasnova and Cadet 2009). Animals given repeated doses of METH suffer significant loss of DA and 5-HT in the striatum, cortex, and olfactory bulb (Deng et al. 2007) and decreases in striatal DAT binding, DAT immunoreactivity and tyrosine hydroxylase (TH) immunoreactivity (Bowyer et al. 2008; Deng et al. 1999), and TH protein levels (O'Callaghan and Miller 1994). Animals that self-administer METH also show deficits in dopaminergic systems (Krasnova et al. 2010). Morphological studies suggest that the reductions in the markers of DA and 5-HT system integrity are related to degeneration of DA and 5-HT axonal terminals (Ricaurte et al. 1982). These neurotoxic consequences can be prevented or attenuated by agents, such as α-methyl-p-tyrosine, that block DA synthesis and decrease DA levels in the striatum (Kuhn et al. 2008). In contrast, treatment with immediate DA precursor, L-3,4-dihydroxyphenylalanine (L-DOPA), that restores cytoplasmic DA levels enhances METH toxicity (Kuhn et al. 2008).

Autoxidation and metabolism of synaptic DA can lead to generation of reactive oxygen species (ROS) including hydrogen peroxide and superoxide radicals, quinones, and semiquinones (Cadet 1988; Cadet and Brannock 1998). The involvement of superoxide radicals in METH toxicity on DA terminals has been tested in transgenic mice that overexpress the human copper–zinc superoxide dismutase (CuZnSOD), a cytosolic enzyme that catalyzes the breakdown of superoxide radicals (Cadet et al. 1994a, b; Jayanthi et al. 1998). These mice that have much higher CuZnSOD enzyme activity in the cytosol than control wild-type animals (Jayanthi et al. 1998) are protected against METH toxicity (Cadet et al. 1994a, b).

METH can also cause neuronal apoptosis in several brain regions, including the striatum, cortex, hippocampus, and olfactory bulb (Deng and Cadet 2000; Deng et al. 2001, 2007; Ladenheim et al. 2000). Because METH administration is associated with ROS production and because ROS can cause apoptosis and DNA damage (Li and Trush 1993), we thought it is likely that METH might induce neuronal apoptosis through ROS-mediated DNA damage. This idea was supported by the observations that METH-induced poly(ADP-ribose) polymerase (PARP) cleavage, increase in caspase-3 activity, and neuronal death were all attenuated in the striata of CuZnSOD transgenic mice (Deng and Cadet 2000). A role for oxidative stress in METH toxicity is supported by data obtained from microarray analyses that have identified changes in the expression of a number of genes that participate in DNA repair, including APEX, PolB, LIG1, and DNA mismatch repair proteins MSH3 and PMS1 after toxic doses of the drug (Cadet et al. 2002).

METH treatment causes alterations in the DAT, including the formation of higher molecular mass DAT-associated complexes (Hadlock et al. 2009). This phenomenon is attenuated by either prevention of METH-induced hyperthermia

or pretreatment with the DA synthesis inhibitor, α-methyl-p-tyrosine (α-MT) (Baucum et al. 2004). Reactive species also are implicated in DAT complex formation because in vitro exposure to the reducing agent, β-mercaptoethanol, reverses this process (Baucum et al. 2004). Concurrent with METH-induced DAT complex formation, METH treatment causes a loss of DAT monomer immunoreactivity and a decrease in DAT function (Baucum et al. 2004).

Another aspect of METH toxicity is the fact that the drug can cause transcription factor NF-E2-related factor 2 (Nrf2) transit cytosolic into nuclear fractions where the protein exerts its regulatory functions (Jayanthi et al. 2009). Nrf2 and metal-responsive transcription factor 1 (MTF-1) are known to play a key role in transcriptional activation of the MT-1/2 genes in response to heavy metals or oxidative stress (Miyazaki et al. 2010), and it has been suggested that astrocytes can act to protect surrounding neurons against excess DA and DA-quinone formation by upregulating quinone-quenching molecules like metallothioneins (MTs) (Miyazaki et al. 2010). Increases in DA might promote nuclear translocation of Nrf2, upregulating MT1/2 expression in astrocytes, suggesting a protective role of Nrf2 against METH toxicity. Accordingly, Granado et al. (2011b) showed that Nrf2 deficiency exacerbated METH-induced damage to dopamine neurons (increase in loss of TH and DAT-containing fibers in striatum) and also potentiated glial activation.

2.2 Role of DAT and VMAT-2 in METH Toxicity

The role of DAT in METH toxicity has been investigated using both pharmacological and genetic means. For example, administration of the DAT inhibitor, methylphenidate, 1 hour after METH treatment, can reverse decreases in vesicular DA uptake, reductions in VMAT-2 ligand binding, and decreases in VMAT-2 immunoreactivity in vesicular subcellular fractions 6 h after injections of the drugs (Sandoval et al. 2003). Modafinil, an anti-narcoleptic drug that has been used off-label to treat psychostimulant addiction, acts as a DAT blocker (Zolkowska et al. 2009) and is neuroprotective against METH toxicity, as documented by attenuation of METH-induced decreases in TH immunoreactivity and DA striatal content (Raineri et al. 2011). Importantly, mice with genetic deletion of DAT are also protected against drug-induced DA depletion, reactive astrocytosis, and ROS production in the striatum (Fumagalli et al. 1998).

A role for VMAT-2 in METH-induced damage to striatal DA terminals is also supported by studies showing that pretreatment with the irreversible inhibitor of vesicular transport, reserpine exacerbates toxicity of the psychostimulant (Kuhn et al. 2008). Additionally, the administration of the alkaloid, lobeline, after injections of METH was able to reverse METH-induced decreases in synaptosomal, membrane-associated, and vesicular VMAT-2 immunoreactivity 24 h after drug treatment while also preventing METH-induced striatal depletion (Eyerman and Yamamoto 2005).

2.3 Role of Dopamine Receptors in METH Toxicity

Several studies have also documented a role for DA receptors in the mediation of METH toxicity (Angulo et al. 2004; Beauvais et al. 2011; Jayanthi et al. 2005; Xu et al. 2005). Specifically, pretreatment with the DA D_1 receptor antagonist, SCH23390 [R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine], given before each of 5 METH injections was able to attenuate drug-induced decreases in TH activity and DA levels in the rat striatum and decreases in tryptophan hydroxylase (TPH) activity and 5-HT levels in the striatum and cortex measured 18–20 h after METH treatment (Sonsalla et al. 1986). The DA D_2 receptor antagonist, sulpiride, also blocked METH-induced toxic effects on DA systems without affording any protection to striatal and cortical 5-HT terminals (Sonsalla et al. 1986). In addition, a single injection of the DA D_1 receptor antagonist, SCH23390, prior to a single high-dose METH injection also attenuated long-term decreases in DA levels (Jayanthi et al. 2005), reductions in DAT binding, and depletion in TH protein levels and reactive astrocytosis (Xu et al. 2005). SCH23390 also protected against METH-induced cell death in the striatum (Jayanthi et al. 2005; Xu et al. 2005). The protection afforded by SCH23390 against METH-mediated cell death appears to depend, in part, on the activation of the Fas/FasL death pathway because pretreatment with SCH23390 caused suppression of METH-induced increases in the expression of FasL and caspase-3 in rat striatal cells (Jayanthi et al. 2005). Jayanthi et al. (2009) also measured the expression of several genes that are regulated during ER stress. These include ATF3, HSP27, Hmox1, HSP40, CHOP/Gadd153 and ATF4, ATF6, BiP/GRP78, and GADD34. The DA D_1 receptor antagonist also attenuated or blocked METH-induced increases in the expression of the majority of these genes. METH injections can also cause substantial increases in the expression of proteins that participate in endoplasmic reticulum- (ER-) and mitochondrial-dependent stress responses, and these changes were also blocked by SCH23390 (Beauvais et al. 2011). However, the D_2-like receptor antagonist, raclopride, was found to have only small to moderate effects on ER stress proteins and only small effects on mitochondrial-dependent cellular stress proteins (Beauvais et al. 2011). Both DA D_1 and D_2 receptors appear to be required for METH toxicity since both D_1R (Ares-Santos et al. 2012) and D_2R (Granado et al. 2011a) knockout mice are protected against METH neurotoxicity.

2.4 Role of Microglia on METH-Induced Toxicity

METH toxicity is accompanied by reactive astrocytosis (Bowyer et al. 1994) and microglial activation (Pubill et al. 2002) in various brain regions. In the normal brain, microglial cells exist in a resting state, but, in response to inflammation or brain damage, these cells increase in size, migrate to the site of the injury, and phagocytize dying and dead cells (Raivich 2005). Although microglial activation is

important for immune responses, their overactivation can result in neurotoxic consequences. This process seems to be involved in the case of METH toxicity. For example, METH induces microgliosis in areas of the brain that suffer from neuronal degeneration (Thomas and Kuhn 2005a; Thomas et al. 2004a, b). Reserpine and clorgyline that exacerbate METH toxicity also potentiate in METH-induced microglial activation in the mouse striatum (Thomas et al. 2008). In contrast, attenuation of METH neurotoxicity by dizocilpine (MK-801), dextromethorphan, and α-methyl-p-tyrosine results in inhibition of METH-induced microglial activation (Kuhn et al. 2008; Thomas and Kuhn 2005b). Anti-inflammatory drugs, ketoprofen and indomethacin, and the second-generation tetracycline minocycline afford protection against METH-induced toxicity and microgliosis (Asanuma et al. 2003, 2004; Zhang et al. 2006). Further studies are necessary to identify the specific mechanisms that underlie METH-induced microgliosis and the manner by which these activated microglial cells might cause degeneration of monoaminergic terminals.

3 Preconditioning Doses of METH Protect Against Toxic Doses of METH

Pretreatment with multiple low-dose injections of METH or with gradually escalating doses of the drug has been shown to provide partial protection against the deleterious effects of a high-dose METH challenge (Cadet et al. 2009, 2011; Danaceau et al. 2007; Graham et al. 2008). These investigators use a regimen that involves gradual increases in METH administration to rats in order to mimic progressively larger doses of the drug used by some human METH addicts. METH preconditioning was associated with complete protection against dopamine depletion caused by a METH challenge in the striatum and cortex (Cadet et al. 2009). Hodges et al. (2011) also reported that this regimen resulted in prolonged protection, with a second challenge of METH causing no further decreases in striatal DA or 5-HT levels in comparison to the single METH challenge. Another study has also reported that, although it was associated with some decreases in striatal DAT, METH self-administration attenuated the persistent deficits in dopaminergic neuronal function and increases in GFAP immunoreactivity caused by a subsequent binge METH exposure (McFadden et al. 2012).

These data suggest that repeated injections of low doses of METH might render the brain refractory to the toxic effects of binge injections of the drug, thus providing protection against toxic METH effects in various brain regions. Neuroprotection induced by METH preconditioning might be related to reductions in METH hyperthermia (McFadden et al 2012). Another possible explanation for the neuroprotective effects of METH is the fact that, by reducing striatal DAT and increasing high-molecular DAT complexes that are nonfunctional, the striatum might have become less susceptible to the toxic effects of a METH challenge (Krasnova et al. 2011). This line of reasoning is consistent with the recent report

that a low, sub-toxic dose of METH was able to attenuate 6-OHDA-induced decreases in ATP levels and mitochondrial dehydrogenase activity (El Ayadi and Zigmond 2011).

4 Summary of Discussion of METH Toxicity

Chronic abuse of METH contributes to depression, psychosis, mood disturbances, and psychomotor dysfunctions in humans (Darke et al. 2008; Homer et al. 2008). METH addicts also suffer from deficits in attention, working memory, and decision-making (Gonzalez et al. 2004; Verdejo-García et al. 2006). Withdrawal from the drug is also associated with anhedonia, irritability, and intense craving for the drug (Darke et al. 2008; Homer et al. 2008). Human addicts also suffer persistent decreases in the levels of DAT in the orbitofrontal cortex, dorsolateral prefrontal cortex, and the caudate–putamen (McCann et al. 1998; Sekine et al. 2003; Volkow et al. 2001a, c). METH abusers also show abnormal glucose metabolism in cortical and subcortical brain areas (Volkow et al. 2001b; Wang et al. 2004). In addition, there is prominent microglial activation in the brains of these individuals (Sekine et al. 2008). Alterations in DA tone produced by METH toxicity on dopamine terminals might not only influence the physiology of the brain but might also alter the complex networks that subserve cognitive and emotional processes. It is thus likely that some of the clinical observations in METH abusers might be secondary to METH-induced toxic and neuroinflammatory responses, suggesting that the development of therapeutic agents that address these processes might be necessary to address the basic neurobiology of METH-induced neuropsychiatric manifestations.

5 MDMA Neurotoxicity

MDMA is a ring-substituted derivative of phenylisopropylamine, structurally similar to METH and the hallucinogen, mescaline (Lyles and Cadet 2003). MDMA impacts peripheral and central nervous system (CNS) functions through its actions on the serotonergic system (Baumann and Rothman 2009). MDMA is a substrate of the serotonin transporter via which it enters monoaminergic neurons and causes release of 5-HT from storage vesicles, followed by 5-HT release into the synaptic cleft by reversal of normal SERT function (Baumann and Rothman 2009). Injections of large doses of MDMA cause massive release of 5-HT from presynaptic vesicles, followed by a rapid decrease in 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels and decreased TPH activity (Lyles and Cadet 2003). There do not appear to be losses of 5-HT uptake sites at early time points after MDMA administration (Lyles and Cadet 2003). MDMA can also cause dose-dependent DA and NE release in the striatum and hippocampus in rats (Lyles and Cadet 2003). Adverse effects of acute MDMA administration, including cardiovascular stimulation and elevated

body temperature, are thought to involve monoamine release from sympathetic nerves in the periphery or nerve terminals in the CNS (Baumann and Rothman 2009). MDMA is selectively neurotoxic to serotonergic nerve terminals in rats, guinea pigs, and nonhuman primates (Green et al. 1995). In mice, however, MDMA appears to affect mainly the nigrostriatal dopaminergic system (Cadet et al. 1995). MDMA users consistently show reduced SERT radionuclide ligand binding across multiple brain regions (Reneman et al. 2006). Some evidence has suggested that these levels began to recover with increased periods of MDMA abstinence. Recently, Erritzoe et al (2011) reported significantly lower SERT binding potential in the neocortex (−56 %), pallidostriatum (−19 %), and amygdala (−32 %), with the extent of binding correlated with lifetime MDMA usage. Kish et al (2010) compared moderate Ecstasy/MDMA users with nonuser controls and reported that SERT binding was significantly reduced in all the cerebral cortices and hippocampus. The degree of these reductions was significantly associated with the extent of past MDMA usage. An additional molecular structural component of the serotonergic signaling pathway is the 5-HT_{2A} receptor. 5-HT_{2A} receptors are densely distributed in human cortex; 5-HT_{2A} receptor levels were reduced in multiple brain regions in the ongoing/acute MDMA user group, possibly because of ongoing MDMA-mediated increases in 5-HT release (Reneman et al. 2002).

Neurochemical and anatomical studies have reported long-term reductions in markers of 5-HT systems in rats. These include decreased levels of 5-HT and of its major metabolite, 5-HIAA; decreased number of 5-HT transporters; and decreased activity of the rate-limiting enzyme of 5-HT synthesis, TPH (Commins et al. 1987; De Souza et al. 1990). Severe reductions in 5-HT and 5-HIAA levels occurred in the rat neocortex, striatum, and hippocampus (De Souza et al. 1990). These abnormalities are reported to last for months or even years after drug administration (Lyles and Cadet 2003). MDMA also perturbs the function of SERT (Green et al. 2003), a marker of the integrity of serotonin neurons (Blakely et al. 1994). By virtue of its moderating synaptic 5-HT levels, SERT is crucial for the process of 5-HT neurotransmission (Green et al. 2003). MDMA downregulates SERT function without altering SERT mRNA or protein expression, and this rapid downregulation is sustained for at least 90 min and is dose dependent (Kivell et al. 2010).

Histologic studies in animals have shown that large doses of MDMA are associated with neurodegeneration particularly affecting the terminal portions of axons and fibers; raphe cell bodies are spared (reviewed by Lyles and Cadet 2003). In rats, histological studies using the Fink–Heimer staining method have also provided evidence for the degeneration of nerve terminals in the striatum and somatosensory cortex after chronic exposure to MDMA (Commins et al. 1987). Ricaurte et al. (2000) and Callahan et al. (2001) have also provided evidence that MDMA can cause reduction of the anterograde transport of [^{3}H] proline in ascending axons originating in the dorsal raphe nuclei, a subset of which comprise ascending 5-HT axons that project to various forebrain regions. A recent study found microtubular injury in frontal cortex after

MDMA treatment. The most apparent alteration in the ultrastructure of the labeled cortical axons of MDMA treated animals was the widespread disorganization of the microtubular system, suggesting a collapse of axonal microtubular system (with intact mitochondria) that leads to axonal swelling (Adori et al. 2011). These authors also found no signs of damage on dorsal raphe cell bodies. In nonhuman primates, reorganization of 5-HT projections has been reported to occur in the brains of nonhuman primates treated with MDMA (Ricaurte et al. 2000).

Formation of toxic MDMA metabolites which generate free radicals and associated oxidative stress and membrane damage has been proposed as causal agents for the long-term MDMA-induced neurodegeneration (reviewed in Lyles and Cadet 2003). Metabolism of MDMA results in the formation of methylenedioxyamphetamine (MDA) by *N*-demethylation and 3,4-dihydroxymethamphetamine (HHMA), the major metabolite, by *O*-demethylation. *O*-demethylation of MDA subsequently results in 3,4-dihydroxyamphetamine (HHA). HHMA and HHA are metabolized by catechol-*O*-methyltransferase (COMT) to HMMA and to 4-hydroxy-3-methoxyamphetamine (HMA) (reviewed by Green et al. 2003). The metabolites of MDMA might induce reactive species through redox cycling. Also, DA-induced oxidative stress in 5-HT terminals, and 5-HT_{2A} and dopamine D_1 receptor-mediated hyperthermia (Shioda et al. 2008) are all factors associated with MDMA neurotoxicity.

6 Cognition in Human MDMA Subjects

The accumulated evidence suggests that heavy MDMA use is associated with cognitive impairments and mood disturbances, in some cases lasting for months after cessation of drug intake (Baumann and Rothman 2009). Several studies have reported long-term deficits in memory and higher cognition, and other psychobiological functions in these substance abusers (Reneman et al. 2006). It is important to note that not all cognitive skills are affected and that there are also MDMA users who show normal neuropsychological functions (Parrott 2006). The role of other psychiatric disorders needs to be considered when evaluating cognitive functions in MDMA abusers. MDMA can also disrupt neuroendocrine regulation that is influenced by 5-HT levels. For example, MDMA users showed significantly reduced prolactin and cortisol responses to the serotonergic agonist d-fenfluramine in comparison with control subjects (Gerra et al. 2000).

7 Protective Mechanisms Against MDMA-Induced Toxicity

Preconditioning with repeated MDMA dosing appears to exert some neuroprotective effects against larger MDMA doses. For example, Bhide et al. (2009) reported that repeated exposure of adult rats to MDMA provides

neuroprotection against a challenge with large doses of MDMA that were shown to deplete 5-HT and decrease in SERT immunoreactivity. Alterations in MDMA pharmacokinetics or MDMA-induced hyperthermia do not appear to contribute to the neuroprotection provided by preconditioning.

There are other agents that also provide protective effects against MDMA toxicity. Chipana et al. (2008) reported that memantine, an alpha-7 nAChR antagonist used for treatment of Alzheimer's disease, was able to provide complete protection against MDMA-induced reduction in cortical [^{3}H]paroxetine binding sites. Fluoxetine, a SERT blocker, also provided long-lasting protection against MDMA-induced loss of SERT detected in vivo using micro-PET (Li et al. 2010).

8 Conclusion

In conclusion, MDMA users have been reported to suffer from cognitive and biochemical abnormalities. There is a need to correlate the changes of the serotonergic markers and other structural neuroimaging data to neuropsychological performance as well as responses to therapeutic interventions. These studies would help to provide better rationale for the development of pharmacological tools to treat METH- and MDMA-substance abusers.

References

Adori, C., Low, P., Andó, R. D., Gutknecht, L., Pap, D., Truszka, F., Takács, J., Kovács, G. G., Lesch, K. P., & Bagdy, G. (2011). Ultrastructural characterization of tryptophan hydroxylase 2-specific cortical serotonergic fibers and dorsal raphe neuronal cell bodies after MDMA treatment in rat. *Psychopharmacology, 213*(2–3), 377–391.

Angulo, J. A., Angulo, N., & Yu, J. (2004). Antagonists of the neurokinin-1 or dopamine D1 receptors confer protection from methamphetamine on dopamine terminals of the mouse striatum. *Annals of the New York Academy of Sciences, 1025*, 171–180.

Ares-Santos, S., Granado, N., Oliva, I., O'Shea, E., Martin, E. D., Colado, M. I., & Moratalla, R. (2012). Dopamine D(1) receptor deletion strongly reduces neurotoxic effects of methamphetamine. *Neurobiology of Disease, 45*(2), 810–820.

Asanuma, M., Tsuji, T., Miyazaki, I., Miyoshi, K., & Ogawa, N. (2003). Methamphetamine-induced neurotoxicity in mouse brain is attenuated by ketoprofen, a non-steroidal anti-inflammatory drug. *Neuroscience Letters, 352*, 13–16.

Asanuma, M., Miyazaki, I., Higashi, Y., Tsuji, T., & Ogawa, N. (2004). Specific gene expression and possible involvement of inflammation in methamphetamine-induced neurotoxicity. *Annals of the New York Academy of Sciences, 1025*, 69–75.

Baucum, A. J., 2nd, Rau, K. S., Riddle, E. L., Hanson, G. R., & Fleckenstein, A. E. (2004). Methamphetamine increases dopamine transporter higher molecular weight complex formation via a dopamine- and hyperthermia-associated mechanism. *Journal of Neuroscience, 24*(13), 3436–3443.

Baumann, M. H., & Rothman, R. B. (2009). Neural and cardiac toxicities associated with 3,4-methylenedioxymethamphetamine (MDMA). *International Review of Neurobiology, 88*, 257–296.

Beauvais, G., Atwell, K., Jayanthi, S., Ladenheim, B., & Cadet, J. L. (2011). Involvement of dopamine receptors in binge methamphetamine-induced activation of endoplasmic reticulum and mitochondrial stress pathways. *PLoS One, 6*(12), e28946.

Berridge, K. C. (2007). The debate over dopamine's role in reward: The case for incentive salience. *Psychopharmacology, 191*(3), 391–431.

Bhide, N. S., Lipton, J. W., Cunningham, J. I., Yamamoto, B. K., & Gudelsky, G. A. (2009). Repeated exposure to MDMA provides neuroprotection against subsequent MDMA-induced serotonin depletion in brain. *Brain Research, 1286*, 32–41.

Blakely, R. D., De Felice, L. J., & Hartzell, H. C. (1994). Molecular physiology of norepinephrine and serotonin transporters. *Journal of Experimental Biology, 196*, 263–281.

Bowyer, J. F., Davies, D. L., Schmued, L., Broening, H. W., Newport, G. D., Slikker, W., Jr., & Holson, R. R. (1994). Further studies of the role of hyperthermia in methamphetamine neurotoxicity. *Journal of Pharmacology and Experimental Therapeutics, 268*, 1571–1580.

Bowyer, J. F., Robinson, B., Ali, S., & Schmued, L. C. (2008). Neurotoxic-related changes in tyrosine hydroxylase, microglia, myelin, and the blood–brain barrier in the caudate-putamen from acute methamphetamine exposure. *Synapse, 62*(3), 193–204.

Cadet, J. L. (1988). A unifying theory of movement and madness: Involvement of free radicals in disorders of the isodendritic core of the brainstem. *Medical Hypotheses, 27*(1), 59–63.

Cadet, J. L., & Brannock, C. (1998). Free radicals and the pathobiology of brain dopamine systems. *Neurochemistry International, 32*(2), 117–131.

Cadet, J. L., Ali, S., & Epstein, C. (1994a). Involvement of oxygen-based radicals in methamphetamine-induced neurotoxicity: Evidence from the use of CuZnSOD transgenic mice. *Annals of the New York Academy of Sciences, 738*, 388–391.

Cadet, J. L., Sheng, P., Ali, S., Rothman, R., Carlson, E., & Epstein, C. (1994b). Attenuation of methamphetamine-induced neurotoxicity in copper/zinc superoxide dismutase transgenic mice. *Journal of Neurochemistry, 62*, 380–383.

Cadet, J. L., Ladenheim, B., Hirata, H., Rothman, R. B., Ali, S., Carlson, E., Epstein, C., & Moran, T. H. (1995). Superoxide radicals mediate the biochemical effects of methylenedioxymethamphetamine (MDMA): Evidence from using CuZn-superoxide dismutase transgenic mice. *Synapse, 21*, 169–176.

Cadet, J. L., McCoy, M. T., & Ladenheim, B. (2002). Distinct gene expression signatures in the striata of wild-type and heterozygous c-fos knockout mice following methamphetamine administration: Evidence from cDNA array analyses. *Synapse, 44*, 211–226.

Cadet, J. L., Krasnova, I. N., Ladenheim, B., Cai, N. S., McCoy, M. T., & Atianjoh, F. E. (2009). Methamphetamine preconditioning: Differential protective effects on monoaminergic systems in the rat brain. *Neurotoxicity Research, 15*(3), 252–259.

Cadet, J. L., Brannock, C., Ladenheim, B., McCoy, M. T., Beauvais, G., Hodges, A. B., Lehrmann, E., Wood, W. H., 3rd, Becker, K. G., & Krasnova, I. N. (2011). Methamphetamine preconditioning causes differential changes in striatal transcriptional responses to large doses of the drug. *Dose–response, 9*(2), 165–181.

Callahan, B. T., Cord, B. J., & Ricaurte, G. A. (2001). Long-term impairment of anterograde axonal transport along fiber projections originating in the rostral raphe nuclei after treatment with fenfluramine or methylenedioxymethamphetamine. *Synapse, 40*(2), 113–121.

Chipana, C., Torres, I., Camarasa, J., Pubill, D., & Escubedo, E. (2008). Memantine protects against amphetamine derivatives-induced neurotoxic damage in rodents. *Neuropharmacology, 54*(8), 1254–1263.

Commins, D. L., Vosmer, G., Virus, R. M., Woolverton, W. L., Schuster, C. R., & Seiden, L. S. (1987). Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. *Journal of Pharmacology and Experimental Therapeutics, 241*, 338–345.

Danaceau, J. P., Deering, C. E., Day, J. E., Smeal, S. J., Johnson-Davis, K. L., Fleckenstein, A. E., & Wilkins, D. G. (2007). Persistence of tolerance to methamphetamine-induced monoamine deficits. *European Journal of Pharmacology, 559*(1), 46–54.

Darke, S., Kaye, S., McKetin, R., & Duflou, J. (2008). Major physical and psychological harms of methamphetamine use. *Drug and Alcohol Review, 27*(3), 253–262.

De Souza, E. B., Battaglia, G., & Insel, T. R. (1990). Neurotoxic effect of MDMA on brain serotonin neurons: Evidence from neurochemical and radioligand binding studies. *Annals of the New York Academy of Sciences, 600*, 682–697.

Deng, X., & Cadet, J. L. (2000). Methamphetamine-induced apoptosis is attenuated in the striata of copper-zinc superoxide dismutase transgenic mice. *Brain Research. Molecular Brain Research, 83*, 121–124.

Deng, X., Ladenheim, B., Tsao, L. I., & Cadet, J. L. (1999). Null mutation of c-fos causes exacerbation of methamphetamine-induced neurotoxicity. *Journal of Neuroscience, 19*(22), 10107–10115.

Deng, X., Wang, Y., Chou, J., & Cadet, J. L. (2001). Methamphetamine causes widespread apoptosis in the mouse brain: Evidence from using an improved TUNEL histochemical method. *Brain Research. Molecular Brain Research, 93*, 64–69.

Deng, X., Ladenheim, B., Jayanthi, S., & Cadet, J. L. (2007). Methamphetamine administration causes death of dopaminergic neurons in the mouse olfactory bulb. *Biological Psychiatry, 61*(11), 1235–1243.

El Ayadi, A., & Zigmond, M. J. (2011). Low concentrations of methamphetamine can protect dopaminergic cells against a larger oxidative stress injury: Mechanistic study. *PLoS One, 6*(10), e24722.

Erritzoe, D., Frokjaer, V. G., Holst, K. K., Christoffersen, M., Johansen, S. S., Svarer, C., Madsen, J., Rasmussen, P. M., Ramsøy, T., Jernigan, T. L., & Knudsen, G. M. (2011). In vivo imaging of cerebral serotonin transporter and serotonin$_{2A}$ receptor binding in 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") and hallucinogen users. *Archives of General Psychiatry, 68*(6), 562–576.

Eyerman, D. J., & Yamamoto, B. K. (2005). Lobeline attenuates methamphetamine-induced changes in vesicular monoamine transporter 2 immunoreactivity and monoamine depletions in the striatum. *Journal of Pharmacology and Experimental Therapeutics, 312*, 160–169.

Fumagalli, F., Gainetdinov, R. R., Valenzano, K. J., & Caron, M. G. (1998). Role of dopamine transporter in methamphetamine-induced neurotoxicity: Evidence from mice lacking the transporter. *Journal of Neuroscience, 18*, 4861–4869.

Gerra, G., Zaimovic, A., Ferri, M., Zambelli, U., Timpano, M., Neri, E., Marzocchi, G. F., Delsignore, R., & Brambilla, F. (2000). Long-lasting effects of (+/−)3,4- methylenedioxy-methamphetamine (ecstasy) on serotonin system function in humans. *Biological Psychiatry, 47*(2), 127–136.

Gonzalez, R., Rippeth, J. D., Carey, C. L., Heaton, R. K., Moore, D. J., Schweinsburg, B. C., Cherner, M., & Grant, I. (2004). Neurocognitive performance of methamphetamine users discordant for history of marijuana exposure. *Drug and Alcohol Dependence, 76*(2), 181–190.

Graham, D. L., Noailles, P. A., & Cadet, J. L. (2008). Differential neurochemical consequences of an escalating dose-binge regimen followed by single-day multiple-dose methamphetamine challenges. *Journal of Neurochemistry, 105*(5), 1873–1885.

Granado, N., Ares-Santos, S., Oliva, I., O'Shea, E., Martin, E. D., Colado, M. I., & Moratalla, R. (2011a). Dopamine D_2-receptor knockout mice are protected against dopaminergic neurotoxicity induced by methamphetamine or MDMA. *Neurobiology of Disease, 42*(3), 391–403.

Granado, N., Lastres-Becker, I., Ares-Santos, S., Oliva, I., Martin, E., Cuadrado, A., & Moratalla, R. (2011b). Nrf2 Deficiency potentiates methamphetamine-induced dopaminergic axonal damage and gliosis in the striatum. *Glia, 59*, 1.

Green, A. R., Cross, A. J., & Goodwin, G. M. (1995). Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy'). *Psychopharmacology, 119*, 247–260.

Green, A. R., Mechan, A. O., Elliott, J. M., O'Shea, E., & Colado, M. I. (2003). The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacological Reviews, 55*(3), 463–508.

Hadlock, G. C., Baucum, A. J., II, King, J. L., Horner, K. A., Cook, G. A., Gibb, J. W., Wilkins, D. G., Hanson, G. R., & Fleckenstein, A. E. (2009). Mechanisms underlying methamphetamine-induced dopamine transporter complex formation. *Journal of Pharmacology and Experimental Therapeutics, 329*(1), 169–174.850–1863.

Hodges, A. B., Ladenheim, B., McCoy, M. T., Beauvais, G., Cai, N., Krasnova, I. N., & Cadet, J. L. (2011). Long-term protective effects of methamphetamine preconditioning against single-day methamphetamine toxic challenges. *Current Neuropharmacology, 9*, 35–39.

Homer, B. D., Solomon, T. M., Moeller, R. W., Mascia, A., DeRaleau, L., & Halkitis, P. N. (2008). Methamphetamine abuse and impairment of social functioning: A review of the underlying neurophysiological causes and behavioral implications. *Psychological Bulletin, 134*(2), 301–310.

Jayanthi, S., Ladenheim, B., & Cadet, J. L. (1998). Methamphetamine-induced changes in antioxidant enzymes and lipid peroxidation in copper/zinc-superoxide dismutase transgenic mice. *Annals of the New York Academy of Sciences, 844*, 92–102.

Jayanthi, S., Deng, X., Ladenheim, B., McCoy, M. T., Cluster, A., Cai, N. S., & Cadet, J. L. (2005). Calcineurin/NFAT-induced up-regulation of the Fas ligand/Fas death pathway is involved in methamphetamine-induced neuronal apoptosis. *Proceeding of the National Academy of Sciences USA, 102*, 868–873.

Jayanthi, S., McCoy, M. T., Beauvais, G., Ladenheim, B., Gilmore, K., Wood, W., III, Becker, K., & Cadet, J. L. (2009). Methamphetamine induces dopamine D_1 receptor-dependent endoplasmic reticulum stress-related molecular events in the rat striatum. *PLoS One, 4*(6), e6092.

Kish, S. J., Lerch, J., Furukawa, Y., Tong, J., McCluskey, T., Wilkins, D., Houle, S., Meyer, J., Mundo, E., Wilson, A. A., Rusjan, P. M., Saint-Cyr, J. A., Guttman, M., Collins, D. L., Shapiro, C., Warsh, J. J., & Boileau, I. (2010). Decreased cerebral cortical serotonin transporter binding in ecstasy users: A positron emission tomography/[^{11}C]DASB and structural brain imaging study. *Brain, 133*, 1779–1797.

Kivell, B., Day, D., Bosch, P., Schenk, S., & Miller, J. (2010). MDMA causes a redistribution of serotonin transporter from the cell surface to the intracellular compartment by a mechanism independent of phospho-p38-mitogen activated protein kinase activation. *Neuroscience, 168*(1), 82–95.

Krasnova, I. N., & Cadet, J. L. (2009). Methamphetamine toxicity and messengers of death. *Brain Research Reviews, 60*(2), 379–407.

Krasnova, I. N., Justinova, Z., Ladenheim, B., Jayanthi, S., McCoy, M. T., Barnes, C., Warner, J. E., Goldberg, S. R., & Cadet, J. L. (2010). Methamphetamine self-administration is associated with persistent biochemical alterations in striatal and cortical dopaminergic terminals in the rat. *PLoS One, 5*(1), e8790.

Krasnova, I. N., Ladenheim, B., Hodges, A. B., Volkow, N. D., & Cadet, J. L. (2011). Chronic methamphetamine administration causes differential regulation of transcription factors in the rat midbrain. *PLoS One, 6*(4), E19179.

Kuhn, D. M., Francescutti-Verbeem, D. M., & Thomas, D. M. (2008). Dopamine disposition in the presynaptic process regulates the severity of methamphetamine-induced neurotoxicity. *Annals of the New York Academy of Sciences, 1139*, 118–126.

Ladenheim, B., Krasnova, I. N., Deng, X., Oyler, J. M., Polettini, A., Moran, T. H., Huestis, M. A., & Cadet, J. L. (2000). Methamphetamine-induced neurotoxicity is attenuated in transgenic mice with a null mutation for interleukin-6. *Molecular Pharmacology, 58*, 1247–1256.

Le Moal, M., & Koob, G. F. (2007). Drug addiction: Pathways to the disease and pathophysiological perspectives. *European Neuropsychopharmacology, 6–7*, 377–393.

Li, Y., & Trush, M. A. (1993). DNA damage resulting from the oxidation of hydroquinone by copper: Role for a Cu(II)/Cu(I) redox cycle and reactive oxygen generation. *Carcinogenesis, 14*, 1303–1311.

Li, I. H., Huang, W. S., Shiue, C. Y., Huang, Y. Y., Liu, R. S., Chyueh, S. C., Hu, S. H., Liao, M. H., Shen, L. H., Liu, J. C., & Ma, K. H. (2010). Study on the neuroprotective effect of fluoxetine against MDMA-induced neurotoxicity on the serotonin transporter in rat brain using micro-PET. *NeuroImage, 49*(2), 1259–1270.

Lyles, J., & Cadet, J. L. (2003). Methylenedioxymethamphetamine (MDMA, ecstasy) neurotoxicity: Cellular and molecular mechanisms. *Brain Research. Brain Research Reviews, 42*(2), 155–168.

McCann, U. D., & Ricaurte, G. A. (2004). Amphetamine neurotoxicity: Accomplishments and remaining challenges. *Neuroscience and Biobehavioral Reviews, 27*(8), 821–826.

McCann, U. D., Wong, D. F., Yokoi, F., Villemagne, V., Dannals, R. F., & Ricaurte, G. A. (1998). Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: Evidence from positron emission tomography studies with [^{11}C]WIN-35,428. *Journal of Neuroscience, 18*(20), 8417–8422.

McFadden, L. M., Hadlock, G. C., Allen, S. C., Vieira-Brock, P. L., Stout, K. A., Ellis, J. D., Hoonakker, A. J., Andrenyak, D. M., Nielsen, S. M., Wilkins, D. G., Hanson, G. R., & Fleckenstein, A. E. (2012). Methamphetamine self-administration causes persistent striatal dopaminergic alterations and mitigates the deficits caused by a subsequent methamphetamine exposure. *Journal of Pharmacology and Experimental Therapeutics, 340*(2), 295–303.

Miyazaki, I., Asanuma, M., Kikkawa, Y., Takeshima, M., Murakami, S., Miyoshi, K., Sogawa, N., & Kita, T. (2010). Astrocyte-derived metallothionein protects dopaminergic neurons from dopamine quinone toxicity. *Glia, 59*(3), 435–451.

O'Callaghan, J. P., & Miller, D. B. (1994). Neurotoxicity profiles of substituted amphetamines in the C57BL/6 J mouse. *Journal of Pharmacology and Experimental Therapeutics, 270*, 741–751.

Parrott, A. C. (2006). MDMA in humans: Factors which affect the neuropsychobiological profiles of recreational ecstasy users, the integrative role of bio-energetic stress. *Journal of Psychopharmacology, 20*, 147–163.

Pubill, D., Verdaguer, E., Sureda, F. X., Camins, A., Pallas, M., Camarasa, J., & Escubedo, E. (2002). Carnosine prevents methamphetamine-induced gliosis but not dopamine terminal loss in rats. *European Journal of Pharmacology, 448*, 165–168.

Raineri, M., Peskin, V., Goitia, B., Taravini, I. R., Giorgeri, S., Urbano, F. J., & Bisagno, V. (2011). Attenuated methamphetamine induced neurotoxicity by modafinil administration in mice. *Synapse, 65*(10), 1087–1098.

Raivich, G. (2005). Like cops on the beat: The active role of resting microglia. *Trends in Neurosciences, 11*, 571–573.

Reneman, L., Endert, E., de Bruin, K., Lavalaye, J., Feenstra, M. G., de Wolff, F. A., & Booij, J. (2002). The acute and chronic effects of MDMA ("ecstasy") on cortical 5-HT_{2A} receptors in rat and human brain. *Neuropsychopharmacology, 26*, 387–396.

Reneman, L., de Win, M. M., van den Brink, W., Booij, J., & den Heeten, G. J. (2006). Neuroimaging findings with MDMA/ecstasy: Technical aspects, conceptual issues and future prospects. *Journal of Psychopharmacology, 20*, 164–175.

Ricaurte, G. A., Guillery, R. W., Seiden, L. S., Schuster, C. R., & Moore, R. Y. (1982). Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Research, 235*(1), 93–103.

Ricaurte, G. A., Yuan, J., & McCann, U. D. (2000). (+/−)3,4-Methylenedioxymethamphetamine ('Ecstasy')-induced serotonin neurotoxicity: Studies in animals. *Neuropsychobiology, 42*(1), 5–10.

Sandoval, V., Riddle, E. L., Hanson, G. R., & Fleckenstein, A. E. (2003). Methylphenidate alters vesicular monoamine transport and prevents methamphetamine-induced dopaminergic deficits. *Journal of Pharmacology and Experimental Therapeutics, 304*(3), 1181–1187.

Sekine, Y., Minabe, Y., Ouchi, Y., Takei, N., Iyo, M., Nakamura, K., Suzuki, K., Tsukada, H., Okada, H., Yoshikawa, E., Futatsubashi, M., & Mori, N. (2003). Association of dopamine transporter loss in the orbitofrontal and dorsolateral prefrontal cortices with methamphetamine-related psychiatric symptoms. *The American Journal of Psychiatry, 160*, 1699–1701.

Sekine, Y., Ouchi, Y., Sugihara, G., Takei, N., Yoshikawa, E., Nakamura, K., Iwata, Y., Tsuchiya, K. J., Suda, S., Suzuki, K., Kawai, M., Takebayashi, K., Yamamoto, S., Matsuzaki, H., Ueki, T., Mori, N., Gold, M. S., & Cadet, J. L. (2008). Methamphetamine causes microglial activation in the brains of human abusers. *Journal of Neuroscience, 28*, 5756–5761.

Shioda, K., Nisijima, K., Yoshino, T., Kuboshima, K., Iwamura, T., Yui, K., & Kato, S. (2008). Risperidone attenuates and reverses hyperthermia induced by 3,4- methylenedioxymethamphetamine (MDMA) in rats. *Neurotoxicology, 29*(6), 1030–1036.

Sonsalla, P. K., Gibb, J. W., & Hanson, G. R. (1986). Roles of D_1 and D_2 dopamine receptor subtypes in mediating the methamphetamine-induced changes in monoamine systems. *Journal of Pharmacology and Experimental Therapeutics, 238*, 932–937.

Sulzer, D., & Rayport, S. (1990). Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: A mechanism of action. *Neuron, 5*(6), 797–808.

Sulzer, D., Sonders, M. S., Poulsen, N. W., & Galli, A. (2005). Mechanisms of neurotransmitter release by amphetamines: A review. *Progress in Neurobiology, 75*(6), 406–433.

Thomas, D. M., & Kuhn, D. M. (2005a). Attenuated microglial activation mediates tolerance to the neurotoxic effects of methamphetamine. *Journal of Neurochemistry, 92*, 790–797.

Thomas, D. M., & Kuhn, D. M. (2005b). MK-801 and dextromethorphan block microglial activation and protect against methamphetamine-induced neurotoxicity. *Brain Research, 1050*, 190–198.

Thomas, D. M., Dowgiert, J., Geddes, T. J., Francescutti-Verbeem, D., Liu, X., & Kuhn, D. M. (2004a). Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. *Neuroscience Letters, 367*, 349–354.

Thomas, D. M., Walker, P. D., Benjamins, J. A., Geddes, T. J., & Kuhn, D. M. (2004b). Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. *Journal of Pharmacology and Experimental Therapeutics, 311*, 1–7.

Thomas, D. M., Francescutti-Verbeem, D. M., & Kuhn, D. M. (2008). The newly synthesized pool of dopamine determines the severity of methamphetamine-induced neurotoxicity. *Journal of Neurochemistry, 105*(3), 605–616.

Verdejo-García, A., Bechara, A., Recknor, E. C., & Pérez-García, M. (2006). Executive dysfunction in substance dependent individuals during drug use and abstinence: An examination of the behavioral, cognitive and emotional correlates of addiction. *Journal of International Neuropsychological Society, 12*(3), 405–415.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Franceschi, D., Sedler, M., Gatley, S. J., Miller, E., Hitzemann, R., Ding, Y. S., & Logan, J. (2001a). Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *Journal of Neuroscience, 21*, 9414–9418.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Franceschi, D., Sedler, M. J., Gatley, S. J., Hitzemann, R., Ding, Y. S., Wong, C., & Logan, J. (2001b). Higher cortical and lower subcortical metabolism in detoxified methamphetamine abusers. *The American Journal of Psychiatry, 58*, 383–389.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Leonido-Yee, M., Franceschi, D., Sedler, M. J., Gatley, S. J., Hitzemann, R., Ding, Y. S., Logan, J., Wong, C., & Miller, E. N. (2001c). Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *The American Journal of Psychiatry, 158*, 377–382.

Wang, G. J., Volkow, N. D., Chang, L., Miller, E., Sedler, M., Hitzemann, R., Zhu, W., Logan, J., Ma, Y., & Fowler, J. S. (2004). Partial recovery of brain metabolism in methamphetamine abusers after protracted abstinence. *The American Journal of Psychiatry, 161*(2), 242–248.

Xu, W., Zhu, J. P., & Angulo, J. A. (2005). Induction of striatal pre- and postsynaptic damage by methamphetamine requires the dopamine receptors. *Synapse, 58*, 110–121.

Zhang, L., Kitaichi, K., Fujimoto, Y., Nakayama, H., Shimizu, E., Iyo, M., & Hashimoto, K. (2006). Protective effects of minocycline on behavioral changes and neurotoxicity in mice after administration of methamphetamine. *Progress in Neuropsychopharmacology and Biological Psychiatry, 30*, 1381–1393.

Zolkowska, D., Jain, R., Rothman, R. B., Partilla, J. S., Roth, B. L., Setola, V., Prisinzano, T. E., & Baumann, M. H. (2009). Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. *Journal of Pharmacology and Experimental Therapeutics, 329*, 738–746.

Glutamate as a Neurotoxin

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Contents

Abstract

Glutamate (Glu) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Excessive glutamate in the extracellular space can trigger passive and active forms of neuronal death in the CNS via excessive activation of glutamate receptors. This phenomenon has been named excitotoxicity.

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Excitotoxicity has been implicated in the pathogenesis of acute and chronic neurodegenerative disorders. However, despite overwhelming preclinical data, human clinical trials in stroke or traumatic brain injury with antiexcitotoxic compounds acting at different levels of the excitotoxic cascade have failed to provide the expected neuroprotective effect. Here we present a focused overview of excitotoxic research including physiology of glutamate receptors and intracellular pathways leading to cell death. We review evidence for involvement of excitotoxicity in human neurodegenerative disorders and the results of several clinical trials. We present the most recent trends in the development of antiexcitotoxic therapies and also briefly refer to the hypothesis that interference with the trophic actions of glutamate in the context of CNS injuries may be one of the reasons why clinical trials with antiexcitotoxic compounds have failed.

Keywords
Death • Excitotoxicity • Ischemia • Neuron • Neurodegenerative disorder • Stroke

1 Introduction

The first hint towards the neurotransmitter role of the amino acid glutamate came in 1935 from work by Krebs, who discovered that glutamate increases metabolism in the isolated retina and that it is concentrated in the cerebral gray matter (Krebs 1935). Hayashi (1952, 1958) discovered the excitatory properties of glutamate on neuronal tissue. Local administration of glutamate on the motor cortex of dogs and primates resulted in motor seizures. Curtis et al. (1959) subsequently demonstrated that glutamate and aspartate, when applied iontophoretically to the cat's spinal cord, depolarized neurons.

Today it is common knowledge that glutamate is the major excitatory neurotransmitter in the nervous system and that it is essential for learning and memory, synaptic plasticity, and neuronal survival and, in early development, for proliferation, migration, and differentiation of neuronal progenitors and immature neurons (Fonnum 1984; Guerrini et al. 1995; Ikonomidou et al. 1999; Komuro and Rakic 1993).

Compartmentalization allows that separate pools within the central nervous system supply glutamate for the metabolic machinery of the cells (metabolic pool) and for intercellular signaling (neurotransmitter pool) (Fonnum 1984). The neurotransmitter pool is located in nerve endings and releases glutamate by a calcium-dependent mechanism. Glutamate is subsequently removed by uptake into the surrounding glial cells and aminated to glutamine.

When released into the synaptic cleft, glutamate acts at the postsynaptic site on receptors (Nakanishi 1992; Hollmann and Heinemann 1994). These include the *N*-methyl-D-aspartate (NMDA), kainate, the 2-amino-3-hydroxy-5-methylisoxazole-propionate (AMPA), and the metabotropic receptors. The first three are coupled to ion

channels and are therefore called ionotropic glutamate receptors. Metabotropic glutamate receptors mediate their actions through G proteins (Hollmann and Heinemann 1994).

Excitotoxicity, a phenomenon discovered in the 1950s and extensively investigated and characterized in the 1970s and 1980s, describes a process in which excess of glutamate and other excitatory amino acid neurotransmitters causes overstimulation of neuronal glutamate receptors leading to degeneration of neurons. Lucas and Newhouse (1957) first described that systemic administration of monosodium glutamate causes degeneration of retinal ganglion cells. Olney and colleagues (Olney 1969, 1971; Olney and Ho 1970; Olney et al. 1972) subsequently observed that excitatory amino acids, when given to infant rodents and primates, cause neurodegeneration in brain areas that lack a blood–brain barrier. The ability of acidic amino acids to destroy neurons correlates with their excitatory potencies (Olney 1969, 1971; Olney and Ho 1970; Olney et al. 1972). This interaction was termed neuroexcitotoxicity. Both ionotropic and metabotropic glutamate receptors can mediate excitotoxicity (McDonald and Schoepp 1992; Schoepp et al. 1995).

2 Glutamate Receptors

2.1 NMDA Receptors

The NMDA receptor (NMDAR) has been named for its affinity for N-methyl-D-aspartate. When activated, the NMDAR allows the influx of cations, most notably calcium. Excessive intracellular calcium concentrations activate intracellular pathways leading to physiological and pathological processes. The NMDAR exhibits a complex gating mechanism, requiring cellular depolarization in addition to the binding of ligands.

NMDA receptors represent tetrameric heteromeric subunit assemblies whose physiological and pharmacological properties depend upon their subunit composition. Three major subunit families, NR1, NR2 (A–D), and NR3 (A and B), have been cloned (Burnashev et al. 1992; Das et al. 1998; Andersen et al. 2001; Chatterton et al. 2002; Matsuda et al. 2002; Monyer et al. 1992). Most NMDA receptors in the CNS are formed from NR1 and NR2 subunits. Alternative splicing results in eight isoforms of NR1. The NR2 subunit family consists of four individual subunits termed NR2A–D (Monyer et al. 1991, 1994; Hollmann and Heinemann 1994). Different NR2 subunits result in different Ca^{2+} permeability of the NMDA receptor channel, different gating properties, and magnesium sensitivity (Monyer et al. 1991, 1994; Ozawa et al. 1998).

Two NR1 subunits can form an ion channel which exhibits all the classical NMDAR properties, i.e., glutamate activation, magnesium block, zinc inactivation, glycine activation, interactions with polyamines, and pH sensitivity. Mayer et al. (1984) showed that depolarization of the NMDAR-expressing neuron is necessary to electrostatically remove a magnesium ion blocking the ion channel pore.

Under physiological conditions, this is accomplished by the activation of other ionotropic glutamate channels. Zinc can inhibit the NMDAR-mediated currents elicited by glutamate (Mayer and Vyklicky 1989). Glycine is a cofactor with glutamate, necessary for NMDAR channel opening (Johnson and Ascher 1987). Glycine shows different affinities at NMDA receptor subtypes. This affinity depends on the NR1 isoform and also the NR2 subunit composition of the receptor complex which allosterically influences the glycine recognition site located on the NR1 subunit (Monyer et al. 1992; Laurie and Seeburg 1994).

Polyamines can potentiate NMDAR currents at low concentrations, increase the frequency of channel opening and glycine affinity, but also reduce currents in a voltage-dependent manner at higher concentrations (Rock and Macdonald 1992; Benveniste and Mayer 1993). The polyamine/NMDAR interaction is dependent upon the subunit composition of the NMDAR channel (Williams 1997).

Protons inhibit NMDAR by interacting with the NR1 subunit on a single lysine residue (Traynelis and Cull-Candy 1990). Thus, alterations in pH can modulate NMDAR function.

NR2 subunits refine NMDAR function and modulate the characteristics of the NR1 ion channel pore. NR2B subunits also bind and link postsynaptic proteins (Sattler et al. 1999) which are parts of postsynaptic densities comprising scaffolding proteins. This allows for the coupling of intracellular enzymes (e.g., neuronal nitric oxide synthase) with ionic second messengers (e.g., Ca^{2+} influx from NMDARs) (Aarts et al. 2002).

A third subunit associated with the NMDA receptor gene family, the NR3 subunit, is expressed in two isoforms: NR3A, which is expressed throughout the CNS, and NR3B, which is expressed primarily in motor neurons (Chatterton et al. 2002). NR1/NR3A and NR1/NR3B complexes are not activated by NMDA or glutamate, but rather elicit an excitatory Ca^{2+} impermeant response via glycine.

2.2 AMPA/Kainate Receptors

AMPA receptors are comprised of four subunits, GluR1–GluR4, and require only glutamate application for activation. They are widely distributed throughout the mammalian central nervous system and mediate fast glutamatergic neurotransmission. The type of subunits within a tetrameric assembly determines biophysical and pharmacological profile of AMPA receptors. GluR1, GluR3, and GluR4 all display strong inwardly rectifying current–voltage and calcium permeability, whereas the GluR2 subunit removes calcium permeability (Hollmann et al. 1991; Burnashev et al. 1995; Geiger et al. 1995). Two splice variants for GluR subunits have been described, flip and flop; these differ in their expression between brain regions and developmental stages. AMPARs are thought to regulate the fast excitation required to remove the magnesium block of nearby NMDARs.

Other non-NMDA receptors are designated GluR5, GluR6, GluR7 (also known as GluK5–7), KA1, and KA2 (also known as GluK1–2). These form high-affinity kainate receptors (Herb et al. 1992, 1996; Hollmann and Heinemann 1994).

The properties of kainate channels are similar to AMPARs, but they are mostly impermeant to calcium ions. Kainate receptors may be localized in both the pre-synapse and post-synapse (Castillo et al. 1997; Vignes and Collingridge 1997), and application of kainate can either stimulate glutamate release (Schmitz et al. 2001) or inhibit presynaptic glutamate release (Frerking et al. 2001). Postsynaptically, kainate channels serve a similar purpose as AMPARs in alleviating magnesium block in NMDARs.

2.3 Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs) are single-peptide seven-transmembrane spanning proteins linked to intracellular G proteins. These are built from eight subunits, mGluR1–8. Recent evidence suggests that G-protein-independent signaling can also occur (Heuss et al. 1999). Within the mGluR family, the sequence identity varies. Classification into three subclasses (I–III) has been proposed (Nakanishi 1992; Pin and Duvoisin 1995). Within each class the amino acid sequence identity is 70 % and between classes 45 %. mGluR1 and mGluR5 belong to class I, mGluR2 and mGluR3 to class II, and mGluR4 and mGluR6–8 to class III. Different transduction mechanisms are activated by each class. Class I activates phospholipase C, increases phosphoinositide turnover and Ca^{2+} release from internal stores, and leads to formation of diacylglycerol which then might activate protein kinase C (Abe et al. 1992; Nakanishi 1992). This group of mGluRs also modulates excitatory postsynaptic potentials at hippocampal synapses via tyrosine kinases in a G-protein-independent fashion (Heuss et al. 1999).

Classes II and III are negatively coupled to adenylate cyclase and reduce the intracellular amount of cAMP. Group II mGluRs include mGluR2s and mGluR3s. Group III mGluRs include mGluR4s, mGluR6s, mGluR7s, and mGluR8s. These mGluRs cause downstream inhibition of voltage-dependent calcium channels (Tanabe et al. 1993; Chavis et al. 1994). These receptors are found pre- and postsynaptically (Ozyurt et al. 1988; Bradley et al. 1996). Since presynaptic calcium is integral to neurotransmission, group II and III mGluRs modulate neurotransmission by functioning as autoreceptors and modulating calcium channel influxes. Group I mGluRs potentiate NMDAR-mediated Ca^{2+} influx (Bruno et al. 1995a). mGluR2, 3 and mGluR4, 6, 7, 8 subunits reduce Ca^{2+} influx via NMDARs (Bruno et al. 1995b).

3 The Neurotoxin Glutamate

The mechanisms that mediate excitotoxicity have been extensively studied in the past five decades. The acute component of glutamate toxicity is mediated through a massive influx of sodium and chloride ions into the cell (Rothman 1984, 1985; Choi et al. 1987). A second component of glutamate neurotoxicity is Ca^{2+} dependent and mediated through activation of Ca^{2+}-sensitive proteases (Choi 1987; Frandsen and Schousboe 1991, 1992; Tymianski et al. 1993a).

The concept of slow excitotoxicity arose from observations made by Novelli and coworkers showing that inhibitors of oxidative phosphorylation or of the Na^+/K^+ pump allow glutamate or NMDA to become neurotoxic (Novelli et al. 1988). Subsequently, Zeevalk and Nicklas showed that cyanide triggers excitotoxic lesions sensitive to NMDA antagonists in the chick embryo retina (Zeevalk and Nicklas 1991). Toxicity induced by metabolic inhibition can be mimicked by membrane depolarization with potassium or by relieving the Mg^{2+} block of the NMDA receptor (Zeevalk and Nicklas 1991). These observations were extended by findings in in vivo models showing that mitochondrial toxins (aminooxyacetic acid, an inhibitor of the maleate–aspartate shunt; 1-methyl-pyridine, a mitochondrial complex I toxin; 3-nitropropionic acid and malonic acid, inhibitors of mitochondrial complex II; the nicotinamide antagonist 3-acetylpyridine) produce axon-sparing, excitotoxic lesions in the mammalian central nervous system which can be blocked by NMDA and AMPA antagonists, glutamate release inhibitors, and prior decortication (Beal et al. 1991, 1993a, b; Greene et al. 1993; Henshaw et al. 1994; McMaster et al. 1991; Urbanska et al. 1991; Srivastava et al. 1993; Schulz et al. 1994; Wüllner et al. 1994). These observations led to the hypothesis that energy impairment at the postsynaptic site of glutamatergic synapses causes membrane depolarization, alleviation of the Mg^{2+} block of the NMDA receptors, and heightened neuronal vulnerability towards physiological concentrations of glutamate (Beal 1992a, b; Ikonomidou and Turski 1996).

4 Mechanisms of Excitotoxicity

In neuronal cultures and in vivo, both active (apoptosis-like) and passive (necrosis-like) forms of cell death are seen depending on the intensity of the excitotoxic insult (Bonfoco et al. 1995; Portera-Cailliau et al. 1997). Heterogenous forms of cell death are also observed in animal models of stroke (Snider et al. 1999), hypoxia/ischemia (Fig. 1), and traumatic brain injury (Singleton and Povlishock 2004; Fig. 1). At one end of the spectrum, neurons displaying necrotic morphology are seen following intense glutamatergic insult (Bonfoco et al. 1995). The mechanisms underlying neuronal necrosis include loss of cellular homeostasis with acute mitochondrial dysfunction leading to massive energy failure. Various cell death pathways which include cysteine proteases, mitochondrial endonucleases, peroxynitrite, PARP-1, and GAPDH eventually accomplish cell death following milder glutamatergic insults. These cell death pathways operate in parallel or in sequence to orchestrate neuronal degeneration, and none of them appears to be dominant.

4.1 Calcium

In neuronal cultures, glutamate neurotoxicity can be separated into two components distinguishable on the basis of differences in time course and ionic dependence. The first component, marked by neuronal swelling, occurs early, is dependent on

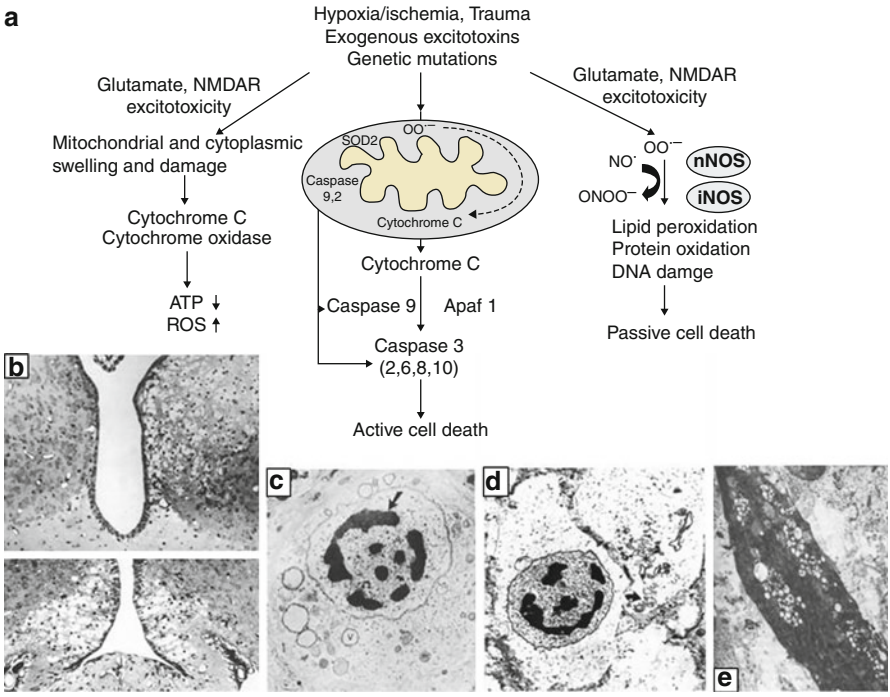

Fig. 1 Pathophysiology of cell death in the context of neurodegenerative disorders. (**a**) Injury generates excessive release of glutamate in the extracellular space which leads to activation of NMDA receptors and massive influx of ions into the cells. Injury also leads to generation of reactive oxygen species (ROS) within the mitochondria, which then signal the release of cytochrome c. Cytochrome c binds to Apaf-1 followed by caspase-9 to form a complex that activates caspase-3 and other caspases. The activation of the *N*-methyl-D-aspartate (*NMDA*) receptor and formation of O_2- and nitric oxide (*NO*) by neuronal nitric oxide (*nNOS*) may lead to release of cytochrome c from the mitochondria or formation of peroxynitrite (*ONOO–*). Resulting hydroxyl radicals can damage lipids, proteins, and DNA and lead to passive cell death. (**b–e**) Examples of types of neuronal degeneration seen in the brain following hypoxia/ischemia or traumatic brain injury (*TBI*) are shown; (**b**) shows neurons undergoing degeneration following hypoxia/ischemia (*upper panel*) in 7-day-old rats or systemic administration of monosodium glutamate in infant mice (*lower panel*). The pattern of degeneration in the two conditions is indistinguishable. (**c**) Electron micrographs depict a neuron undergoing excitotoxic death in the P7 rat brain at 4 h after hypoxia/ischemia and (**d**) after traumatic brain injury. Both cells display disrupted nuclear and cytoplasmic membranes, swollen mitochondria, and cytoplasmic debris. In (**e**) a neuron displaying dark cytoplasm filled with vacuoles is shown 4 h following traumatic brain injury in a 7-day-old rat brain. This neuron is degenerating in a more delayed fashion than shown in (**b**) and (**c**) (Adapted and modified with permission from Liebert Publications from Ikonomidou and Kaindl 2011)

extracellular Na and Cl, and is thus possibly "excitotoxic." The second component, marked by gradual neuronal disintegration, occurs late, is dependent on extracellular Ca^{2+}, and is thus mediated by a transmembrane influx of Ca. While either component alone is ultimately capable of producing irreversible neuronal injury, the Ca-dependent mechanism predominates at lower exposures to glutamate.

Glutamate exposure likely leads to a Ca influx both through glutamate-activated cation channels and through voltage-dependent Ca channels activated by membrane depolarization (Choi 1985, 1987).

Subsequent studies by Choi (1987) suggested that NMDARs may be primarily responsible for this calcium entry. Tymianski et al. (1993a) demonstrated that the path of calcium influx, and not the calcium load, was important in the NMDAR-mediated neurodegenerative process. Indeed, higher lethality can be achieved with lower calcium influxes via NMDARs compared to higher calcium influxes via other calcium-permeant channels (Sattler et al. 1998). Further work showed that NMDARs are spatially linked to neuronal nitric oxide synthase (nNOS) which can produce toxic levels of nitric oxide (NO) (Sattler et al. 1999).

The majority of intracellular calcium is sequestered into the mitochondria (Thayer and Wang 1995; Wang and Thayer 1996). Three phases of intracellular calcium concentration changes in cultured hippocampal neurons exposed to glutamate have been described: an initial phase of increased intracellular calcium lasting 5–10 min, a 2-h latent phase with normal calcium concentrations, and a gradual sustained rise in intracellular calcium associated with cell death (Randall and Thayer 1992).

Calcium activates several mechanisms to orchestrate cell death, including activation of nitric oxide synthase, proteases, and mitochondrial injury.

4.2 Nitric Oxide, Peroxynitrite, and Free Radicals

The findings by Dawson et al. (1991) that nitric oxide synthase (NOS) inhibitors prevent excitotoxic neuronal death in vitro provided first evidence for the role of NO in glutamate-mediated neurodegeneration. Neuronal NOS (nNOS) is the isoform causing excitotoxic injury (Dawson et al. 1996). The postsynaptic density protein of 95 kDa (PSD-95) structurally links nNOS and NMDARs (Sattler et al. 1999). The postsynaptic density protein PSD-95 binds to the C-terminus of the NR2B subunit via a PDZ1 domain and the N-terminus of nNOS via a PDZ2 domain. In addition to targeting multiple intracellular molecules (Stamler et al. 1992), NO also reacts with superoxide to form peroxynitrite, which causes cell death via protein and lipid peroxidation, protein nitration, and DNA injury (Radi et al. 1991a, b) or direct interaction with GAPDH (Hara et al. 2005). GAPDH is a ubiquitous housekeeping enzyme that participates in glycolysis. NO can nitrosylate GAPDH and bind Siah1, an ubiquitin ligase. The formed heterodimer translocates to the nucleus via Siah1 nuclear translocation domain and enhances p300/CBP-associated acetylation of nuclear proteins (Sen et al. 2009). As GAPDH is an active participant in glycolysis, it is possible that the interaction of GAPDH with nitric oxide also causes a functional loss (Molina et al. 1992) which can result in neuronal death via energy failure.

During normal cellular respiration, superoxide is produced and converted by superoxide dismutase into oxygen and hydrogen peroxide. Peroxynitrite, formed from NO and superoxide via a diffusion limited reaction (Huie and Padmaja 1993),

can cause protein nitrosylation (van der Vliet et al. 1998; Schrammel et al. 2003), lipid peroxidation (Radi et al. 1991a, b), direct DNA damage, and protein dysfunction. Peroxynitrite can also inhibit the mitochondrial electron transport chain at complex I and complex II (Radi et al. 1994), the normal function of cytochrome c (Nakagawa et al. 2001) as well as manganese and iron superoxide dismutase in scavenging superoxide (Ischiropoulos et al. 1992) via protein nitration (Yamakura et al. 1998). Peroxynitrite-mediated DNA damage can cause overactivation of the nuclear repairing enzyme poly(ADP)-ribose polymerase (PARP-1) (Zhang et al. 1994; Zingarelli et al. 1996). Evidence that free radicals play a role in glutamate excitotoxicity came from studies showing that superoxide dismutate and antioxidant compounds such as nitrone-based scavengers, free radical spin traps, and 21-aminosteroids/lazaroids confer neuroprotection (Dykens 1994; Gonzalez-Zulueta et al. 1998). Using paramagnetic resonance imaging, Lafon-Cazal et al. (1993) demonstrated a dose-dependent increase in superoxide production following increasing concentrations of NMDA. Further work in different in vitro systems demonstrated that free radical production in the context of excitotoxicity is secondary to calcium influx via NMDAR (Dykens 1994; Dugan et al. 1995; Reynolds and Hastings 1995).

4.3 Zinc

Zinc is present in glutamatergic neurons in a vesicle-associated pool (Martinez-Guijarro et al. 1991), which can contain up to 300 μM concentrations of zinc (Yokoyama et al. 1986). Initial in vivo observations in kainate-treated rodents (Frederickson et al. 1989) that depletion of zinc at presynaptic terminals and simultaneous zinc accumulation in degenerating neurons occurred link zinc to glutamate neurotoxicity. Presynaptic zinc depletion, postsynaptic zinc accumulation, and neuroprotection with zinc chelation have been observed in models of cerebral ischemia (Tonder et al. 1990; Koh et al. 1996) and traumatic brain injury (Suh et al. 2000).

Zinc entry into neurons has been linked to voltage-gated calcium channels, sodium exchangers, NMDARs and AMPA/kainate receptors (Sensi et al. 1997), and TRMP7 channels (Inoue et al. 2010). Increasing intracellular zinc levels result in glycolytic dysfunction as a result of GAPDH inhibition (Sheline et al. 2000), interference with the mitochondrial electron transport chain (Nicholls and Malviya 1968), inhibition of the citric acid cycle (Brown et al. 2000), and increasing levels of reactive oxygen species (Sensi et al. 1999) via overactivation of superoxide-generating enzymes (Noh and Koh 2000) or LOX-12 (Zhang et al. 2004). Increasing levels of zinc also lead to p38 phosphorylation and ERK 1/2 activation which can trigger cell death (McLaughlin et al. 2001; Du et al. 2002).

Although the role of zinc in excitotoxic injury in animal and cell culture models is well documented, it is unclear whether modulating zinc pathways has any potential for treating human diseases.

4.4 Caspases and Calpains

Caspase inhibition prevents NMDA-mediated neuronal death in cerebrocortical (Tenneti et al. 1998) and cerebellar cultures (Du et al. 1997). Tenneti and Lipton (2000) described cytosolic activation of caspases at 20 min post-NMDA application with caspase activity in the nucleus at 18–24 h post-insult. Nuclear caspase activity is indicative of cleaved ICAD, a protein inhibiting the activity of caspase-3-activated DNase (CAD) and results in DNA fragmentation and cell death (Enari et al. 1998).

Brorson et al. (1995b) demonstrated modest neuroprotection in hippocampal cell cultures from NMDA insults with calpain inhibitors. The role of calpains remains less clear and may actually play a reparative role in axons with low, sublethal doses of NMDA (Faddis et al. 1997). Mu-calpain proteolytic activity is necessary in the cleavage and release of apoptosis-inducing factor (AIF) from mitochondria in a cell-free system (Polster et al. 2005). Further, calpain inhibition prevented AIF translocation and subsequent neuronal death in neuronal cultures subjected to oxygen–glucose deprivation (Cao et al. 2007), whereas other studies revealed opposite findings (Wang et al. 2009).

The existing evidence suggests that overactivation of PARP-1 secondary to an excitotoxic stimulus releases PAR polymers into the cytoplasm, which in turn activate calpains by an unclear mechanism. This, in some neuronal systems, leads to AIF release from mitochondria and subsequent cell death.

5 Excitotoxicity in Ischemia and Trauma

Jorgensen and Diemer (1982) first recognized a similar pattern of neuroanatomical degeneration in rats exposed to cerebral hypoxia/ischemia compared to rats treated systemically with monosodium glutamate (Olney 1969). Increased extracellular glutamate concentrations in response to ischemia were measured by microdialysis (Globus et al. 1988). In developing rats, sensitivity to NMDA neurotoxicity was found to parallel developmental vulnerability to hypoxic/ischemic injury (Ikonomidou et al. 1989), and hypoxia/ischemia was described to cause acute neuronal loss in the developing brain with ultrastructural characteristics identical to those caused by glutamate in infant rodent (Ikonomidou et al. 1989; Fig. 1). In adult global ischemia models, increases in glutamate concentrations were observed in the striatum, hippocampus, cortex, and thalamus (Globus et al. 1990). Focal ischemic models demonstrated similar patterns of elevated extracellular glutamate (Hillered et al. 1989).

Glutamate receptor antagonists were found to protect from ischemic injury. Both competitive and noncompetitive NMDA antagonists are effective in focal models of ischemia, and non-NMDA antagonists appear more effective in global cerebral ischemia models. Group I metabotropic glutamate receptor antagonists and group II agonists have been shown to be neuroprotective in gerbils subjected to transient global ischemia (Kingston et al. 1999).

Traumatic brain injury was also shown to result in marked increases in extracellular glutamate concentrations in animals (Faden et al. 1989; Nilsson et al. 1990) and in humans (Baker et al. 1993; Koura et al. 1998) and produce neuropathological changes similar to those induced by glutamate (Fig. 1). Glutamate receptor antagonists can confer neuroprotection in the context of traumatic injuries. NMDAR channel blockers reduced edema following fluid percussion brain injury in rats (McIntosh et al. 1989), whereas riluzole, a sodium channel blocker and glutamate release inhibitor, also provided histological neuroprotection (Zhang et al. 1998). Kynurenate and indole-2-carboxylic acid reduced cerebral edema and improved cognitive and motor dysfunction induced by trauma while protecting against hippocampal cell loss induced by fluid percussion injury (Hicks et al. 1994).

6 Excitotoxicity and Chronic Neurodegenerative Diseases

Morphological changes produced by excitotoxic compounds in the brain and spinal cord resemble in many aspects those observed in the context of chronic neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. Therefore, it has been suggested that excitotoxic mechanisms may partly mediate these disorders (Beal 1992b). Especially slow excitotoxicity, triggered by the impairment of mitochondrial energy metabolism, has been implicated.

Although the genetic basis for some of these diseases has been defined, the actual mechanism(s) leading to neurodegeneration are still not understood. Slow excitotoxicity may indeed constitute a contributing factor that could be amenable to pharmacological treatment.

Parkinson's disease (PD) is associated with degeneration of dopaminergic neurons in the ventral mesencephalon. Clinical symptoms consist of tremor, rigidity, bradykinesia, postural deficits, and impaired gait. A gradual decline in the number of dopaminergic neurons of the substantia nigra pars compacta and dopamine content with age is common in the general population. In PD there may be an accelerated rate of cell death, so that a critical level of dopaminergic cell loss (50–70 % of neurons) is reached during normal life span and neurological symptoms of PD become obvious (Bernheimer et al. 1973; McGeer et al. 1989; Fearnley and Lees 1991). It is also possible that an exogenous insult, such as an environmental toxin, may cause a partial loss of substantia nigra dopaminergic neurons which will be followed by further "physiological" decline with age (Calne and Langston 1983). Careful neuropathological studies, serial positron emission tomography (PET), and single photon emission spectroscopy (SPECT) demonstrate a more rapid decline of dopaminergic neurons in the substantia nigra of Parkinson patients as compared to control aging subjects (McGeer et al. 1989; Fearnley and Lees 1991; Brooks 1998).

Initial observations linking excitotoxicity to PD were made by Spencer and colleagues (Spencer 1987). They reported that the excitatory amino acid α-amino-beta-methylaminopropionic acid (BMAA) is linked to Guam ALS–parkinsonism dementia syndrome.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and its metabolite MPP^+ (1-methyl-4-phenylpyridinium ion) have been used to model PD, since it was discovered that MPTP can cause PD in man (Davis et al. 1979; Langston et al. 1983). The first report suggesting that excitotoxicity may play a role in the pathogenesis of MPTP-induced Parkinson's disease was made by Turski and colleagues (1991), who demonstrated that neurotoxicity of MPP^+, the active metabolite of MPTP, can be blocked in the substantia nigra with both competitive and noncompetitive NMDA receptor antagonists. Such results could be reproduced by other groups (Santiago et al. 1992; Brouillet and Beal 1993; Chan et al. 1993; Srivastava et al. 1993; Jones-Humble et al. 1994). Subsequent studies in primates confirmed that NMDA antagonists attenuated MPTP-induced depletion of substantia nigra dopaminergic neurons (Zuddas et al. 1992; Lange et al. 1993). Glutamate receptor blockers and neuronal NOS inhibitors have been reported to attenuate degeneration of dopamine neurons in MPTP-treated primates (Blum et al. 2001).

Considerable evidence implicates mitochondrial dysfunction in PD. Studies of the electron transport enzymes have been carried out in lymphocytes, platelets, muscle tissue, and postmortem brain tissue from PD patients (Schapira et al. 1990a, b; Di Monte et al. 1991; Hattori et al. 1991; Mann et al. 1992; Nakagawa-Hattori et al. 1992; DiDonato et al. 1993; Janetzky et al. 1994). Decrease in complex I activity was found in the brain, muscle tissue, and platelets of PD patients compared to control subjects. A nuclear magnetic resonance spectroscopy study to measure occipital lobe lactate concentration revealed a highly significant increase of lactate concentrations in PD patients with the largest increases in PD patients with dementia (Bowen et al. 1994). Similar findings indicating increased lactate levels were reported from the striatum of PD patients (Chen et al. 1994; Taylor et al. 1994).

Impaired mitochondrial function may increase vulnerability of affected neurons towards physiological concentrations of glutamate (slow excitotoxicity). Given the fact that the substantia nigra receives rich glutamatergic inputs from the neocortex and the subthalamic nucleus, contribution of slow excitotoxicity to the pathogenesis of this disease in the context of energy failure becomes a very attractive hypothesis.

Antiparkinsonian activity was observed with the noncompetitive NMDA antagonist dextromethorphan (Saez and Tanner, 1993) and in initial trials with the glutamate release inhibitor lamotrigine (Zipp et al. 1993), but these findings could not be confirmed in subsequent studies (Zipp et al. 1995; Montastruc et al. 1997). So far, the only compounds with antiglutamatergic properties that are used for the treatment of patients with PD are amantadine, memantine, and budipine (Schwab et al. 1969; Rabey et al. 1992; Adler et al. 1997). They are used to ameliorate clinical symptoms, but there is no clinical evidence indicating that they influence the course of the disease.

There have been reports that the AMPA receptor antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfonyl-benzo[f]quinoxaline) improves symptoms and enhances the effect of L-3,4-dihydroxyphenylalanine (L-DOPA) in MPTP-treated monkeys (Klockgether et al. 1991; Wachtel et al. 1992), but these findings could not

be replicated (Luquin et al. 1993; Papa et al. 1993; Zadow et al. 1994; Gossel et al. 1995). Due to such inconsistencies, AMPA receptor antagonists have not made their way into the clinical treatment of PD.

Huntington's disease (HD) is an autosomal dominant disease that gives rise to progressive, selective (localized) neural cell death associated with choreic movements and dementia. The disease is associated with increases in the length of a CAG triplet repeat present in a gene called "huntingtin" located on chromosome 4p16.3. The classic signs of Huntington's disease are progressive chorea, rigidity, and dementia, frequently associated with seizures. A characteristic atrophy of the caudate nucleus is seen radiographically. There is a prodromal phase of mild psychotic and behavioral symptoms which precedes frank chorea by up to 10 years.

The disease was initially modeled by injecting quinolinic acid (an NMDA agonist) into the striatum, leading to loss of GABAergic (GABA, γ-aminobutyric acid) output neurons and sparing interneurons containing somatostatin-NPY (Schwarcz and Kohler 1983; Schwarcz et al. 1983). This neuropathological pattern resembles that seen in Huntington's disease brains (Schwarcz et al. 1983; Beal et al. 1986). Based on these findings, an excitotoxicity hypothesis for the pathogenesis of Huntington's disease was formulated.

Administration of the mitochondrial toxin 3-nitropropionic acid (3-NP) to rodents produces pathology resembling Huntington's disease (Brouillet et al. 1995; Palfi et al. 1996; Borlongan et al. 1997). Interestingly, striatal toxicity of 3-NP can be ameliorated by NMDA receptor antagonists, such as memantine (Wenk et al. 1996), and glutamate release inhibitors, such as riluzole (Guyot et al. 1997). Similarly, striatal toxicity of malonate, a complex II–III inhibitor, was prevented by NMDA receptor antagonists MK-801 and memantine and by the glutamate release inhibitor lamotrigine (Greene and Greenamyre 1995). Thus, it has been postulated that mitochondrial dysfunction in Huntington's patients may be a trigger that constitutes striatal neurons prone to excitotoxicity.

The mechanism through which the widely expressed mutant HD gene mediates slowly progressing striatal neurotoxicity is unknown. Glutamate receptor-mediated excitotoxicity has been hypothesized to contribute to HD pathogenesis. However, inconsistent with this hypothesis, Hansson et al. (1999) showed that transgenic HD mice expressing exon 1 of the human HD gene with an expanded number of CAG repeats are strongly protected from acute striatal excitotoxic lesions. Intrastriatal infusions of quinolinic acid, the agonist of the NMDAR, caused massive striatal neuronal death in wild-type mice, but no damage in transgenic HD littermates. The remarkable neuroprotection in transgenic HD mice occurred at the stage when they had not developed any neurologic symptoms caused by the mutant HD gene. At this stage, there was no change in the number of striatal neurons and astrocytes in untreated transgenic mice, although the striatal volume was decreased by 17 %. Hansson et al. (1999) proposed that the presence of exon 1 of the mutant HD gene induces profound changes in striatal neurons that render these cells resistant to excessive NMDA receptor activation.

The antiglutamatergic compound remacemide, tested in a double-blind placebo-controlled study in HD patients, showed only a trend towards symptomatological

improvement (Kieburtz et al. 1996). Ketamine failed to show any symptomatological improvement (Murman et al. 1997). Thus, there is so far no evidence that symptomatological improvements should be expected by the use of antiglutamatergic compounds in HD patients.

Alzheimer's disease (AD) is by far the most common cause of dementia. Terry and Davies (1980) recommended the term senile dementia of the Alzheimer type (SDAT). Senile (neuritic) plaques and neurofibrillary tangles comprise the major neuropathological lesions in AD brains. Neuritic plaques are spherical lesions and are found in moderate or large numbers in limbic structures and association neocortex. They contain extracellular deposits of amyloid-β protein. Degenerating axons and dendrites are present within and around the amyloid deposits (Terry and Davies 1980; Selkoe 1999).

There are some indications that glutamate and the mechanism of excitotoxicity may be involved in pathogenesis and progression of AD. Constituents of senile plaques stimulate production by microglia of an unknown NMDA agonist (Giulian et al. 1995) as well as production of nitric oxide (NO), known to enhance glutamate release and inhibit its uptake (Lees 1993; Goodwin et al. 1995). β-Amyloid enhances glutamate toxicity (Brorson et al. 1995a; Mattson and Goodman 1995), enhances depolarization dependent glutamate release (Arias et al. 1995), and inhibits its glial uptake (Harris et al. 1996).

There is a decrease in astroglial glutamate EAA2 carrier in the frontal cortex of AD patients. Colocalization of glutamate neurons and neurofibrillary tangles was reported in AD brains (Braak et al. 1993; Francis et al. 1992). Finally, intracerebroventricular administration of β-amyloid produced long-lasting depression of EPSP that was prevented by the NMDA antagonist CPP (Cullen et al. 1996), whereas subcutaneous administration of memantine prevented pathological alterations in the hippocampus produced by direct injection of β-amyloid.

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in adults. It is characterized by selective degeneration of upper and lower motor neurons, progressive weakness and paralysis of all muscles, and spasticity (Cleveland and Rothstein 2001). Over 90 % of cases occur sporadically; in 5–10 % of cases the disease is inherited in a dominant mode (familial ALS).

Several pathogenetic mechanisms have been suggested. These include oxidative damage, neurofilament disorganization and subsequent axonal damage, toxicity from intracellular aggregates, and excitotoxicity (Cleveland and Rothstein 2001).

There is unquestionable and landmark evidence that familial ALS and some forms of sporadic ALS are caused by mutations in the Cu/Zn superoxide dismutase (SOD1) gene (Gaudette et al. 2000; Andersen 2001; Andersen et al. 2001). Multiple mutations have been identified so far and almost all provoke a dominantly inherited disease.

Debate on the involvement of excitotoxicity in ALS is based on studies suggesting that the metabolism of glutamate is abnormal in patients with ALS. Rothstein et al. (1992) postulated that the high-affinity glutamate transporter is the site of the defect at least in some forms of the disease. The inhibition of glutamate transport is toxic to neurons, due to persistent elevation of extracellular

glutamate. A possible mechanism for the elevated cerebrospinal fluid concentrations of glutamate and aspartate in patients with ALS could be deficient transport into cells. Studying synaptosomes from neural tissue obtained from 13 patients with ALS as well as from controls, Rothstein et al. (1992) found that ALS patients showed a marked diminution in the maximal velocity of transport for high-affinity glutamate uptake in synaptosomes from the spinal cord, motor cortex, and somatosensory cortex, but not in those from visual cortex, striatum, or hippocampus. This diminution was the result of a pronounced loss of the astroglial EAAT2 protein (Rothstein et al. 1995). Transport of other molecules (gamma-aminobutyric acid and phenylalanine) was normal in patients with ALS.

The fact is that in about one third of ALS patients, glutamate concentrations are increased in the cerebrospinal fluid (CSF) (Shaw et al. 1995), whereas intracellular glutamate concentrations are decreased (Perry et al. 1987; Young 1990; Ludolph et al. 1988). CSF from ALS patients is toxic to hippocampal neurons via stimulation of AMPA receptors (Couratier et al. 1993). The glutamate release inhibitor riluzole attenuated damage induced by ALS CSF (Couratier et al. 1994; Terro et al. 1996). These findings, along with experimental evidence implicating selective vulnerability of spinal motoneurons to AMPA/kainate-receptor agonists (Hugon et al. 1989; Ikonomidou et al. 1996; Williams et al. 1997), implicate that glutamate acting on AMPA/kainate receptors may be pathogenetically involved in motor neuron degeneration in ALS.

Excitotoxicity has been implicated in two other neurological disorders that resemble ALS, the Guam ALS–parkinsonism dementia syndrome and a disease described in the Kii peninsula of Japan. These two disorders have been linked to food excitotoxins (Spencer et al. 1986, 1993).

Along with the finding that SOD1 mutants, when expressed in mice, lead to functional loss of EAAT2 (Nagano et al. 1996), it seems likely that deficient glutamate uptake may contribute to the pathogenesis of some forms of ALS. Riluzole, a glutamate release inhibitor, achieved a modest effect at increasing survival in two independent trials (Bensimon et al. 1994; Lacomblez et al. 1996) and has been approved for the treatment of ALS, although patients do not experience slowing in disease progression.

7 Clinical Experience with Drugs Targeting Excitotoxicity in Stroke and Traumatic Brain Injury

Overall clinical experience has been extremely disappointing. Even though animal models demonstrate dramatic histological and behavioral neuroprotection, no pharmacological intervention targeting excitotoxicity has yet been shown to provide benefit in humans (Muir 2006).

Noncompetitive NMDAR antagonists have been shown in animal models to provide histological and behavioral neuroprotection following focal ischemia (Block and Schwarz 1996; Ozyurt et al. 1988; Seif et al. 1990; Steinberg et al. 1988). Cerestat (CNS 1102) was tested in a multicenter trial in 628 stroke patients

with low-dose, high-dose, and placebo treatments. The trial was stopped because of lack of effect and a trend towards higher mortality with high-dose Cerestat compared to placebo (Albers et al. 2001). Memantine has shown a much better side effect profile in humans (Areosa et al. 2005) and is currently approved for use only in moderate to severe Alzheimer's disease (Reisberg et al. 2003). No stroke trials with memantine have been performed.

The competitive NMDA antagonist Selfotel (CGS 19755) was shown to reduce hippocampal damage in gerbil models of ischemia (Boast et al. 1988). Phase III trials in stroke and in severe head injury were abandoned due to concerns about higher mortality in the treatment arms (Davis et al. 2000; Morris et al. 1999).

The glycine antagonist Gavestinel reached phase III clinical trials, but no difference in morbidity or mortality rates following treatment in stroke was evident (Lees et al. 2000). Ifenprodil and eliprodil, drugs binding at the NMDAR polyamine site, underwent phase III clinical trials but showed no efficacy.

AMPA receptor antagonists initially appeared more effective and showed a longer time-window in preventing neuronal loss in animal models of global ischemia (Sheardown et al. 1990; Buchan et al. 1991). YM872 demonstrated substantial neuroprotective effects in animal models of focal ischemia (Takahashi et al. 1998) and traumatic brain injury models (Furukawa et al. 2003), but phase III trials in ischemic stroke showed no efficacy in 2006.

Lamotrigine inhibits presynaptic voltage-dependent sodium channels and reduces overall excitability and neurotransmitter release (Leach et al. 1986). In animal models, lamotrigine failed to provide neuroprotection in focal ischemia (Tymianski et al. 1993a, b) but showed a reduction of CA1 hippocampal loss of 50 % following global ischemia (Crumrine et al. 1997). BW619C89, a derivative of lamotrigine, underwent phase II trials in 16 of 21 stroke patients (Muir et al. 2000). Clinical development was halted in 2001. Riluzole has also been shown to reduce infarct volumes and improve behavioral outcomes in ischemic (Malgouris et al. 1989; Wahl et al. 1993) and traumatic brain injury (Wahl et al. 1997; Zhang et al. 1998), but no clinical trials for stroke or traumatic brain injury have been performed. Riluzole is licensed for use in ALS (Miller et al. 2007).

8 Clinical Trials with Free Radical Scavengers

The nitrone-based antioxidant NXY-059 was tested in stroke. While the SAINT1 trial examining the efficacy of NXY-059 in clinical stroke was promising (Lees et al. 2000), the SAINT2 trial showed no clinical benefit with treatment (Shuaib et al. 2007). Human clinical trials with the 21-aminosteroid Tirilazad® in head injury resulted in decreased mortality only in males with severe head injury patients and subarachnoid hemorrhages (Marshall et al. 1998). Subsequent trials with Tirilazad in the treatment of subarachnoid hemorrhages (Lanzino and Kassell 1999; Lanzino et al. 1999) showed a reduction in mortality only with the highest grades on arrival, but meta-analysis of Tirilazad in the treatment of subarachnoid aneurysmal hemorrhage demonstrated no clinical outcome differences, but reduced

symptomatic vasospasm (Jang et al. 2009). A Cochrane Review demonstrated increased death or disability in stroke victims treated with Tirilazad compared to placebo in a review of six clinical trials without any statistically significant difference in overall mortality (Bath et al. 2001). Studies in ischemic stroke using Tirilazad have since been abandoned.

In a large multicenter stroke trial, the free radical trapping agent NXY-059, when given within 6 h after the onset of acute ischemic stroke, significantly improved the primary outcome (reduced disability at 90 days), but it did not significantly improve other outcome measures, including neurologic functioning as measured by the NIHSS score (Lees et al. 2006).

Ebselen is a glutathione peroxidase which can interact with peroxynitrite and inhibit enzymes involved in inflammation (Schewe 1995). Clinical trials in Japan demonstrated no significant differences in a 3-month clinical outcome in patients with complete middle cerebral artery occlusion (Yamaguchi et al. 1998) and no significant improvement in clinical outcome despite a reduction in infarct size (Ogawa et al. 1999).

A novel class of drugs named membrane-associated guanylate kinase (MAGUK) inhibitors are currently undergoing phase II clinical trials. The MAGUK inhibitor NA1 uncouples nNOS and NMDARs and has demonstrated neuroprotection in various animal models of stroke (Aarts et al. 2002; Sun et al. 2008).

9 Conclusion

Despite the overwhelming evidence that a variety of neurotoxic mechanisms triggered by excessive glutamate concentrations mediate brain injury in human neurological disorders, all treatment attempts with antiglutamatergic agents in humans have failed. One hypothesis for this failure is that glutamate has a dual function in the injured brain, a toxic one and a trophic one, and that the latter has been ignored.

Synaptic transmission mediated by NMDA receptors is essential for neuronal survival. Blockade of NMDA receptors for only a few hours triggers apoptosis in the developing brain (Ikonomidou et al. 1999; Pohl et al. 1999). NMDA antagonists, when administered during a critical period following traumatic brain injury or in the context of slowly ongoing neurodegeneration, markedly exacerbate damage in adult brain (Ikonomidou et al. 2000).

The Ca^{2+} pool in the immediate vicinity of synaptic NMDA receptors is the on switch for extracellular signal-regulated kinase (ERK1/2)-mediated synapse-to-nucleus signaling (Hardingham et al. 2001). One important function of this Ca^{2+} microdomain near NMDA receptors is to prolong phosphorylation of the transcription factor cyclic AMP response element-binding protein (CREB) induced by synaptic stimuli, thereby enhancing CREB-mediated gene expression. CREB controls transcription of prosurvival genes such as brain-derived neurotrophic factor (BDNF), vasoactive intestinal peptide (VIP), bcl-2, and mcl-1 (Fink et al. 1991; Tao et al. 1998; Riccio et al. 1999). It is therefore postulated that survival-promoting properties of

NMDAR activation derive from the transcription of such prosurvival genes. Neurons surviving ischemic insults (penumbra) demonstrate elevated concentrations of BDNF, bcl-2, and activated CREB, suggesting sustained induction of prosurvival signals (Chen et al. 1995; Tanaka et al. 1999, 2000; Walton and Dragunow 2000). From these findings the logical conclusion is that suppression of NMDA receptor-initiated survival-promoting signals with NMDA antagonists may facilitate death of such cells. Blockade of NMDA-mediated synaptic transmission must therefore be detrimental in situations, in which support by endogenous measures is required, as it happens after stroke or traumatic brain injury or in chronic neurodegenerative disorders (Ikonomidou and Turski 2002).

The long-term elevation of glutamate concentration in humans (days to weeks) after traumatic brain injury has been considered neurotoxic and interpreted as an opportunity for delayed therapy with NMDA antagonists (long time-window for therapy initiation). However, this interpretation may be wrong and such mild elevations of glutamate concentration may represent a self-defense mechanism of the injured brain.

Glutamate may indeed be involved in the initial acute neurodestructive phase after traumatic or ischemic injury, but after this period, it assumes its normal physiological functions, which include promotion of neuronal survival. The hypothesis was that mild increases of glutamate concentrations starting shortly after brain injury are necessary for supporting survival of neurons endangered to die after the insult and for maintaining their physiological functions. Conforming with this hypothesis, it was possible to demonstrate that NMDA antagonists are detrimental to neurons subjected to traumatic brain injury when administration starts after the initial profound elevation of extracellular glutamate concentrations has subsided, that is, 1–7 h after trauma (Ikonomidou et al. 2000). Antecedent treatment with NMDA antagonists in this same setting prevented traumatic neuronal death. These findings strongly argue for the hypothesis that glutamate indeed kills neurons over a brief period after injuries but starts to facilitate repair immediately thereafter. In contrast to its killer function, repair mediated by glutamate appears to be a long-lasting phenomenon. This is in obvious agreement with physiological function of glutamate in the nervous system during development.

Thus, to be successful in preventing excitotoxic brain injury, we would need to specifically target the neurodestructive pathways activated by glutamate while preserving the ones that promote survival. One promising strategy is in the horizon. PSD-95 inhibitors have been shown to confer neuroprotection in stroke models in rodents (Aarts et al. 2002; Sun et al. 2008). The 20-mer peptide Tat-NR2B9c, which acts by perturbing the protein–protein interactions of PSD-95, a synaptic protein that links NMDA receptors to neurotoxic signaling pathways, was most recently shown to confer histological and behavioral neuroprotection in gyrencephalic primates (Cook et al. 2012). This work rejuvenates hopes that more sophisticated and exclusive blockade of neurotoxic but not trophic mechanisms triggered by glutamate may one day provide a mode of treatment that will enable us to limit brain injuries in human patients.

References

Aarts, M., Liu, Y., Liu, L., Besshoh, S., Arundine, M., Gurd, J. W., Wang, Y. T., Salter, M. W., & Tymianski, M. (2002). Treatment of ischemic brain damage by perturbing NMDA receptor-PSD-95 protein interactions. *Science, 298*, 846–850.

Abe, T., Sugihara, H., Nawa, H., Shigemoto, R., Mizuno, N., & Nakanishi, S. (1992). Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca^{2+} signal transduction. *The Journal of Biological Chemistry, 267*, 13361–13368.

Adler, C. H., Stern, M. B., Vernon, G., & Hurtig, H. I. (1997). Amantadine in advanced Parkinson's disease: Good use of an old drug. *Journal of Neurology, 244*, 336–337.

Albers, G. W., Goldstein, L. B., Hall, D., Lesko, L. M., & Aptiganel Acute Stroke Investigators. (2001). Aptiganel hydrochloride in acute ischemic stroke: A randomized controlled trial. *Journal of the American Medical Association, 286*, 2673–2682.

Andersen, P. M. (2001). Genetics of sporadic ALS. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 2*, S37–S41.

Andersen, P. M., Moritam, M., & Brown, R. H., Jr. (2001). Nucleotide sequence, genomic organization, and chromosomal localization of genes encoding the human NMDA receptor subunits NR3A and NR3B. *Genomics, 78*, 178–184.

Areosa, S. A., Sherriff, F., & McShane, R. (2005). Memantine for dementia. *Cochrane Database Syst Rev, 2,* CD003154.

Arias, C., Arrieta, I., & Tapia, R. (1995). beta-Amyloid peptide fragment 25-35 potentiates the calcium-dependent release of excitatory amino acids from depolarized hippocampal slices. *Journal of Neuroscience Research, 41*, 561–566.

Baker, A. J., Moulton, R. J., MacMillan, V. H., & Shedden, P. M. (1993). Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans. *Journal of Neurosurgery, 79*, 369–372.

Bath, P. M., Iddenden, R., Bath, F. J., Orgogozo, J. M., & Tirilazad International Steering Committee. (2001). Tirilazad for acute ischaemic stroke. *Cochrane Database Syst Rev, 4*, CD002087.

Beal, M. F. (1992a). Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? *Annals of Neurology, 31*, 119–130.

Beal, M. F. (1992b). Role of excitotoxicity in human neurological disease. *Current Opinion in Neurology, 2*, 657–662.

Beal, M. F., Kowall, N. W., Ellison, D. W., Mazurek, M. F., Swartz, K. J., & Martin, J. B. (1986). Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature, 321*, 168–171.

Beal, M. F., Ferrante, R. J., Swartz, K. J., & Kowall, N. W. (1991). Chronic quinolinic acid lesions in rats closely resemble Huntington's disease. *The Journal of Neuroscience, 11*, 1649–1659.

Beal, M. F., Brouillet, E., Jenkins, B., Henshaw, R., Rosen, B., & Hyman, B. T. (1993a). Age-dependent striatal excitotoxic lesions produced by the endogenous mitochondrial inhibitor malonate. *Journal of Neurochemistry, 61*, 1147–1150.

Beal, M. F., Brouillet, E., Jenkins, B. G., Ferrante, R. J., Kowall, N. W., Miller, J. M., Storey, E., Srivastava, R., Rosen, B. R., & Hyman, B. T. (1993b). Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. *The Journal of Neuroscience, 13*, 4181–4192.

Bensimon, G., Lacomblez, L., Meininger, V., & The ALS/Riluzole Study Group. (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. *The New England Journal of Medicine, 330*, 585–591.

Benveniste, M., & Mayer, M. L. (1993). Multiple effects of spermine on N-methyl-D-aspartic acid receptor responses of rat cultured hippocampal neurones. *The Journal of Physiology, 464*, 131–163.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *Journal of Neurological Sciences, 20*, 415–455.

Block, F., & Schwarz, M. (1996). Memantine reduces functional and morphological consequences induced by global ischemia in rats. *Neuroscience Letters, 208*(1), 41–44.

Blum, D., Torch, S., Lambeng, N., Nissou, M., Benabid, A. L., Sadoul, R., & Verna, J. M. (2001). Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: Contribution to the apoptotic theory in Parkinson's disease. *Progress in Neurobiology, 65*, 135–172.

Boast, C. A., Gerhardt, S. C., Pastor, G., Lehmann, J., Etienne, P. E., & Liebman, J. M. (1988). The N-methyl-D-aspartate antagonists CGS 19755 and CPP reduce ischemic brain damage in gerbils. *Brain Research, 442*, 345–348.

Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P., & Lipton, S. A. (1995). Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 7162–7166.

Borlongan, C. V., Koutouzis, T. K., & Sanberg, P. R. (1997). 3-Nitropropionic acid animal model and Huntington's disease. *Neuroscience and Biobehavioral Reviews, 21*, 289–293.

Bowen, B. C., Block, R. E., Sanchez-Ramos, J., Pattany, P. M., Lampman, D. A., Murdoch, J. B., & Quencer, R. M. (1994). Proton MR spectroscopy of the brain in 14 patients with Parkinson's disease. *American Journal of Neuroradiology, 16*, 61–68.

Braak, H., Braak, E., & Bohl, J. (1993). Staging of Alzheimer-related cortical destruction. *European Neurology, 33*, 403–408.

Bradley, S. R., Levey, A. I., Hersch, S. M., & Conn, P. J. (1996). Immunocytochemical localization of group III metabotropic glutamate receptors in the hippocampus with subtype-specific antibodies. *The Journal of Neuroscience, 16*, 2044–2056.

Brooks, D. J. (1998). The early diagnosis of Parkinson's disease. *Annals of Neurology, 44*(Suppl), S10–S18.

Brorson, J. R., Bindokas, V. P., Iwama, T., Marcuccilli, C. J., Chisholm, J. C., & Miller, R. J. (1995a). The Ca^{2+} influx induced by beta-amyloid peptide 25-35 in cultured hippocampal neurons results from network excitation. *Journal of Neurobiology, 26*, 325–338.

Brorson, J. R., Marcuccilli, C. J., & Miller, R. J. (1995b). Delayed antagonism of calpain reduces excitotoxicity in cultured neurons. *Stroke, 26*, 1259–1266.

Brouillet, E., & Beal, M. F. (1993). NMDA antagonists partially protect against MPTP induced neurotoxicity in mice. *Neuroreport, 4*, 387–390.

Brouillet, E., Hantraye, P., Ferrante, R. J., Dolan, R., Leroy-Willig, A., Kowall, N. W., & Beal, M. F. (1995). Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 7105–7109.

Brown, A. M., Kristal, B. S., Effron, M. S., Shestopalov, A. I., Ullucci, P. A., Sheu, K. F., Blass, J. P., & Cooper, A. J. (2000). Zn^{2+} inhibits alpha-ketoglutarate-stimulated mitochondrial respiration and the isolated alpha-ketoglutarate dehydrogenase complex. *The Journal of Biological Chemistry, 275*, 13441–13447.

Bruno, V., Battaglia, G., Copani, A., Giffard, R. G., Raciti, G., Raffaele, R., Shinozaki, H., & Nicoletti, F. (1995a). Activation of class II or III metabotropic glutamate receptors protects cultured cortical neurons against excitotoxic degeneration. *The European Journal of Neuroscience, 7*, 1906–1913.

Bruno, V., Copani, A., Knopfel, T., Kuhn, R., Casabona, G., Dell'Albani, P., Condorelli, D. F., & Nicoletti, F. (1995b). Activation of metabotropic glutamate receptors coupled to inositol phospholipid hydrolysis amplifies NMDA-induced neuronal degeneration in cultured cortical cells. *Neuropharmacology, 34*, 1089–1098.

Buchan, A. M., Xue, D., Huang, Z. G., Smith, K. H., & Lesiuk, H. (1991). Delayed AMPA receptor blockade reduces cerebral infarction induced by focal ischemia. *Neuroreport, 2*, 473–476.

Burnashev, N., Schoepfer, R., Monyer, H., Ruppersberg, J. P., Gunther, W., Seeburg, P. H., & Sakmann, B. (1992). Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptors. *Science, 257*, 1415–1419.

Burnashev, N., Zhou, Z., Neher, E., & Sakmann, B. (1995). Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. *The Journal of Physiology, 485*(Pt 2), 403–418.

Calne, D. B., & Langston, J. W. (1983). Aetiology of Parkinson's disease. *Lancet, 2*(8365–66), 1457–1459.

Cao, G., Xing, J., Xiao, X., Liou, A. K., Gao, Y., Yin, X. M., Clark, R. S., Graham, S. H., & Chen, J. (2007). Critical role of calpain I in mitochondrial release of apoptosis-inducing factor in ischemic neuronal injury. *The Journal of Neuroscience, 27*, 9278–9293.

Castillo, P. E., Malenka, R. C., & Nicoll, R. A. (1997). Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. *Nature, 388*, 182–186.

Chan, P., Langston, J. W., & Monte, D. A. (1993). MK-801 temporarily prevents MPTP-induced acute dopamine depletion and MPP^+ elimination in the mouse striatum. *The Journal of Pharmacology and Experimental Therapeutics, 267*, 1515–1520.

Chatterton, J. E., Awobuluyi, M., Premkumar, L. S., Takahashi, H., Talantova, M., Shin, Y., Cui, J., Tu, S., Sevarino, K. A., Nakanishi, N., Tong, G., Lipton, S. A., & Zhang, D. (2002). Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature, 415*, 793–798.

Chavis, P., Shinozaki, H., Bockaert, J., & Fagni, L. (1994). The metabotropic glutamate receptor types 2/3 inhibit L-type calcium channels via a pertussis toxin-sensitive G-protein in cultured cerebellar granule cells. *The Journal of Neuroscience, 14*, 7067–7076.

Chen, Y. I., Jenkins, B. G., & Rosen, B. R. (1994). Evidence for impairment of energy metabolism in Parkinson's disease using in vivo localized MR spectroscopy. *Proceedings of International Society for Magnetic Resonance in Medicine, 1*, 194.

Chen, J., Graham, S. H., Chan, P. H., Lan, J., Zhou, R. L., & Simon, R. P. (1995). BCL-2 is expressed in neurons that survive focal ischemia in the rat. *Neuroreport, 6*, 394–398.

Choi, D. W. (1985). Glutamate neurotoxicity in cortical cell culture is calcium dependent. *Neuroscience Letters, 58*, 293–297.

Choi, D. W. (1987). Ionic dependence of glutamate neurotoxicity. *The Journal of Neuroscience, 7*, 369–379.

Choi, D. W., Maulucci-Gedde, M., & Kriegstein, A. R. (1987). Glutamate neurotoxicity in cortical cell culture. *The Journal of Neuroscience, 7*, 357–368.

Cleveland, D. W., & Rothstein, J. D. (2001). From Charcot to Lou Gehrig: Deciphering selective motor neuron death in ALS. *Nature Reviews Neuroscience, 2*(11), 806–819.

Cook, D. J., Teves, L., & Tymianski, M. (2012). Treatment of stroke with PSD-05 inhibitor in the gyrencephalic primate brain. *Nature, 483*, 213–218.

Couratier, P., Hugon, J., Sindou, P., Vallat, J. M., & Dumas, M. (1993). Cell culture evidence for neuronal degeneration in amyotrophic lateral sclerosis being linked to glutamate AMPA/kainate receptors. *Lancet, 341*, 265–268.

Couratier, P., Sindou, P., Esclaire, F., & Hugon, J. (1994). Neuroprotective effects of riluzole in ALS CSF toxicity. *Neuroreport, 5*, 1012–1014.

Crumrine, R. C., Bergstrand, K., Cooper, A. T., Faison, W. L., & Cooper, B. R. (1997). Lamotrigine protects hippocampal CA1 neurons from ischemic damage after cardiac arrest. *Stroke, 28*, 2230–2236.

Cullen, W. K., Wu, J. Q., Anwyl, R., & Rowan, M. J. (1996). beta-Amyloid produces a delayed NMDA receptor-dependent reduction in synaptic transmission in rat hippocampus. *Neuroreport, 8*, 87–92.

Curtis, D. R., Phillis, J. W., & Watkins, J. C. (1959). Chemical excitation of spinal neurones. *Nature, 183*, 611–612.

Das, S., Sasaki, Y. F., Rothe, T., Premkumar, L. S., Takasu, M., Crandall, J. E., Dikkes, P., Conner, D. A., Rayudu, P. V., Cheung, W., Chen, H. S., Lipton, S. A., & Nakanishi, N. (1998).

Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature, 393*, 377–381.

Davis, G. C., Williams, A. C., Markey, S. P., Ebert, M. H., Caine, E. D., Reichert, C. M., & Kopin, I. J. (1979). Chronic parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Research, 1*, 249–254.

Davis, S. M., Lees, K. R., Albers, G. W., Diener, H. C., Markabi, S., Karlsson, G., & Norris, J. (2000). Selfotel in acute ischemic stroke: Possible neurotoxic effects of an NMDA antagonist. *Stroke, 31*, 347–354.

Dawson, V. L., Dawson, T. M., London, E. D., Bredt, D. S., & Snyder, S. H. (1991). Nitric oxide mediates glutamate neurotoxicity in primary cortical culture. *Proceedings of the National Academy of Sciences of the United States of America, 88*, 6368–6371.

Dawson, V. L., Kizushi, V. M., Huang, P. L., Snyder, S. H., & Dawson, T. M. (1996). Resistance to neurotoxicity in cortical cultures from neuronal nitric oxide synthase-deficient mice. *The Journal of Neuroscience, 16*, 2479–2487.

Di Monte, D., Tetrud, J. W., & Langston, J. W. (1991). Blood lactate in Parkinson's disease and senescence. *Biochemical and Biophysical Research Communications, 170*, 483–489.

DiDonato, S., Zeviani, M., Giovannini, P., Savarese, N., Rimoldi, M., Mariotti, C., Girotti, F., & Caraceni, T. (1993). Respiratory chain and mitochondrial DNA in muscle and brain in Parkinson's disease patients. *Neurology, 43*, 2262–2268.

Du, Y., Bales, K. R., Dodel, R. C., Hamilton-Byrd, E., Horn, J. W., Czilli, D. L., Simmons, L. K., Ni, B., & Paul, S. M. (1997). Activation of a caspase 3-related cysteine protease is required for glutamate-mediated apoptosis of cultured cerebellar granule neurons. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 11657–11662.

Du, S., McLaughlin, B., Pal, S., & Aizenman, E. (2002). In vitro neurotoxicity of methylisothiazolinone, a commonly used industrial and household biocide, proceeds via a zinc and extracellular signal-regulated kinase mitogen-activated protein kinase-dependent pathway. *The Journal of Neuroscience, 22*, 7408–7416.

Dugan, L. L., Sensi, S. L., Canzoniero, L. M., Handran, S. D., Rothman, S. M., Lin, T. S., Goldberg, M. P., & Choi, D. W. (1995). Mitochondrial production of reactive oxygen species in cortical neurons following exposure to N-methyl-D-aspartate. *The Journal of Neuroscience, 15*, 6377–6388.

Dykens, J. A. (1994). Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated Ca^{2+} and Na^{+}: Implications for neurodegeneration. *Journal of Neurochemistry, 63*, 584–591.

Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A., & Nagata, S. (1998). A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature, 391*, 43–50.

Faddis, B. T., Hasbani, M. J., & Goldberg, M. P. (1997). Calpain activation contributes to dendritic remodeling after brief excitotoxic injury in vitro. *The Journal of Neuroscience, 17*, 951–959.

Faden, A. I., Demediuk, P., Panter, S. S., & Vink, R. (1989). The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science, 244*, 798–800.

Fearnley, J., & Lees, A. (1991). Parkinson's disease: Neuropathology. *Brain, 114*, 2283–2301.

Fink, J. S., Verhave, M., Walton, K., Mandel, G., & Goodman, R. H. (1991). Cyclic AMP- and phorbol ester-induced transcriptional activation are mediated by the same enhancer element in the human vasoactive intestinal peptide gene. *The Journal of Biological Chemistry, 266*, 3882–3887.

Fonnum, F. (1984). Glutamate: A neurotransmitter in mammalian brain. *Journal of Neurochemistry, 42*, 1–11.

Francis, P. T., Pangalos, M. N., & Bowen, D. M. (1992). Animal and drug modelling for Alzheimer synaptic pathology. *Progress in Neurobiology, 39*, 517–545.

Frandsen, A., & Schousboe, A. (1991). Dantrolene prevents glutamate cytotoxicity and Ca^{2+}-release from intracellular stores in cultures cerebral cortical neurons. *Journal of Neurochemistry, 56*, 1075–1078.

Frandsen, A., & Schousboe, A. (1992). Mobilization of dantrolene-sensitive intracellular calcium pools is involved in the cytotoxicity induced by quisqualate and N-methyl-D-aspartate but not 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate and kainate in cultured cerebral cortical neurons. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 2590–2594.

Frederickson, C. J., Hernandez, M. D., & McGinty, J. F. (1989). Translocation of zinc may contribute to seizure-induced death of neurons. *Brain Research, 480*, 317–321.

Frerking, M., Schmitz, D., Zhou, Q., Johansen, J., & Nicoll, R. A. (2001). Kainate receptors depress excitatory synaptic transmission at CA3 → CA1 synapses in the hippocampus via a direct presynaptic action. *The Journal of Neuroscience, 21*, 2958–2966.

Furukawa, T., Hoshino, S., Kobayashi, S., Asakura, T., Takahashi, M., Atsumi, T., & Teramoto, A. (2003). The glutamate AMPA receptor antagonist, YM872, attenuates cortical tissue loss, regional cerebral edema, and neurological motor deficits after experimental brain injury in rats. *Journal of Neurotrauma, 20*, 269–278.

Gaudette, M., Hirano, M., & Siddique, T. (2000). Current status of SOD1 mutations in familial amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 1*, 83–89.

Geiger, J. R., Melcher, T., Koh, D. S., Sakmann, B., Seeburg, P. H., Jonas, P., & Monyer, H. (1995). Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron, 15*, 193–204.

Giulian, D., Haverkamp, L. J., Li, J., Karshin, W. L., Yu, J., Tom, D., Li, X., & Kirkpatrick, J. B. (1995). Senile plaques stimulate microglia to release a neurotoxin found in Alzheimer brain. *Neurochemistry International, 27*, 119–137.

Globus, M. Y., Busto, R., Dietrich, W. D., Martinez, E., Valdes, I., & Ginsberg, M. D. (1988). Intra-ischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. *Neuroscience Letters, 91*, 36–40.

Globus, M. Y., Busto, R., Martinez, E., Valdés, I., & Dietrich, W. D. (1990). Ischemia induces release of glutamate in regions spared from histopathologic damage in the rat. *Stroke, 21*, III43–III46.

Gonzalez-Zulueta, M., Ensz, L. M., Mukhina, G., Lebovitz, R. M., Zwacka, R. M., Engelhardt, J. F., Oberley, L. W., Dawson, V. L., & Dawson, T. M. (1998). Manganese superoxide dismutase protects nNOS neurons from NMDA and nitric oxide-mediated neurotoxicity. *The Journal of Neuroscience, 18*, 2040–2055.

Goodwin, J. L., Uemura, E., & Cunnick, J. E. (1995). Microglial release of nitric oxide by the synergistic action of beta-amyloid and IFN-gamma. *Brain Research, 692*, 207–214.

Gossel, M., Schmidt, W. J., Loscher, W., Zajaczkowski, W., & Danysz, W. J. (1995). Effect of coadministration of glutamate receptor antagonists and dopaminergic agonists on locomotion in monoamine-depleted rats. *Journal of Neural Transmission, 19*, 27–39.

Greene, J. G., & Greenamyre, J. T. (1995). Manipulation of membrane potential modulates malonate-induced striatal excitotoxicity in vivo. *Journal of Neurochemistry, 64*, 2332–2338.

Greene, J. G., Porter, R. H. P., Eller, R. V., & Greenamyre, J. T. (1993). Inhibition of succinate dehydrogenase by malonic acid produces an "excitotoxic" lesion in rat striatum. *Journal of Neurochemistry, 61*, 1151–1154.

Guerrini, L., Blasi, F., & Denis, D. (1995). Synaptic activation of NF-kappa B by glutamate in cerebellar granule neurons in vitro. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 9077–9081.

Guyot, M. C., Palfi, S., Stutzmann, J. M., Maziere, M., Hantraye, P., & Brouillet, E. (1997). Riluzole protects from motor deficits and striatal degeneration produced by systemic 3-nitropropionic acid intoxication in rats. *Neuroscience, 81*, 141–149.

Hansson, O., Petersen, A., Leist, M., Nicotera, P., Castilho, R. F., & Brundin, P. (1999). Transgenic mice expressing a Huntington's disease mutation are resistant to quinolinic acid-induced striatal excitotoxicity. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 8727–8732.

Hara, M. R., Agrawal, N., Kim, S. F., Cascio, M. B., Fujimuro, M., Ozeki, Y., Takahashi, M., Cheah, J. H., Tankou, S. K., Hester, L. D., Ferris, C. D., Hayward, S. D., Snyder, S. H., & Sawa, A. (2005). S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nature Cell Biology, 7*, 665–674.

Hardingham, G. E., Arnold, F. J. L., & Bading, H. (2001). A calcium microdomain near NMDA receptors: On switch for ERK-dependent synapse-to-nucleus communication. *Nature Neuroscience, 4*, 565–566.

Harris, M. E., Wang, Y., Pedigo, N. W., Jr., Hensley, K., Butterfield, D. A., & Carney, J. M. (1996). Amyloid beta peptide (25–35) inhibits Na^+ -dependent glutamate uptake in rat hippocampal astrocyte cultures. *Journal of Neurochemistry, 67*, 277–286.

Hattori, N., Tanaka, M., Ozawa, T., & Mizuno, Y. (1991). Immunohistochemical studies on complexes I, II, III and IV of mitochondria in Parkinson's disease. *Annals of Neurology, 30*, 563–571.

Hayashi, T. (1952). A physiological study of epileptic seizures following cortical stimulation in animals and its application to human clinics. *Japanese Journal of Pharmacology, 3*, 46–64.

Hayashi, T. (1958). Inhibition and excitation due to g-aminobutyric acid in the central nervous system. *Nature, 182*, 1076–1077.

Henshaw, R., Jenkins, B. G., Schulz, J. B., Ferrante, R. J., Kowall, N. W., Rosen, B. R., & Beal, M. F. (1994). Malonate produces striatal lesions by indirect NMDA receptor activation. *Brain Research, 647*, 161–166.

Herb, A., Burnashev, N., Werner, P., Sakmann, B., Wisden, W., & Seeburg, P. H. (1992). The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. *Neuron, 8*, 775–785.

Herb, A., Higuchi, M., Sprengel, R., & Seeburg, P. H. (1996). Q/R site editing in kainate receptor GluR5 and GluR6 pre-mRNAs requires distant intronic sequences. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 1875–1880.

Heuss, C., Scanziani, M., Gahwiler, B. H., & Gerber, U. (1999). G-protein-independent signaling mediated by metabotropic glutamate receptors. *Nature Neuroscience, 2*, 1070–1077.

Hicks, R. R., Smith, D. H., Gennarelli, T. A., & McIntosh, T. (1994). Kynurenate is neuroprotective following experimental brain injury in the rat. *Brain Research, 655*, 91–96.

Hillered, L., Hallstrom, A., Segersvard, S., Persson, L., & Ungerstedt, U. (1989). Dynamics of extracellular metabolites in the striatum after middle cerebral artery occlusion in the rat monitored by intracerebral microdialysis. *Journal of Cerebral Blood Flow and Metabolism, 9*, 607–616.

Hollmann, M., & Heinemann, S. (1994). Cloned glutamate receptors. *Annual Review of Neuroscience, 17*, 31–108.

Hollmann, M., Hartley, M., & Heinemann, S. (1991). Ca^{2+} permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. *Science, 252*, 851–853.

Hugon, J., Vallat, J. M., Spencer, P. S., Leboutet, M. J., & Barthe, D. (1989). Kainic acid induces early and delayed degenerative neuronal changes in rat spinal cord. *Neuroscience Letters, 104*, 258–262.

Huie, R. E., & Padmaja, S. (1993). The reaction of no with superoxide. *Free Radical Research Communications, 18*, 195–199.

Ikonomidou, C., & Kaindl, A. M. (2011). Neuronal death and oxidative stress in the developing brain. *Antioxidants and Redox Signaling, 14*, 1535–1550.

Ikonomidou, C., & Turski, L. (1996). Neurodegenerative disorders: Clues from glutamate and energy metabolism. *Critical Reviews in Neurobiology, 10*, 239–263.

Ikonomidou, C., & Turski, L. (2002). Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? *Lancet Neurology, 1*, 383–386.

Ikonomidou, C., Mosinger, J. L., Salles, K. S., Labruyere, J., & Olney, J. W. (1989). Sensitivity of the developing rat brain to hypobaric/ischemic damage parallels sensitivity to N-methyl-aspartate neurotoxicity. *The Journal of Neuroscience, 9*, 2809–2818.

Ikonomidou, C., Qin, Y.-Q., Labruyere, J., & Olney, J. W. (1996). Motor neuron degeneration induced by excitotoxin agonists has features in common with those seen in the SOD-1

transgenic mouse model of amyotrophic lateral sclerosis. *Journal of Neuropathology and Experimental Neurology, 55*, 211–224.

Ikonomidou, C., Bosch, F., Miksa, M., Vöckler, J., Bittigau, P., Dikranian, K., Tenkova, T., Turski, L., & Olney, J. W. (1999). Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science, 283*, 70–74.

Ikonomidou, C., Stefovska, V., & Turski, L. (2000). Neuronal death enhanced by N-methyl-D-aspartate antagonists. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 12885–12890.

Inoue, K., Branigan, D., & Xiong, Z. G. (2010). Zinc-induced neurotoxicity mediated by transient receptor potential melastatin 7 channels. *The Journal of Biological Chemistry, 285*, 7430–7439.

Ischiropoulos, H., Zhu, L., Chen, J., Tsai, M., Martin, J. C., Smith, C. D., & Beckman, J. S. (1992). Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Archives of Biochemistry and Biophysics, 298*, 431–437.

Janetzky, B., Hauck, S., Youdim, M. B., Riederer, P., Jellinger, K., Pantucek, F., Zochling, R., Boissl, K. W., & Reichmann, H. (1994). Unaltered aconitase activity, but decreased complex I activity in substantia nigra pars compacta of patients with Parkinson's disease. *Neuroscience Letters, 169*, 126–128.

Jang, Y. G., Ilodigwe, D., & Macdonald, R. L. (2009). Metaanalysis of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage. *Neurocritical Care, 10*, 141–147.

Johnson, J. W., & Ascher, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature, 325*, 529–531.

Jones-Humble, S. A., Morgan, P. F., & Cooper, B. R. (1994). The novel anticonvulsant lamotrigine prevents dopamine depletion in C57 black mice in the MPTP animal model of Parkinson's disease. *Life Sciences, 54*, 245–252.

Jorgensen, M. B., & Diemer, N. H. (1982). Selective neuron loss after cerebral ischemia in the rat: Possible role of transmitter glutamate. *Acta Neurologica Scandinavica, 66*, 536–546.

Kieburtz, K., Feigin, A., McDermott, M., Como, P., Abwender, D., Zimmermann, C., Hickey, C., Orme, C., Claude, K., Sotack, J., Greenamyre, J. T., Dunn, C., & Shoulson, I. (1996). A controlled trial of remacemide hydrochloride in Huntington's disease. *Movement Disorders, 11*, 273–277.

Kingston, A. E., O'Neill, M. J., Bond, A., Bruno, V., Battaglia, G., Nicoletti, F., Harris, J. R., Clark, B. P., Monn, J. A., Lodge, D., & Schoepp, D. D. (1999). Neuroprotective actions of novel and potent ligands of group I and group II metabotropic glutamate receptors. *Annals of the New York Academy of Sciences, 890*, 438–449.

Klockgether, T., Turski, L., Honore, T., Zhang, Z. M., Gash, D. M., Kurlan, R., & Greenamyre, J. T. (1991). The AMPA receptor antagonist NBQX has antiparkinsonian effects in monoamine-depleted rats and MPTP-treated monkeys. *Annals of Neurology, 30*, 717–723.

Koh, J. Y., Suh, S. W., Gwag, B. J., He, Y. Y., Hsu, C. Y., & Choi, D. W. (1996). The role of zinc in selective neuronal death after transient global cerebral ischemia. *Science, 272*, 1013–1016.

Komuro, H., & Rakic, P. (1993). Modulation of neuronal migration by NMDA receptors. *Science, 260*, 95–97.

Koura, S. S., Doppenberg, E. M., Marmarou, A., Choi, S., Young, H. F., & Bullock, R. (1998). Relationship between excitatory amino acid release and outcome after severe human head injury. *Acta Neurochirurgica. Supplement (Wien), 71*, 244–246.

Krebs, H. A. (1935). Metabolism of amino acids. III Deamination of amino acids. *Biochemical Journal, 29*, 1520–1644.

Lacomblez, L., Bensimon, G., Leigh, P. N., Guillet, P., & Meininger, V. (1996). Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet, 347*, 1425–1431.

Lafon-Cazal, M., Pietri, S., Culcasi, M., & Bockaert, J. (1993). NMDA-dependent superoxide production and neurotoxicity. *Nature, 364*, 535–537.

Lange, K. W., Loschmann, P. A., Sofic, E., Burg, M., Horowski, R., Kalveram, K. T., Wachtel, H., & Riederer, P. (1993). The competitive NMDA antagonists CPP protect substantia nigra neurons

from MPTP-induced degeneration in primates. *Naunyn-Schmiedeberg's Archives of Pharmacology, 348*, 586–592.

Langston, J. W., Ballard, P., Tetrud, J. W., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science, 219*, 979–980.

Lanzino, G., & Kassell, N. F. (1999). Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage. Part II. A cooperative study in North America. *Journal of Neurosurgery, 90*, 1018–1024.

Lanzino, G., Kassell, N. F., Dorsch, N. W., Pasqualin, A., Brandt, L., Schmiedek, P., Truskowski, L. L., & Alves, W. M. (1999). Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage. Part I. A cooperative study in Europe, Australia, New Zealand, and South Africa. *Journal of Neurosurgery, 90*, 1011–1017.

Laurie, D. J., & Seeburg, P. H. (1994). Ligand affinities at recombinant N-methyl-D-aspartate receptors depend on subunit composition. *European Journal of Pharmacology, 268*(3), 335–345.

Leach, M. J., Marden, C. M., & Miller, A. A. (1986). Pharmacological studies on lamotrigine, a novel potential antiepileptic drug: II. Neurochemical studies on the mechanism of action. *Epilepsia, 27*, 490–497.

Lees, G. J. (1993). The possible contribution of microglia and macrophages to delayed neuronal death after ischemia. *Journal of Neurological Sciences, 114*, 119–122.

Lees, K. R., Asplund, K., Carolei, A., Davis, S. M., Diener, H. C., Kaste, M., Orgogozo, J. M., & Whitehead, J. (2000). Glycine antagonist (gavestinel) in neuroprotection (GAIN International) in patients with acute stroke: A randomised controlled trial. GAIN International Investigators. *Lancet, 355*, 1949–1954.

Lees, K. R., Zivin, J. A., Ashwood, T., Davalos, A., Davis, S. M., Diener, H. C., Grotta, J., Lyden, P., Shuaib, A., Hårdemark, H. G., Wasiewski, W. W., & Stroke-Acute Ischemic NXY Treatment (SAINT I) Trial Investigators. (2006). NXY-059 for acute ischemic stroke. *The New England Journal of Medicine, 354*, 588–600.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium-L-glutamate on the inner layers of the retina. *Archives of Ophthalmology, 58*, 193–201.

Ludolph, A. C., Meyer, T., Riepe, M. W., & Vökel, H. (1988). Amyotrophic lateral sclerosis and glutamate. *Restorative Neurology and Neuroscience, 13*, 59–67.

Luquin, M. R., Obeso, J. A., Laguna, J., Guillen, J., & Martineslage, J. M. (1993). The AMPA receptor antagonist NBQX does not alter the motor response induced by selective dopamine agonists in MPTP-treated monkeys. *European Journal of Pharmacology, 235*, 297–300.

Malgouris, C., Bardot, F., Daniel, M., Pellis, F., Rataud, J., Uzan, A., Blanchard, J. C., & Laduron, P. M. (1989). Riluzole, a novel antiglutamate, prevents memory loss and hippocampal neuronal damage in ischemic gerbils. *The Journal of Neuroscience, 9*, 3720–3727.

Mann, V. M., Cooper, J. M., Krige, D., Daniel, S. E., Schapira, A. H., & Marsden, C. D. (1992). Brain, skeletal muscle and platelet homogenate mitochondrial function in Parkinson's disease. *Brain, 115*, 333–342.

Marshall, L. F., Maas, A. I., Marshall, S. B., Bricolo, A., Fearnside, M., Iannotti, F., Klauber, M. R., Lagarrigue, J., Lobato, R., Persson, L., Pickard, J. D., Piek, J., Servadei, F., Wellis, G. N., Morris, G. F., Means, E. D., & Musch, B. (1998). A multicenter trial on the efficacy of using tirilazad mesylate in cases of head injury. *Journal of Neurosurgery, 89*, 519–525.

Martinez-Guijarro, F. J., Soriano, E., Del Rio, J. A., & Lopez-Garcia, C. (1991). Zinc-positive boutons in the cerebral cortex of lizards show glutamate immunoreactivity. *Journal of Neurocytology, 20*, 834–843.

Matsuda, K., Kamiya, Y., Matsuda, S., & Yuzaki, M. (2002). Cloning and characterization of a novel NMDA receptor subunit NR3B: A dominant subunit that reduces calcium permeability. *Brain Research. Molecular Brain Research, 100*, 43–52.

Mattson, M. P., & Goodman, Y. (1995). Different amyloidogenic peptides share a similar mechanism of neurotoxicity involving reactive oxygen species and calcium. *Brain Research, 676*, 219–224.

Mayer, M. L., & Vyklicky, L., Jr. (1989). The action of zinc on synaptic transmission and neuronal excitability in cultures of mouse hippocampus. *The Journal of Physiology, 415*, 351–365.

Mayer, M. L., Westbrook, G. L., & Guthrie, P. B. (1984). Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature, 309*, 261–263.

McDonald, J. W., & Schoepp, D. D. (1992). The metabotropic excitatory amino acid receptors agonist 1S,3R-ACPD selectively potentiates N-methyl-D-aspartate-induced brain injury. *European Journal of Pharmacology, 215*, 353–354.

McGeer, P. L., Itagaki, S., Akiyama, H., & McGeer, E. G. (1989). Rate of cell death in parkinsonism indicates active neuropathological process. *Annals of Neurology, 24*, 574–576.

McIntosh, T. K., Vink, R., Soares, H., Hayes, R., & Simon, R. (1989). Effects of the N-methyl-D-aspartate receptor blocker MK-801 on neurologic function after experimental brain injury. *Journal of Neurotrauma, 6*, 247–259.

McLaughlin, B., Pal, S., Tran, M. P., Parsons, A. A., Barone, F. C., Erhardt, J. A., & Aizenman, E. (2001). p38 activation is required upstream of potassium current enhancement and caspase cleavage in thiol oxidant-induced neuronal apoptosis. *The Journal of Neuroscience, 21*, 3303–3311.

McMaster, O. G., Du, F., French, E. D., & Schwarcz, R. (1991). Focal injection of aminooxyacetic acid produces seizures and lesions in rat hippocampus: Evidence for mediation by NMDA receptors. *Experimental Neurology, 113*, 378–385.

Miller, R. G., Mitchell, J. D., Lyon, M., & Moore, D. H. (2007). Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst Rev, 1*, CD001447.

Molina y Vedia, L., McDonald, B., Reep, B., Brüne, B., Di Silvio, M., Billiar, T. R., & Lapetina, E. G. (1992). Nitric oxide-induced S-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase inhibits enzymatic activity and increases endogenous ADP-ribosylation. *The Journal of Biological Chemistry, 267*, 24929–24932.

Montastruc, J. L., Rascol, O., & Senard, J. M. (1997). Glutamate antagonists and Parkinson's disease: A review of clinical data. *Neuroscience and Biobehavioral Reviews, 21*, 477–480.

Monyer, H., Seeburg, P. H., & Wisden, W. (1991). Glutamate-operated channels: Developmentally early and mature forms arise by alternative splicing. *Neuron, 6*, 799–810.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., & Seeburg, P. H. (1992). Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science, 256*, 1217–1221.

Monyer, H., Burnashev, N., Laurie, D. J., Sakmann, B., & Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron, 12*, 529–540.

Morris, G. F., Bullock, R., Marshall, S. B., Marmarou, A., Maas, A., & Marshall, L. F. (1999). Failure of the competitive N-methyl-D-aspartate antagonist Selfotel (CGS 19755) in the treatment of severe head injury: Results of two phase III clinical trials. The Selfotel Investigators. *Journal of Neurosurgery, 91*, 737–743.

Muir, K. W. (2006). Glutamate-based therapeutic approaches: Clinical trials with NMDA antagonists. *Current Opinion in Pharmacology, 6*, 53–60.

Muir, K. W., Holzapfel, L., & Lees, K. R. (2000). Phase II clinical trial of sipatrigine (619C89) by continuous infusion in acute stroke. *Cerebrovascular Diseases, 10*, 431–436.

Murman, D. L., Giordani, B., Mellow, A. M., Johanns, J. R., Little, R. J., Hariharan, M., & Foster, N. L. (1997). Cognitive, behavioral, and motor effects of the NMDA antagonist ketamine in Huntington's disease. *Neurology, 49*, 153–161.

Nagano, I., Wong, P. C., & Rothstein, J. D. (1996). Nitration of glutamate transporters in transgenic mice with a familial amyotrophic lateral sclerosis-linked SOD1 mutation. *Annals of Neurology, 40*, 542.

Nakagawa, D., Ohshima, Y., Takusagawa, M., Ikota, N., Takahashi, Y., Shimizu, S., & Ozawa, T. (2001). Functional modification of cytochrome c by peroxynitrite in an electron transfer reaction. *Chemical & Pharmaceutical Bulletin (Tokyo), 49*, 1547–1554.

Nakagawa-Hattori, Y., Yoshino, H., Kondo, T., Mizuno, Y., & Horai, S. (1992). Is Parkinson's disease a mitochondrial disorder? *Journal of Neurological Sciences, 107*, 29–33.

Nakanishi, S. (1992). Molecular diversity of glutamate receptors and implications for brain function. *Science, 258*, 597–603.

Nicholls, P., & Malviya, A. N. (1968). Inhibition of nonphosphorylating electron transfer by zinc. The problem of delineating interaction sites. *Biochemistry, 7*, 305–310.

Nilsson, P., Hillered, L., Ponten, U., Pontén, U., & Ungerstedt, U. (1990). Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. *Journal of Cerebral Blood Flow and Metabolism, 10*, 631–637.

Noh, K. M., & Koh, J. Y. (2000). Induction and activation by zinc of NADPH oxidase in cultured cortical neurons and astrocytes. *The Journal of Neuroscience, 20*, RC111.

Novelli, A., Reilly, J. A., Lysko, P. G., & Henneberry, R. C. (1988). Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptors when intracellular energy levels are reduced. *Brain Research, 451*, 205–212.

Ogawa, A., Yoshimoto, T., Kikuchi, H., Sano, K., Saito, I., Yamaguchi, T., & Yasuhara, H. (1999). Ebselen in acute middle cerebral artery occlusion: A placebo-controlled, double-blind clinical trial. *Cerebrovascular Diseases, 9*, 112–118.

Olney, J. W. (1969). Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science, 164*, 719–721.

Olney, J. W. (1971). Glutamate-induced neuronal necrosis in the infant mouse hypothalamus: An electron microscopic study. *Journal of Neuropathology and Experimental Neurology, 30*, 75–90.

Olney, J. W., & Ho, O. L. (1970). Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature, 227*, 609–610.

Olney, J. W., Sharpe, L. G., & Feigin, R. D. (1972). Glutamate-induced brain damage in infant primates. *Journal of Neuropathology and Experimental Neurology, 31*, 464–488.

Ozawa, S., Kamiya, H., & Tsuzuki, K. (1998). Glutamate receptors in the mammalian central nervous system. *Progress in Neurobiology, 54*, 581–618.

Ozyurt, E., Graham, D. I., Woodruff, G. N., & McCulloch, J. (1988). Protective effect of the glutamate antagonist, MK-801 in focal cerebral ischemia in the cat. *Journal of Cerebral Blood Flow and Metabolism, 8*, 138–143.

Palfi, S. P., Ferrante, R. J., Brouillet, E., Beal, M. F., Dolan, R., Guyot, M. C., Peschanski, M., & Hantraye, P. (1996). Chronic 3-nitropropionic acid treatment in baboons replicates the cognitive and motor deficits of Huntington's disease. *The Journal of Neuroscience, 16*, 3019–3025.

Papa, S. M., Engber, T. M., Boldry, R. C., & Chase, T. N. (1993). Opposite effects of NMDA and AMPA receptor blockade on catalepsy induced by dopamine receptor antagonists. *European Journal of Pharmacology, 232*, 247–253.

Perry, T. L., Hansen, S., & Jones, K. (1987). Brain glutamate deficiency in amyotrophic lateral sclerosis. *Neurology, 37*, 1845–1848.

Pin, J. P., & Duvoisin, R. (1995). The metabotropic glutamate receptors: Structure and functions. *Neuropharmacology, 34*, 1–26.

Pohl, D., Bittigau, P., Ishimaru, M. J., Stadthaus, D., Hübner, C., Olney, J. W., Turski, L., & Ikonomidou, C. (1999). NMDA antagonists and apoptotic cell death triggered by head trauma in developing rat brain. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 2508–2513.

Polster, B. M., Basanez, G., Etxebarria, A., Hardwick, J. M., & Nicholls, D. G. (2005). Calpain I induces cleavage and release of apoptosis-inducing factor from isolated mitochondria. *The Journal of Biological Chemistry, 280*, 6447–6454.

Portera-Cailliau, C., Price, D. L., & Martin, L. J. (1997). Non-NMDA and NMDA receptor-mediated excitotoxic neuronal deaths in adult brain are morphologically distinct: Further evidence for an apoptosis-necrosis continuum. *The Journal of Comparative Neurology, 378*, 88–104.

Rabey, J. M., Nissipeanu, P., & Korczyn, A. D. (1992). Efficacy of memantine, an NMDA receptor antagonist, in the treatment of Parkinson's disease. *Journal of Neural Transmission, 4*, 277–282.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991a). Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics, 288*, 484–487.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991b). Peroxynitrite oxidation of sulfhydryls. *The Journal of Biological Chemistry, 266*, 4244–4250.

Radi, R., Rodriguez, M., Castro, L., & Telleri, R. (1994). Inhibition of mitochondrial electron transport by peroxynitrite. *Archives of Biochemistry and Biophysics, 308*, 89–95.

Randall, R. D., & Thayer, S. A. (1992). Glutamate-induced calcium transient triggers delayed calcium overload and neurotoxicity in rat hippocampal neurons. *The Journal of Neuroscience, 12*, 1882–1895.

Reisberg, B., Doody, R., Stöffler, A., Schmitt, F., Ferris, S., Möbius, H. J., & Memantine Study Group. (2003). Memantine in moderate-to-severe Alzheimer's disease. *The New England Journal of Medicine, 348*, 1333–1341.

Reynolds, I. J., & Hastings, T. G. (1995). Glutamate induces the production of reactive oxygen species in cultured forebrain neurons following NMDA receptor activation. *The Journal of Neuroscience, 15*, 3318–3327.

Riccio, A., Ahn, S., Davenport, C. M., Blendy, J. A., & Ginty, D. D. (1999). Mediation by a CREB family transcription factors of NGF-dependent survival of sympathetic neurons. *Science, 286*, 2358–2361.

Rock, D. M., & Macdonald, R. L. (1992). The polyamine spermine has multiple actions on N-methyl-D-aspartate receptor single-channel currents in cultured cortical neurons. *Molecular Pharmacology, 41*, 83–88.

Rothman, S. (1984). Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. *The Journal of Neuroscience, 4*, 1884–1891.

Rothman, S. M. (1985). The neurotoxicity of excitatory amino acids is produced by passive chloride influx. *The Journal of Neuroscience, 5*, 1483–1489.

Rothstein, J. D., Martin, L. J., & Kuncl, R. W. (1992). Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *New England Journal of Medicine, 326*, 1464–1468.

Rothstein, J. D., Van Kammen, M., Levey, A. I., Martin, L. J., & Kuncl, R. W. (1995). Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Annals of Neurology, 38*, 73–84.

Santiago, M., Venero, J. L., Machado, A., & Cano, J. (1992). In vivo protection of striatum from MPP^+ neurotoxicity by N-methyl-D-aspartate antagonists. *Brain Research, 586*, 203–207.

Sattler, R., Charlton, M. P., Hafner, M., & Tymianski, M. (1998). Distinct influx pathways, not calcium load, determine neuronal vulnerability to calcium neurotoxicity. *Journal of Neurochemistry, 71*, 2349–2364.

Sattler, R., Xiong, Z., Lu, W. Y., Hafner, M., MacDonald, J. F., & Tymianski, M. (1999). Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science, 284*, 1845–1848.

Schapira, A. H., Cooper, J. M., Dexter, D., Clark, J. B., Jenner, P., & Marsden, C. D. (1990a). Mitochondrial complex I deficiency in Parkinson's disease. *Journal of Neurochemistry, 54*, 823–827.

Schapira, A. H., Mann, V. M., Cooper, J. M., Dexter, D., Daniel, S. E., Jenner, P., Clark, J. B., & Marsden, C. D. (1990b). Anatomic and disease specificity of NADH CoQ_1 reductase (complex I) deficiency in Parkinson's disease. *Journal of Neurochemistry, 55*, 2142–2145.

Schewe, T. (1995). Molecular actions of ebselen – an antiinflammatory antioxidant. *General Pharmacology, 26*, 1153–1169.

Schmitz, D., Mellor, J., Frerking, M., & Nicoll, R. A. (2001). Presynaptic kainate receptors at hippocampal mossy fiber synapses. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 11003–11008.

Schoepp, D. D., Tizzano, J. P., Wright, R. A., & Fix, A. S. (1995). Reversible and irreversible neuronal injury induced by intrahippocampal infusion of the mGluR agonist 1S,3R-ACPD in the rat. *Neurodegeneration, 4*, 71–80.

Schrammel, A., Gorren, A. C., Schmidt, K., Pfeiffer, S., & Mayer, B. (2003). S-nitrosation of glutathione by nitric oxide, peroxynitrite, and (*)NO/O(2)(*-). *Free Radical Biology & Medicine, 34*, 1078–1088.

Schulz, J. B., Henshaw, D. R., Jenkins, B. G., Ferrante, R. J., Kowall, N. W., Rosen, B. R., & Beal, M. F. (1994). 3-Acetylpyridine produces age-dependent neuronal lesions by an excitotoxic and free radical mediated mechanism. *Journal of Cerebral Blood Flow and Metabolism, 14*, 1024–1029.

Schwab, R. S., England, A. C., Poskranzer, D. C., & Young, R. R. (1969). Amantadine in the treatment of Parkinson's disease. *Journal of the American Medical Association, 208*, 1168–1170.

Schwarcz, R., & Kohler, C. (1983). Differential vulnerability of central neurons of the rat to quinolinic acid. *Neuroscience Letters, 38*, 85–90.

Schwarcz, R., Whetsell, W. O., & Mangano, R. M. (1983). Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. *Science, 219*, 316–318.

Seif el, N. M., Peruche, B., Rossberg, C., Rossberg, C., Mennel, H. D., & Krieglstein, J. (1990). Neuroprotective effect of memantine demonstrated in vivo and in vitro. *European Journal of Pharmacology, 185*, 19–24.

Selkoe, D. J. (1999). Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature Supplement*, 399, A23–A31.

Sen, N., Hara, M. R., Ahmad, A. S., Cascio, M. B., Kamiya, A., Ehmsen, J. T., Agrawal, N., Hester, L., Doré, S., Snyder, S. H., & Sawa, A. (2009). GOSPEL: A neuroprotective protein that binds to GAPDH upon S-nitrosylation. *Neuron, 63*, 81–91.

Sensi, S. L., Canzoniero, L. M., Yu, S. P., Ying, H. S., Koh, J. Y., Kerchner, G. A., & Choi, D. W. (1997). Measurement of intracellular free zinc in living cortical neurons: Routes of entry. *The Journal of Neuroscience, 17*, 9554–9564.

Sensi, S. L., Yin, H. Z., Carriedo, S. G., Rao, S. S., & Weiss, J. H. (1999). Preferential Zn^{2+} influx through Ca^{2+}-permeable AMPA/kainate channels triggers prolonged mitochondrial superoxide production. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 2414–2419.

Shaw, P. J., Forrest, V., Ince, P. G., Richardson, J. P., & Wastell, H. J. (1995). Studies on cellular free radical protection mechanisms in the anterior horn from patients with amyotrophic lateral sclerosis. *Neurodegeneration, 4*, 209–216.

Sheardown, M. J., Nielsen, E. O., Hansen, A. J., Jacobsen, P., & Honoré, T. (1990). 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: A neuroprotectant for cerebral ischemia. *Science, 247*, 571–574.

Sheline, C. T., Behrens, M. M., & Choi, D. W. (2000). Zinc-induced cortical neuronal death: Contribution of energy failure attributable to loss of NAD(+) and inhibition of glycolysis. *The Journal of Neuroscience, 20*, 3139–3146.

Shuaib, A., Lees, K. R., Lyden, P., Grotta, J., Davalos, A., Davis, S. M., Diener, H. C., Ashwood, T., Wasiewski, W. W., Emeribe, U., & SAINT II Trial Investigators. (2007). NXY-059 for the treatment of acute ischemic stroke. *The New England Journal of Medicine, 357*, 562–571.

Singleton, R. H., & Povlishock, J. T. (2004). Identification and characterization of heterogeneous neuronal injury and death in regions of diffuse brain injury: Evidence for multiple independent injury phenotypes. *The Journal of Neuroscience, 24*, 3543–3553.

Snider, B. J., Gottron, F. J., & Choi, D. W. (1999). Apoptosis and necrosis in cerebrovascular disease. *Annals of the New York Academy of Sciences, 893*, 243–253.

Spencer, P. S. (1987). Guam ALS/parkinsonism-dementia: A long-latency neurotoxic disorder caused by "slow toxin(s)" in food? *The Canadian Journal of Neurological Sciences, 14*(Suppl 3), 347–357.

Spencer, P. S., Roy, D. N., Ludolph, A., Hugon, J., Dwivedi, M. P., & Schaumburg, H. H. (1986). Lathyrism: Evidence for the role of the neuroexcitatory amino acid BOAA. *Lancet, 2*, 1066–1067.

Spencer, P. S., Kisby, G. E., Ross, S. M., Roy, D. N., Hugon, J., Ludolph, A. C., & Nunn, P. B. (1993). Guam ALS-PDC: Possible causes. *Science, 262*, 825–826.

Srivastava, R., Brouillet, E., Beal, M. F., Storey, E., & Hyman, B. T. (1993). Blockade of 1-methyl-4-phenylpyridinium ion (MPP^+) nigral toxicity in the rat by prior decortication or MK-801 treatment. *Neurobiology of Aging, 14*, 295–301.

Stamler, J. S., Simon, D. I., Osborne, J. A., Mullins, M. E., Jaraki, O., Michel, T., Singel, D. J., & Loscalzo, J. (1992). S-nitrosylation of proteins with nitric oxide: Synthesis and characterization of biologically active compounds. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 444–448.

Steinberg, G. K., George, C. P., DeLaPaz, R., Shibata, D. K., & Gross, T. (1988). Dextromethorphan protects against cerebral injury following transient focal ischemia in rabbits. *Stroke, 19*, 1112–1118.

Suh, S. W., Chen, J. W., Motamedi, M., Bell, B., Listiak, K., Pons, N. F., Danscher, G., & Frederickson, C. J. (2000). Evidence that synaptically-released zinc contributes to neuronal injury after traumatic brain injury. *Brain Research, 852*, 268–273.

Sun, H. S., Doucette, T. A., Liu, Y., Fang, Y., Teves, L., Aarts, M., Ryan, C. L., Bernard, P. B., Lau, A., Forder, J. P., Salter, M. W., Wang, Y. T., Tasker, R. A., & Tymianski, M. (2008). Effectiveness of PSD95 inhibitors in permanent and transient focal ischemia in the rat. *Stroke, 39*, 2544–2553.

Takahashi, M., Ni, J. W., Kawasaki-Yatsugi, S., Toya, T., Yatsugi, S. I., Shimizu-Sasamata, M., Koshiya, K., Shishikura, J. I., Sakamoto, S., & Yamaguchi, T. (1998). YM872, a novel selective alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor antagonist, reduces brain damage after permanent focal cerebral ischemia in cats. *The Journal of Pharmacology and Experimental Therapeutics, 284*, 467–473.

Tanabe, Y., Nomura, A., Masu, M., Shigemoto, R., Mizuno, N., & Nakanishi, S. (1993). Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4. *The Journal of Neuroscience, 13*, 1372–1378.

Tanaka, K., Nagata, E., Suzuki, S., Dembo, T., Nogawa, S., & Fukuuchi, Y. (1999). Immunohistochemical analysis of cyclic AMP response element binding protein phosphorylation in focal cerebral ischemia in rats. *Brain Research, 818*, 520–526.

Tanaka, K., Nogawa, S., Ito, D., Suzuki, S., Dembo, T., Kosakai, A., & Fukuuchi, Y. (2000). Activated phosphorylation of cyclic AMP response element binding protein is associated with preservation of striatal neurons after focal cerebral ischemia in the rat. *Neuroscience, 100*, 345–354.

Tao, X., Finkbeiner, S., Arnold, D. B., Shaywitz, A. J., & Greenberg, M. E. (1998). Ca^{2+} influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron, 20*, 709–726.

Taylor, D. J., Krige, D., Barnes, P. R., Kemp, G. J., Carroll, M. T., Mann, V. M., Cooper, J. M., Marsden, C. D., & Schapira, A. H. (1994). A ^{31}P magnetic resonance spectroscopy study of mitochondrial function in skeletal muscle of patients with Parkinson's disease. *Journal of Neurological Sciences, 125*, 77–81.

Tenneti, L., & Lipton, S. A. (2000). Involvement of activated caspase-3-like proteases in N-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. *Journal of Neurochemistry, 74*, 134–142.

Tenneti, L., D'Emilia, D. M., Troy, C. M., & Lipton, S. A. (1998). Role of caspases in N-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. *Journal of Neurochemistry, 71*, 946–959.

Terro, F., Lasort, M., Vlader, F., Ludolph, A., & Hugon, J. (1996). Antioxidant drugs block in vitro the neurotoxicity of CSF from patients with amyotrophic lateral sclerosis. *Neuroreport, 7*, 1970–1972.

Terry, R. D., & Davies, P. (1980). Dementia of the Alzheimer type. *Annual Review of Neuroscience, 3*, 77–95.

Thayer, S. A., & Wang, G. J. (1995). Glutamate-induced calcium loads: Effects on energy metabolism and neuronal viability. *Clinical and Experimental Pharmacology and Physiology, 22*, 303–304.

Tonder, N., Johansen, F. F., Frederickson, C. J., Zimmer, J., & Diemer, N. H. (1990). Possible role of zinc in the selective degeneration of dentate hilar neurons after cerebral ischemia in the adult rat. *Neuroscience Letters, 109*, 247–252.

Traynelis, S. F., & Cull-Candy, S. G. (1990). Proton inhibition of N-methyl-D-aspartate receptors in cerebellar neurons. *Nature, 345*, 347–350.

Turski, L., Bressler, K., Rettig, K. J., Loschmann, P. A., & Wachtel, H. (1991). Protection of substantia nigra from MPP^+ neurotoxicity by N-methyl-D-aspartate antagonists. *Nature, 349*, 414–418.

Tymianski, M., Charlton, M. P., Carlen, P. L., & Tator, C. H. (1993a). Source specificity of early calcium neurotoxicity in cultured embryonic spinal neurons. *The Journal of Neuroscience, 13*, 2085–2104.

Tymianski, M., Wallace, M. C., Spigelman, I., Uno, M., Carlen, P. L., Tator, C. H., & Charlton, M. P. (1993b). Cell-permeant Ca^{2+}-chelators reduce early excitotoxic and ischemic neuronal injury in vitro and in vivo. *Neuron, 11*, 221–235.

Urbanska, E., Ikonomidou, C., Sieklucka, M., & Turski, W. A. (1991). Aminooxyacetic acid produces excitotoxic lesions in the rat striatum. *Synapse, 9*, 129–135.

van der Vliet, A., Hoen, P. A., Wong, P. S., Bast, A., & Cross, C. E. (1998). Formation of S-nitrosothiols via direct nucleophilic nitrosation of thiols by peroxynitrite with elimination of hydrogen peroxide. *The Journal of Biological Chemistry, 273*, 30255–30262.

Vignes, M., & Collingridge, G. L. (1997). The synaptic activation of kainate receptors. *Nature, 388*, 179–182.

Wachtel, H., Kunow, M., & Loschmann, P. A. (1992). NBQX (6-nitro-sulfamoyl-benzo-quinoxaline-dione) and CPP (3-carboxy-piperazin-propyl phosphonic acid) potentiate dopamine agonist induced rotations in substantia nigra lesioned rats. *Neuroscience Letters, 142*, 179–182.

Wahl, F., Allix, M., Plotkine, M., & Boulu, R. G. (1993). Effect of riluzole on focal cerebral ischemia in rats. *European Journal of Pharmacology, 230*, 209–214.

Wahl, F., Renou, E., Mary, V., & Stutzmann, J. M. (1997). Riluzole reduces brain lesions and improves neurological function in rats after a traumatic brain injury. *Brain Research, 756*, 247–255.

Walton, M. R., & Dragunow, M. (2000). Is CREB a key to neuronal survival? *Trends in Neurosciences, 23*, 48–53.

Wang, G. J., & Thayer, S. A. (1996). Sequestration of glutamate-induced Ca^{2+} loads by mitochondria in cultured rat hippocampal neurons. *Journal of Neurophysiology, 76*, 1611–1621.

Wang, Y., Kim, N. S., Li, X., Greer, P. A., Koehler, R. C., Dawson, V. L., & Dawson, T. M. (2009). Calpain activation is not required for AIF translocation in PARP-1-dependent cell death (parthanatos). *Journal of Neurochemistry, 110*, 687–696.

Wenk, G. L., Danysz, W., & Mobley, S. L. (1996). MK-801, memantine and amantadine show neuroprotective activity in the nucleus basalis magnocellularis. *European Journal of Pharmacology, 293*, 267–270.

Williams, K. (1997). Interactions of polyamines with ion channels. *Biochemical Journal, 325* (Pt 2), 289–297.

Williams, T. L., Day, N. C., Kamboj, R. K., Ince, P. G., & Shaw, P. J. (1997). Calcium-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors: A molecular determinant of selective vulnerability in amyotrophic lateral sclerosis. *Annals of Neurology, 35*, 200–207.

Wüllner, U., Young, A. B., Penney, J. B., & Beal, M. F. (1994). 3-Nitropropionic acid toxicity in the striatum. *Journal of Neurochemistry, 63*, 1772–1781.

Yamaguchi, T., Sano, K., Takakura, K., Saito, I., Shinohara, Y., Asano, T., & Yasuhara, H. (1998). Ebselen in acute ischemic stroke: A placebo-controlled, double-blind clinical trial. Ebselen Study Group. *Stroke, 29*, 12–17.

Yamakura, F., Taka, H., Fujimura, T., & Murayama, K. (1998). Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. *The Journal of Biological Chemistry, 273*, 14085–14089.

Yokoyama, M., Koh, J., & Choi, D. W. (1986). Brief exposure to zinc is toxic to cortical neurons. *Neuroscience Letters, 71*, 351–355.

Young, A. B. (1990). What's the excitement about excitatory amino acids in amyotrophic lateral sclerosis? *Annals of Neurology, 28*, 9–11.

Zadow, B., & Schmidt, W. J. (1994). The AMPA antagonists NBQX and GYKI 52466 do not counteract neuroleptic-induced catalepsy. *Naunyn Schmiedeberg's Archives of Pharmacology, 349*, 61–65.

Zeevalk, G. D., & Nicklas, W. J. (1991). Mechanisms underlying initiation of excitotoxicity associated with metabolic inhibition. *The Journal of Pharmacology and Experimental Therapeutics, 257*, 870–878.

Zhang, J., Dawson, V. L., Dawson, T. M., & Snyder, S. H. (1994). Nitric oxide activation of poly (ADP-ribose)synthetase in neurotoxicity. *Science, 263*, 687–689.

Zhang, C., Raghupathi, R., Saatman, K. E., Smith, D. H., Stutzmann, J. M., Wahl, F., & McIntosh, T. K. (1998). Riluzole attenuates cortical lesion size, but not hippocampal neuronal loss, following traumatic brain injury in the rat. *Journal of Neuroscience Research, 52*, 342–349.

Zhang, Y., Wang, H., Li, J., Jimenez, D. A., Levitan, E. S., Aizenman, E., & Rosenberg, P. A. (2004). Peroxynitrite-induced neuronal apoptosis is mediated by intracellular zinc release and 12-lipoxygenase activation. *The Journal of Neuroscience, 24*, 10616–10627.

Zingarelli, B., O'Connor, M., Wong, H., Salzman, A. L., & Szabó, C. (1996). Peroxynitrite-mediated DNA strand breakage activates poly-adenosine diphosphate ribosyl synthetase and causes cellular energy depletion in macrophages stimulated with bacterial lipopolysaccharide. *Journal of Immunology, 156*, 350–358.

Zipp, F., Baas, H., & Fischer, P. A. (1993). Lamotrigine–antiparkinsonian activity by blockade of glutamate release? *Journal of Neural Transmission, 5*, 67–75.

Zipp, F., Burklin, F., Stecker, K., Baas, H., & Fischer, P. A. (1995). Lamotrigine in Parkinson's disease–a double blind study. *Journal of Neural Transmission, 10*, 199–206.

Zuddas, A., Oberto, G., Vaglini, F., Fascetti, F., Fornai, F., & Corsini, G. U. (1992). MK-801 prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in primates. *Journal of Neurochemistry, 59*, 733–739.

Domoic Acid as a Neurotoxin

Anabel Pérez-Gómez and R. Andrew Tasker

Contents

Abstract

Domoic acid (DOM) is a naturally occurring excitatory amino acid with structural similarity, and similar but not identical pharmacological profile, to kainic acid. DOM is most commonly associated with toxic blooms of marine phytoplankton resulting in contamination of shellfish as well as other species. This brief review summarizes the known toxicological properties of DOM both in vitro and in vivo in a variety of model systems. This chapter also summarizes information on clinical cases of intoxication in both wildlife and humans, as well as highlighting the growing evidence that DOM is a potent neurodevelopmental toxin with relevance to both food safety issues and the etiology of neurological diseases.

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1 Chemistry, Physicochemical Properties, and Pharmacology

Domoic acid is a naturally occurring excitatory amino acid (EAA) that has been linked to numerous outbreaks of toxicity in humans (Teitelbaum et al. 1990; Cendes et al. 1995) as well as a variety of other species (for review see Doucette and Tasker 2008; Lefebvre and Robertson 2010). The parent compound is a tricarboxylic amino acid produced by certain marine organisms, the best characterized of which are the red alga *Chondria armata* and the planktonic diatoms of *Nitzschia spp*. (for review see Bates and Horner 1998; Bates and Trainer 2006).

Chemically domoic acid is (2S,3S,4S)-2-carboxy-4-1-methyl-5(R)-carboxyl-l (Z)-3(E)-hexadienyl pyrrolidine-3-acetic acid ($C_{15}H_{21}NO_6$) and has an anhydrous molecular weight of 311.14, a melting point of 215–216 °C (dihydrate), and a UV (ethanol) absorption spectrum with a maximum of 242 nm although this is pH dependent. Structurally domoic acid is very similar to another known toxin, kainic acid, and both are analogues of the amino acids glutamate and proline. The structures of domoic and kainic acid are shown in Fig. 1.

In addition to the parent compound, a number of isomers of domoic acid have been identified. Isodomoic acids A, B, and C are present in small amounts in the red alga *Chondria armata* (Maeda et al. 1986) as are isodomoic acids G and H (Zaman et al. 1997). The isomers isodomoic acids D, E, and F as well as the 5′ epimer have been identified in small amounts from both plankton cells and shellfish tissue (Wright et al. 1990; Walter et al. 1994) although it seems likely that these isomers form on exposure to UV light rather than being metabolic products produced by the plankton (Quilliam 2003). Although the binding of domoic acid isomers in vitro to kainate receptors (Rs) was reported a number of years ago (Hampson et al. 1992), there have been few investigations to date of the pharmacology and toxicology of domoate isomers, with the exception of four publications out of New Zealand (Sawant et al. 2007, 2008; Munday et al. 2008; Sawant et al. 2010).

Both DOM and its analogue kainic acid are excitotoxic and mediate their toxic effects through activation of glutamate receptors (GluRs) with the participation and co-activation of both *N*-methyl-D-aspartate (NMDA) receptors and non-NMDA-R subtypes (AMPA [alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid]/kainate) (Novelli et al. 1992; Larm et al. 1997; Berman and Murray 1997; Berman et al. 2002; Hampson and Manalo 1998; Clements et al. 1998; Tasker and Strain 1998; Tasker et al. 2005). While DOM stimulates AMPA/kainate-Rs (AMPA/KA-Rs) directly, NMDA-R activation appears to be a consequence of either AMPA/KA-R-mediated stimulation of EAA efflux (Novelli et al. 1992; Berman and Murray 1997; Clements et al. 1998; Berman et al. 2002) and/or to reduce tonic

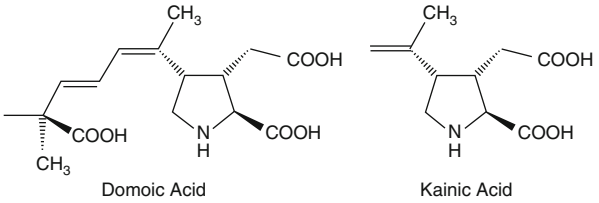

Fig. 1 Structures of domoic acid and kainic acid

inhibition by gamma-aminobutyric acid (GABA) as a result of activation of presynaptic kainate-Rs (KA-Rs) on GABAergic interneurons (Duran et al. 1995; Cunha et al. 1997; Rodriguez-Moreno et al. 2000). Although similar in many respects, DOM and kainic acid are pharmacologically dissociable in some systems because DOM interacts preferentially with the so-called low-affinity kainate subunits designated GluK1 and GluK2 (previously referred to as GluR5 and GluR6) (Verdoorn et al. 1994; Tasker et al. 1996). The complexity of DOM pharmacology combined with the relative lack of knowledge about the physiological properties of KA-Rs probably explains some of the discrepancies reported in the literature, particularly between in vitro and in vivo preparations (see below).

2 DOM Toxicity In Vitro

DOM-induced neurotoxicity has been evaluated in a variety of in vitro systems including immature and mature primary cultures of neurons from cerebellum, cortex, and hippocampus, organotypic hippocampal cultures, and neuroblastoma cell lines. DOM-induced neuronal death is dependent on both exposure duration and concentration, with an EC_{50} reported to range from 3 to 10 μM after 24 h exposure in cerebellar granule cell cultures from both rat and mouse (Novelli et al. 1992; Fernandez-Sanchez and Novelli 1993; Larm et al. 1997; Giordano et al. 2006; Perez-Gomez et al. 2010). The EC_{50} values in cortical neurons range from 4.2 μM in rat (Qiu et al. 2006) to higher than 20 μM in mouse (Jensen et al. 1999) after 24 h treatment, and in mixed hippocampal cultures, an EC_{50} of 7.5 μM has been reported (Roy and Sapolsky 2003). An EC_{50} of 3 μM was obtained for the NG108-15 neuroblastoma cell line (Canete and Diogene 2008), and finally, the treatment of organotypic hippocampal cell cultures (OHSCs) with DOM resulted in selective but not specific toxicity to CA1 pyramidal neurons with an EC_{50} of 3.5 μM after 24 h of exposure (Perez-Gomez and Tasker 2012) and 6 μM after 48 h exposure (Jakobsen et al. 2002). DOM effects in vitro confirm results obtained in vivo showing that DOM is 3–20-fold more potent than KA itself (Stewart et al. 1990; Tasker et al. 1991).

The mechanism of action responsible for DOM toxicity in vitro appears to be more complex than would be predicted from the compound's pharmacological profile as an AMPA/KA-R agonist. DOM in vitro elicits a non-desensitizing response via activation of low-affinity AMPA-Rs (Diaz-Trelles et al. 2003), while activation of KA-Rs does not seem to play a crucial role (Berman and Murray 1997;

Jakobsen et al. 2002; Diaz-Trelles et al. 2003; Gressens et al. 2005). DOM has also been shown to evoke the release of both glutamate and aspartate in cerebellar granule cells (CGCs) (Berman and Murray 1997) as well as in isolated chick retina explants (Nduaka et al. 1999). This appears to occur via a non-vesicular mechanism that is dependent upon reversal of the high-affinity glutamate transporter and cell swelling and, therefore, synergistically potentiates glutamate-/aspartate-mediated neurotoxicity (Berman and Murray 1997). Pharmacologically, both NMDA-R and non-NMDA-R have been implicated in the toxic actions of DOM, in that DOM-induced injury seems to be mediated in part by NMDA-Rs that are activated secondarily as a consequence of AMPA-R-mediated stimulation of EAA release (Berman and Murray 1997). Accordingly, DOM has been shown to act synergistically with NMDA in producing acute cytotoxicity in CGCs (Novelli et al. 1992; Berman and Murray 1996).

In vitro studies have shown increased DOM toxicity with increasing maturation of the central nervous system (Qiu et al. 2006; Hogberg and Bal-Price 2011). This could be due to lower functional expression of the GluRs in the younger cultures. It has also been proposed that more calcium-permeable AMPA-Rs or AMPA-R isoforms could be activated in the mature cultures in comparison to the younger cultures (Qiu et al. 2006), causing glutamate release that subsequently could activate the NMDA-R, promoting neurotoxicity (Berman and Murray 1997; Berman et al. 2002).

Application of DOM produces a rapid and concentration-dependent increase in intracellular calcium in a variety of neuronal preparations, including embryonic brain stem cells (Konig et al. 1994), dorsal root ganglion neurons (Joseph et al. 2011), cultured neurons of chick embryonic retina (Duran et al. 1995), and primary cultures of cortical (Beani et al. 2000), hippocampal (Xi and Ramsdell 1996), cerebellar granule (Perez-Gomez et al. 2010), and Purkinje neurons (Gruol et al. 1997). Vale-Gonzalez et al. (2006) described a prominent intracellular acidification resulting from a rise in intracellular calcium. Some studies have proven that DOM-stimulated increases in cytosolic-free calcium are dependent upon the activation of L-type voltage-sensitive calcium channels (VSCC) following activation of non-NMDA-R (Xi and Ramsdell. 1996; Berman et al. 2002). However, the contribution of a calcium increase through VSCC to DOM toxicity remains controversial as some data suggested that calcium may reduce rather than potentiate the non-desensitizing AMPA-R response to DOM in CGCs, thereby decreasing neurotoxicity as well as the generation of reactive oxygen species (Fernandez-Sanchez and Novelli 1993; Diaz-Trelles et al. 2003; Perez-Gomez et al. 2010).

In some in vitro preparations, DOM-induced neuronal cell death appears to be primarily necrotic in nature. No signs of the classical caspase-dependent apoptotic pathway were detected in organotypic hippocampal slices using different concentrations and time of exposures to DOM (Perez-Gomez and Tasker. 2012) (Fig. 2). In mixed hippocampal cell cultures, DOM neurotoxicity was also found to be necrotic when examined both biochemically and morphologically (Roy and Sapolsky. 2003), and protection afforded by two virally derived caspase inhibitors (p35 and crmA) was ascribed to their ability to sequester free radicals and to stabilize the mitochondrial

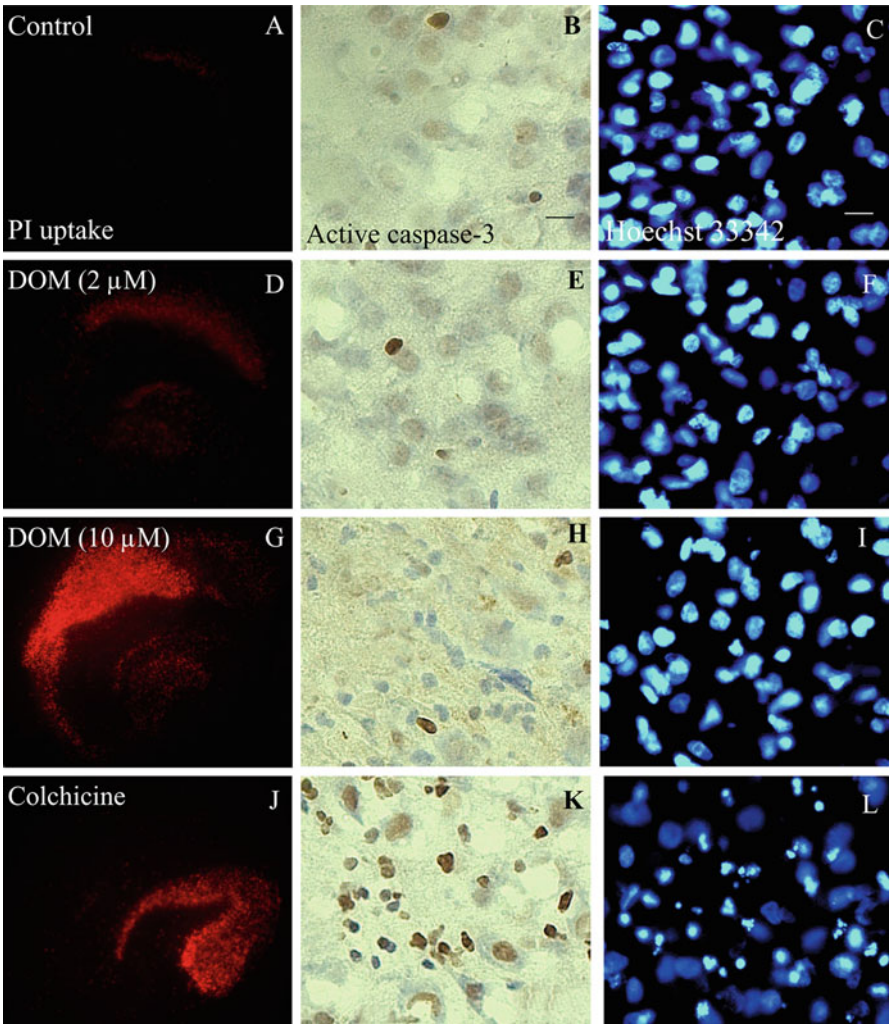

Fig. 2 DOM-induced cell death in organotypic hippocampal slice cultures is primarily necrotic. Propidium iodide exclusion (**a**, **d**, **g**) demonstrates that cell death in OHSCs is concentration dependent but is not accompanied by an increase in the apoptosis marker activated caspase-3 (**e**, **h**) relative to control (**b**) or a change in the intensity of Hoechst 33342 staining for condensed chromatin (**f**, **i**) relative to control (**c**). In contrast administration of colchicine as a positive control results in comparable cell death (**j**) accompanied by changes in both caspase-3 and Hoechst 33342 staining (**k**, **l**)

membrane potential, respectively. However, acute in vitro studies in pure mouse CGCs have shown that DOM can cause either necrotic or apoptotic cell death, depending on its concentration. A high concentration of DOM (1 h, 10 μM) activates both the AMPA/KA-R and NMDA-R which causes a rapid accumulation of intracellular calcium; promotes GSH efflux and a concomitant decrease in

intracellular GSH, inducing an increased lipid peroxidation; and leads to necrotic neuronal death (Giordano et al. 2006). On the other hand, AMPA/KA-Rs (not NMDA-R) have been shown to mediate low-concentration (<0.1 μM) DOM-induced apoptosis (Giordano et al. 2007) with DOM inducing oxidative stress, leading to mitochondrial dysfunction and activation of caspase-3. Similar results were obtained using the motor neuron-like cell line NSC34, where DOM dramatically increased reactive oxygen species production, reduced mitochondrial function and cell viability, and was accompanied by an increase of cell apoptosis (Xu et al. 2008). A moderate increase in degraded α-spectrin concentration was found in organotypic rat brain slices (Erin and Billingsley 2004). The importance of oxidative stress in DOM-induced apoptosis was confirmed in mouse CGCs by the fact that activation of muscarinic receptors provides protection against DOM toxicity in part by an increase in the antioxidant capacity of the cells (Giordano et al. 2009). In addition, and by using the same experimental system (mouse CGCs), Giordano et al. (2008) have shown that DOM also causes an increase in the phosphorylation of p38 and Jun N-terminal (JNK) kinases, which are preferentially activated by cell stress-inducing signals (Raman et al. 2007).

While many reports have described the effects of high concentrations of DOM on neuronal systems in vitro, the effects of low-concentration DOM have also been tested in a variety of in vitro systems, leading to interesting results. Indeed, long-term exposure of primary cortical neurons to low-concentration DOM (50 nM, 4 weeks), which acutely did not induce any effects on electrical activity in the mature cultures, significantly increased the spontaneous activity of neurons when compared to non-treated cultures (Hogberg et al. 2011). The authors concluded that DOM should be recognized as a developmental neurotoxicant as the induced changes in electrical activity could lead to serious disturbance of overall neuronal activity in the developing brain. This conclusion is consistent with the findings of a number of in vivo studies in perinatal animals (see subsequent section) as well as with a recent report by Perez-Gomez and Tasker (2012) in which organotypic hippocampal slice cultures were exposed to concentrations below those required to induce permanent neurotoxicity. In this study, low-concentration DOM produced an increase of proliferation and neurogenesis in the dentate gyrus and CA1 subfields that may contribute to the development of neuroplastic changes and abnormal circuits relevant to disease. Along similar lines, Perez-Gomez et al. (2010) demonstrated that mild, nontoxic exposures to the Na^+/K^+-ATPase inhibitor palytoxin synergistically sensitized the vulnerability of CGCs to normally nontoxic concentrations of DOM, leaving NMDA receptor-mediated excitotoxic response unaltered. The results obtained demonstrate not only a crucial role for Na^+/K^+-ATPase activity in determining neuronal vulnerability to DOM-mediated excitotoxicity, but, as palytoxin and DOM are both seafood contaminants found in geographically overlapping regions, the results raise reasonable concern about possible risks for human health associated to the ingestion of low (i.e., below regulatory limits), subtoxic amounts of these phycotoxins in food.

A few studies have been conducted exploring the possibility that DOM affects not only neurons but also other cells of the central nervous system such as

astrocytes or microglia. It has been shown that exposure of astrocytic cultures to DOM did not induce any cell death (Novelli et al. 1992; Ross et al. 2000; Giordano et al. 2006), but, to a certain extent, it changed some glial functions. Injury to the astrocytes was characterized by ultrastructural changes and altered expression of chemokines/cytokines, tyrosine kinases (Trk), and apoptotic genes (Gill et al. 2008). Changes in the mRNA expression of glial fibrillary acidic protein (GFAP) (downregulation) and S100β (upregulation) (Hogberg and Bal-Price 2011) as well as effects on membrane function of rat glial cells (Liu et al. 2008) and modest increases in intracellular calcium (Giordano et al. 2006) have been reported after DOM treatment. Involvement of glial cells could enhance DOM-induced neurotoxicity as both glutamate uptake and its metabolism to glutamine were significantly inhibited (Ross et al. 2000).

In accordance with the results in astrocytes, DOM does not seem to induce toxicity to microglial cells in vitro (Mayer et al. 2001). The hypothesis that DOM might lead to the activation of microglia has been tested as well, and results seem to depend on the duration of the exposure as well as on the type of culture used. In organotypic hippocampal slice cultures, low-dose DOM insult (2 μM, 24 h) induced a significant increase in the number of reactive microglial cells 7 days after treatment (Perez-Gomez and Tasker 2012). On the other hand, DOM treatment (4–24 h, 1 mM) does not appear to activate neonatal rat microglial cultures and the concomitant expression and release of pro- and anti-inflammatory mediators (Mayer et al. 2007).

3 DOM Toxicity In Vivo

3.1 Absorption, Distribution, and Elimination

DOM is usually administered parenterally (i.p. or s.c.) in most experimental paradigms, but the more common route of exposure for intoxication of wildlife and humans (see Sects. 4.1 and 4.2 below) is by oral ingestion. Surprisingly, however, there have been very few objective studies on the absorption of this toxin following oral administration. Based on these limited observations, it appears that DOM absorption from the GI tract is erratic and bioavailability is approximately 5–10 % whether studied in mice and rats (Iverson et al. 1989, 1990) or in cynomolgus monkeys (Iverson et al. 1990; Tryphonas et al. 1990b; Truelove et al. 1996). Bioavailability following parenteral administration (i.p. or i.v.) on the other hand appears to be complete.

Once absorbed DOM appears to be largely restricted to the vascular compartment with an interpolated apparent steady-state volume of distribution (Vd_{ss}) of approximately 0.20 l/kg in all species studied to date (Suzuki and Hierlihy 1993; Truelove and Iverson 1994). This is consistent with the very hydrophilic nature of the molecule, as is the comparatively poor blood–brain barrier penetration of DOM in normal adult rats, where mean transfer constants ranging from 1.60 to 1.82 ml/g/s $\times$ 10^6 have been reported (Preston and Hynie 1991) (in the same study

sucrose transfer constants ranged from 1.00 to 1.24). These data argue against the existence of a carrier protein and suggest that the highly charged state of DOM at physiological pH results in poor CNS penetration kinetics, which seems at variance with the known CNS toxicity associated with DOM (see below). On closer examination, however, the data suggest that DOM is an extremely potent neurotoxin within the CNS, an assumption that is confirmed by studies reporting extremely low ED_{50} values when DOM is administered directly into the CNS via intracerebroventricular (i.c.v.) injection (Sawant et al. 2008; Vranyac-Tramoundanas et al. 2011) or is administered to very young rats prior to blood–brain barrier closure (Xi et al. 1997; Doucette et al. 2000).

DOM appears to be eliminated almost exclusively by renal excretion. Preston and Hynie (1991) reported that a single i.v. dose of radiolabelled DOM was almost completely eliminated from the serum of intact rats within 30 min, but that nephrectomy resulted in a significantly reduced clearance, such that detectable serum concentrations of DOM were still present at 60 min postinjection (the last time point studied). In a more robust study of renal pharmacokinetics, Suzuki and Hierlihy (1993) reported that serum clearance of DOM was almost entirely due to renal excretion, with similar kinetics over a wide range of dosages (0.5 ng/kg–2.0 mg/kg i.v.). Analysis of serum concentration-time curves produced elimination rate constants (k) of 0.025–0.035 min^{-1} (equivalent to an elimination half-life of approximately 13 min). These authors also reported total body clearance values of 7.75–10.82 ml/min/kg and renal clearance values ranging from 8.80 to 12.20 ml/min/kg. Renal excretion in this study appeared to be mainly by glomerular filtration because kinetics were not altered by the presence of probenecid. Interestingly, these data in rats may help to explain why comparatively few of the humans who ate contaminated shellfish in 1987 presented with symptoms of toxicity (see Sect. 4.2 below).

3.2 Acute Behavioral Toxicity

Following the 1987 outbreak of human toxicity following consumption of shellfish contaminated with DOM (see Sect. 4.2 below), a number of studies were published describing the acute toxicity of DOM in mice, rats, and, to a lesser extent, nonhuman primates. A complete review of these studies is beyond the scope of this chapter, but the reader is referred to Doucette and Tasker (2008) and Tasker et al. (2011) for greater detail. One important thing to note when reviewing the early (pre-1995) literature is that it was intended to describe toxicity resulting from contaminated shellfish. Consequently, most of these investigators did not have access to purified DOM, and/or the precise nature of the toxin(s) causing amnesic shellfish poisoning (see Sect. 4.2) was unknown. For these reasons toxicity data are derived largely from intraperitoneal (i.p.) injections of crude homogenates of toxic mussels (Wright et al. 1990) or of toxin-containing methanol/water extracts of whole mussels (Iverson et al. 1989, 1990; Glavin et al. 1989, 1990; Grimmelt et al. 1990; Bose et al. 1990; Tasker et al. 1991; Strain and Tasker 1991) or mussel

hepatopancreas (Bose et al. 1990; Glavin et al. 1990). Variability in sample preparation probably accounts for some of the variability in dose–response data in these chapters. In fact, Novelli et al. (1992) reported that domoic acid toxicity in mussel extracts could be altered by the presence of other amino acids, implying that even identically prepared extracts could have small differences in toxicity if different batches of shellfish are used. This is consistent with detailed dose–response data compiled by Tasker et al. (1991) in which these authors reported differences in the dose–response curves generated using DOM from extracts of toxic mussels (concentrations confirmed by HPLC) and extracts of "clean" mussels that were "spiked" with known concentrations of purified DOM.

Despite the above-mentioned limitations in early studies, subsequently confirmed in later studies using chemically purified DOM, the acute behavioral toxicity elicited by DOM is remarkably consistent both within and between species. Systemic injections of DOM elicit a graded series of behavioral changes prior to seizure onset whether studied in mice (Iverson et al. 1989; Tasker et al. 1991), rats (Tryphonas and Iverson 1990; Scallet et al. 2004), or nonhuman primates (Tryphonas et al. 1990a, b; Scallet et al. 1993; Schmued et al. 1995; Slikker et al. 1998). The sequence of events is both highly reproducible and predictably dose-related. Following injection, animals will initially demonstrate hypoactivity and sedation (accompanied by gagging and vomiting in nonhuman primates, but not seen in rodents) followed by akinesia, rigidity, stereotypies (repetitive scapular scratching in rodents), loss of postural control, convulsions (forelimb only, progressing to full tonic-clonic), and death. Both the extent to which the animal progresses through this sequence and the speed of progression are dose dependent (Tasker et al. 1991). Further, the profile of acute behavioral toxicity does not appear to be appreciably different in male or female animals, although it has been suggested that different strains of mice may demonstrate altered sensitivity to DOM (Peng et al. 1997).

Predictably, behavioral toxicity following acute injections of DOM correlates with abnormal hippocampal discharges and limbic seizures as recorded electroencephalographically (Nakajima and Potvin 1992; Fujita et al. 1996; Scallet et al. 2004). Moreover, and consistent with the effects observed in human intoxication (see Sect. 4.2), acute injections of DOM in rats have also been reported to alter neurobehavioral performance in tests of passive avoidance, auditory startle, or conditioned avoidance response (Sobotka et al. 1996) or of learning and memory (Sutherland et al. 1990; Petrie et al. 1992; Nakajima and Potvin 1992; Clayton et al. 1999).

3.3 Histopathology

Despite the differences in study protocol, there is an overall agreement regarding the histopathology of the acute brain lesions associated with DOM toxicity. DOM induces neurodegenerative changes consisting of neuronal shrinkage, vacuolization of the cytoplasm, cell drop out, edema, and microvacuolation of the neuropil. Light

and electron microscopic observations in animal studies show two types of neuronal degeneration associated with DOM toxicity: swollen, vacuolated neurons are described intermingled with some shrunken and darkly stained electron dense neurons (Tryphonas et al. 1990a, b; Strain and Tasker 1991; Ananth et al. 2001; Pulido 2008). These findings are consistent with the view that the neuronal degeneration induced by DOM is mostly necrotic, at least in the short term. Studies using markers of cell injury and apoptosis also support this view (Ananth et al. 2001) as do studies in organotypic hippocampal slice cultures (see Sect. 2 above). These changes have preferential distribution within structures of the limbic system (Tryphonas et al. 1990a, b; Tryphonas and Iverson 1990; Strain and Tasker,1991), and the hippocampus among other brain regions appears to be a specific target site having high sensitivity to DOM toxicity, particularly the pyramidal neurons in the CA3 region, followed by the dentate gyrus and CA1 region (Tryphonas, et al. 1990a, b; Sutherland et al. 1990; Strain and Tasker 1991). With some exception, the CA2 region is reported as the least affected (Slikker et al. 1998). Other regions affected include the piriform and entorhinal cortices, olfactory bulbs, nucleus accumbens, arcuate nucleus, area postrema, and the retina (Iverson et al. 1989; Tryphonas et al. 1990a, b; Tryphonas and Iverson 1990; Schmued et al. 1995). The anatomical extent of brain lesions induced by DOM has also been identified by magnetic resonance imaging microscopy in both human (Cendes et al. 1995) and rats (Lester et al. 1999) and is consistent with the histopathology data.

In addition to neuronal degeneration, there is also evidence suggesting involvement and injury of glial cells, including astrocytes and microglia (Mayer 2000; Mayer et al. 2001; Gill et al. 2008; Pulido 2008) which is also consistent with results obtained in organotypic slice cultures (see Sect. 2 above).

3.4 DOM as a Developmental Neurotoxin

Although much of the early DOM literature dealt with acute, and to a lesser extent chronic, toxicity in adult animals (see Sects. 3.2 and 3.3 above), evidence and interest in the effects of DOM on the developing nervous system and DOM's potential role as a developmental neurotoxin have been appearing at an escalating pace over the past several years (for review see Costa et al. 2010). Despite an early report of negative consequences to DOM exposure in utero (Dakshinamurti et al. 1993), the issue of gestational toxicity lays somewhat dormant for many years but has recently been explored in greater detail with publications on maternal-fetal transfer rates (Maucher and Ramsdell 2007) and fetal kinetics (Maucher-Fuquay et al. 2012) as well as reports of toxicity following exposure in utero in rats (Tanemura et al. 2009) and sea lions (Ramsdell and Zabka 2008).

Because rat brain development is delayed relative to humans, early postnatal life in the rat corresponds roughly to late third-trimester brain development in humans, a particularly sensitive period of brain development. Pioneering studies by Xi et al. (1997) and Doucette et al. (2000) demonstrated convincingly that the neonatal rat brain was exquisitely sensitive to the toxic effects of DOM. Further, this difference

in potency relative to adult rats changes throughout early life, with dose–response relationships for DOM toxicity shifting progressively to the right between 0 and 22 days of age (Doucette et al. 2000), an effect that is presumed to be due to maturation of the blood–brain barrier. During the second postnatal week of life in the rat (i.e., the middle of the brain growth spurt; Dobbing and Smart 1974), doses of DOM as low as 20 μg/kg can alter neurobehavioral development (Doucette et al. 2003). Of particular interest, and concern, are an ever-increasing number of publications showing that exposure of rat pups to low doses of DOM during early development can result in later-onset symptoms of disease in the resulting adult animal. Best characterized to date are the effects of low-dose neonatal DOM in producing a neurodevelopmental model of temporal lobe epilepsy characterized by changes in behavior (Doucette et al. 2004), hippocampal morphology (Doucette et al. 2004; Bernard et al. 2007; Gill et al. 2010b), seizure threshold (Gill et al. 2010a), and cognition (Gill et al. 2012). More recently, low doses of DOM during development have also been shown to produce apparently permanent changes in both sleep patterns (Gill et al. 2009) and attentional processing performance (e.g., prepulse inhibition, latent inhibition) (Adams et al. 2008; Marriott et al. 2012) that are commonly seen in schizophrenia and related neuropsychiatric disorders. These observations and the resulting slowly developing disease models can prove useful in dissecting the complex sequence of changes that underlies neurological diseases, but given that current regulatory limits on DOM are based on acute toxicity in adult animals, they also raise serious questions about the potential of low concentrations of DOM in the environment or in food being a developmental neurotoxin that may warrant additional scrutiny (Stewart 2010).

4 Clinical Toxicity

4.1 Toxicity in Wildlife

DOM and to some extent DOM isomers have been found in oceans worldwide, so it is not surprising that there have been many reports of DOM toxicity in wildlife species. Reviews of much of this data can be found in Doucette and Tasker (2008) and Lefebvre and Robertson (2010) as well as other sources. While production of DOM in the aquatic environment has historically been associated with the red alga *Chondria armata* (Takemoto and Daigo 1960), the source of DOM as an environmental toxicant is through blooms of planktonic species including *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia australis* as well as many others. For further detail, the reader is directed to any of several excellent reviews including Bates and Horner (1998) and Bates and Trainer (2006). Plankton species are a food source for many aquatic invertebrates particularly bivalve molluscs (e.g., mussels, clams, oysters) as well as planktivorous fish (e.g., anchovies, sardines) and mammals (e.g., filter-feeding whales). While toxicity has been reported in all of these species, it is also clear that DOM toxicity extends higher up to the food chain and affects invertebrates that consume contaminated shellfish (e.g., crabs) as well as piscivorous birds

(e.g., cormorants, pelicans) and marine mammals that eat fish and/or shellfish (e.g., sea lions, sea otters, piscivorous whales) (Lefebvre et al. 1999, 2000, 2001, 2002).

The symptoms associated with DOM toxicity in these species vary, but it is generally believed that most invertebrate species are unaffected (presumably due to their rudimentary nervous system), whereas fish, birds, and mammals display classic signs of neurotoxicity. Indeed it is believed, although not confirmed, that seabirds intoxicated with DOM in California were the inspiration for Alfred Hitchcock's famous movie The Birds (© Universal Pictures, 1963). Less romantic, but far better documented, accounts of DOM intoxication in wildlife have been reported by a number of groups, including an excellent series of reports on intoxication of California sea lions (*Zalophus californianus*) by both US west coast and east coast scientists (e.g., Scholin et al. 2000; Ch'ng et al. 2002; Gulland et al. 2002; Silvagni et al. 2005; Goldstein et al. 2008).

4.2 Toxicity in Humans

Late in 1987, an outbreak of a newly recognized acute illness caused by eating blue mussels and characterized by gastrointestinal and unusual neurological symptoms occurred in Canada. More than 107 people (47 men and 60 women) were affected, most from Quebec. A case was defined as the presence of gastrointestinal symptoms (vomiting, abdominal cramps, diarrhea) within 24 h and neurological symptoms within 48 h (severe headache and memory loss). The etiologic agent was found to be DOM, an excitatory neurotransmitter amino acid, produced by *Nitzschia pungens f. multiseries*. Both *N. pungens* and DOM were present in the digestive glands of the causative cultivated mussels, harvested from the eastern coast of Prince Edward Island, and shipped to other parts of Canada (Perl et al. 1990; Teitelbaum et al. 1990).

Of the 107 persons that met the case definition, 19 were hospitalized from 4 to 101 days, 12 of these were admitted to intensive care, and 3 died in hospital at 12–18 days after admission. Another patient died after 3 months. Ninety nine of the patients answered a questionnaire, in which they provided information on the symptoms and on the amount of mussels consumed. Of these patients, 49 were between 40 and 59 years old and 38 patients were 60 years or older. Symptoms of illness included nausea (77 %), vomiting (76 %), abdominal cramps (51 %), diarrhea (42 %), headache (43 %), and memory loss (25 %). None of the younger patients (20–39 yrs) suffered memory loss, and their only symptoms were of a gastrointestinal nature. Hospital charts were available for 16 of the 19 hospitalized patients, indicating that all severely ill patients less than 65 years old had preexisting illnesses, including diabetes (3), chronic renal disease (2), and hypertension with a history of transient ischemic attacks (1) considered to be predisposing factors. All patients admitted to intensive care had serious neurologic dysfunction, including coma (9), mutism (11), and seizures (8). Seven patients had unstable blood pressure or cardiac arrhythmias. For nine patients and one person who did not become ill, analytical information on the unconsumed portion of the

Table 1 Estimated exposure and clinical course of patients who ingested DOM in contaminated mussels during the 1987 outbreak of amnesic shellfish poisoning in Canada

Patient	Age	Estimated DOM consumed (mg)	Clinical symptoms and treatment			
			GI	Memory loss	Hospitalized	ICU
1	72	60	Y	-	-	-
2	62	70	Y	Y	-	-
3	70	80	Y	-	-	-
4	61	90	Y	-	-	-
5	67	110	Y	-	-	-
6	71	110	Y	-	-	-
7	74	270	Y	Y	Y	-
8	68	290	Y	Y	Y	Y
9	84	290	Y	Y	Y	Y

Data adapted from Perl et al. (1990)
GI refers to gastrointestinal symptoms; *ICU* refers to intensive care unit

mussels and recall information on portion size were available, and this was used to estimate exposure. The concentration of DOM in these mussels was determined by mouse bioassay (characteristic hind leg scratching) and ranged between 31 and 128 mg/100 g. Increasing exposure correlated with the clinical course of events (Table 1). All patients reported gastrointestinal illness, but only one of six patients who consumed between 60 and 110 mg DOM suffered memory loss and none required hospitalization. All three patients who had consumed 270–290 mg DOM suffered neurological symptoms and were hospitalized. One person who consumed only 20 mg DOM did not become ill. The cognitive impairment observed in this new disease, attributed to DOM, appeared to be persistent and led to the term "amnesic shellfish poisoning" (Perl et al. 1990; Todd 1990). For a detailed description of neurologic and neuropsychiatric profiles of patients subsequent to the initial event, the reader is referred to reports by Gjedde and Evens (1990) and Cendes et al. (1995).

Neuropathological examination of the four patients who died indicated neuronal necrosis and astrocytosis particularly in the hippocampus and the amygdaloid nucleus. All four victims also had lesions in the claustrum, secondary olfactory areas, the septal area, and the nucleus accumbens septi. Two had prominent thalamic damage, especially in the dorsal medial nucleus. The subfrontal cortex was also damaged in three of the patients. The authors noted that the pattern of damage in the hippocampus appeared to parallel that seen in animals which suffered neurotoxic reactions after administration of kainic acid. Cerebrovascular disease was evident in two patients: an acute hemorrhagic infarct in the left posterior perisylvian cerebral in one victim and, in the other, cerebellar infarcts likely related to a myocardial infarct 3 days prior to death. Neurofibrillary tangles, and senile plaques, typical features of Alzheimer's disease, were not observed in these patients (Teitelbaum et al. 1990).

Although the 1987 incident in Montreal is the only confirmed case of human intoxication with DOM, an outbreak of DOM poisoning may have occurred in

October/November 1991 in Washington State, USA. Approximately two-dozen people became ill after ingesting razor clams harvested along the Washington and Oregon coasts. Although gastrointestinal or neurological symptoms were observed within 36 h of ingestion, no other symptoms consistent with DOM intoxication were reported. A total of 21 possible DOM victims and 43 non-victims were interviewed. Based on the concentrations of DOM measured in razor clams, the mean total DOM consumption by the 21 victims would have been 17 mg (estimated to be 0.28 mg/kg b.w.) and 8 mg by the unaffected individuals (0.13 mg/kg b.w.). A total of 13 patients developed neurological symptoms, but all recovered. Unfortunately the incident was not well reported and was unsubstantiated (Todd 1993; Jeffrey et. al. 2004).

5 Conclusion

Revived from relative obscurity into the limelight as a result of the 1987 incident of human intoxication, DOM has become an environmental and experimental toxin of considerable importance worldwide. As an environmental contaminant affecting both the food supply and wildlife species, DOM and DOM isomers are now routinely monitored in many jurisdictions. As an experimental tool in neurotoxicology and neuroscience, DOM has a unique place as a slowly desensitizing AMPA/KA-R agonist that shows high potency and efficacy in a wide variety of experimental systems. Perhaps the most interesting thing, and of great potential concern, however, is the emerging evidence of DOM as a neurodevelopmental toxin and a precipitating factor in the onset and progression of various forms of neurological disease. Whether these properties can be exploited in the form of disease models and/or warrant improved vigilance on the part of global regulatory bodies, continued study of the many fascinating properties of DOM and isomers is warranted.

References

Adams, A. L., Doucette, T. A., & Ryan, C. L. (2008). Altered pre-pulse inhibition in adult rats treated neonatally with domoic acid. *Amino Acids, 35*, 157–160.

Ananth, C., Dheen, S. T., Gopalakrishnakone, P., & Kaur, C. (2001). Domoic acid-induced neuronal damage in the rat hippocampus: Changes in apoptosis related genes (Bcl-2, Bax, Caspase-3) and microglial response. *Journal of Neuroscience Research, 66*, 177–190.

Bates, S. S., & Horner, R. A. (1998). Bloom dynamics and physiology of domoic-acid-producing *Pseudo-nitzschia* species. In D. M. Anderson, A. D. Cembella, & G. M. Hallegraeff (Eds.), *Physiological ecology of harmful algal blooms* (pp. 267–292). Heidelberg: Springer.

Bates, S. S., & Trainer, V. L. (2006). The ecology of harmful diatoms. In E. Granéli & J. Turner (Eds.), *Ecology of harmful algae* (Ecological studies, Vol. 189, pp. 81–93). Heidelberg: Springer.

Beani, L., Bianchi, C., Guerrini, F., Marani, L., Pistocchi, R., Tomasini, M. C., Ceredi, A., Milandri, A., Poletti, R., & Boni, L. (2000). High sensitivity bioassay of paralytic (PSP) and amnesic (ASP) algal toxins based on the fluorimetric detection of [Ca(2+)](i) in rat cortical primary cultures. *Toxicon, 38*, 1283–1297.

Berman, F. W., & Murray, T. F. (1996). Characterization of glutamate toxicity in cultured rat cerebellar granule neurons at reduced temperature. *Journal of Biochemical Toxicology, 11*, 111–119.

Berman, F. W., & Murray, T. F. (1997). Domoic acid neurotoxicity in cultured cerebellar granule neurons is mediated predominantly by NMDA receptors that are activated as a consequence of excitatory amino acid release. *Journal of Neurochemistry, 69*, 693–703.

Berman, F. W., LePage, K. T., & Murray, T. F. (2002). Domoic acid neurotoxicity in cultured cerebellar granule neurons is controlled preferentially by the NMDA receptor Ca(2+) influx pathway. *Brain Research, 924*, 20–29.

Bernard, P. B., Macdonald, D. S., Gill, D. A., Ryan, C. L., & Tasker, R. A. (2007). Hippocampal mossy fiber sprouting and elevated trkB receptor expression following systemic administration of low dose domoic acid during neonatal development. *Hippocampus, 17*, 1121–1133.

Bose, R., Pinsky, C., & Glavin, G. B. (1990). Sensitive murine model and putative antidotes for behavioral toxicosis from contaminated mussel extracts. *Canada Diseases Weekly Report, 16* (SIE), 91–100.

Canete, E., & Diogene, J. (2008). Comparative study of the use of neuroblastoma cells (Neuro-2a) and neuroblastomaxglioma hybrid cells (NG108-15) for the toxic effect quantification of marine toxins. *Toxicon, 52*, 541–550.

Cendes, F., Andermann, F., Carpenter, S., Zatorre, R. J., & Cashman, N. R. (1995). Temporal lobe epilepsy caused by domoic acid intoxication: Evidence for glutamate receptor-mediated excitotoxicity in humans. *Annals of Neurology, 37*, 123–126.

Ch'ng, M. M., Leighfield, T. A., Busman, M. A., Gulland, F., Matassa, K., Chechowitz, M., Rowles, T., & Van Dolah, F. M. (2002). *Involvement of domoic acid in marine mammal morbidities and mortalities on the west coast of the U.S. during the year 2000*. Report to the Working Group on Marine Mammal Unusual Mortality Events.

Clayton, E. C., Peng, Y.-G., & Ramsdell, J. S. (1999). Working memory deficits induced by single but not repeated exposures to domoic acid. *Toxicon, 37*, 1025–1039.

Clements, J. D., Feltz, A., Sahara, Y., & Westbrook, G. L. (1998). Activation kinetics of AMPA receptor channels reveal the number of functional agonist binding sites. *Journal of Neuroscience, 18*, 119–127.

Costa, L. G., Giordan, G., & Faustman, E. M. (2010). Domoic acid as a developmental neurotoxin. *Neurotoxicology, 31*, 409–423.

Cunha, R. A., Constantino, M. D., & Ribeiro, J. A. (1997). Inhibition of [^{3}H]γ-aminobutyric acid release by kainate receptor activation in rat hippocampal synaptosomes. *European Journal of Pharmacology, 323*, 167–172.

Dakshimamurti, K., Sharma, S. K., Sundaram, M., & Wantanabe, T. (1993). Hippocampal changes in developing postnatal mice following intrauterine exposure to domoic acid. *Journal of Neuroscience, 13*, 4486–4495.

Diaz-Trelles, R., Novelli, A., & Fernandez-Sanchez, M. T. (2003). RNA synthesis-dependent potentiation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor-mediated toxicity by antihistamine terfenadine in cultured rat cerebellar neurons. *Neuroscience Letters, 345*, 136–140.

Dobbing, J., & Smart, J. L. (1974). Vulnerability of developing brain and behaviour. *British Medical Bulletin, 30*, 164–168.

Doucette, T. A., & Tasker, R. A. (2008). Domoic acid: Detection methods, pharmacology and toxicology. In L. M. Botano (Ed.), *Seafood and freshwater toxins: Pharmacology, physiology and detection* (pp. 397–430). Boca Raton: CRC Press.

Doucette, T. A., Strain, S. M., Allen, G. V., Ryan, C. L., & Tasker, R. A. R. (2000). Comparative behavioural toxicity of domoic acid and kainic acid in neonatal rats. *Neurotoxicology and Teratology, 22*, 863–869.

Doucette, T. A., Bernard, P. B., Yuill, P. C., Tasker, R. A., & Ryan, C. L. (2003). Low doses of non-NMDA glutamate receptor agonists alter neurobehavioural development in the rat. *Neurotoxicology & Teratology, 25*, 473–479.

Doucette, T. A., Bernard, P. B., Husum, H., Perry, M. A., Ryan, C. L., & Tasker, R. A. (2004). Low doses of domoic acid during postnatal development produce permanent changes in rat behaviour and hippocampal morphology. *Neurotoxicity Research, 6*, 555–563.

Duran, R., Reis, R. A., Almeida, O. M., de-Mello, M. C., & de-Mello, F. G. (1995). Domoic acid induces neurotoxicity and ip3 mobilization in cultured cells of embryonic chick retina. *Brazilian Journal of Medical and Biological Research, 28*, 100–107.

Erin, N., & Billingsley, M. L. (2004). Domoic acid enhances Bcl-2-calcineurin-inositol-1,4,5-trisphosphate receptor interactions and delayed neuronal death in rat brain slices. *Brain Research, 1014*, 45–52.

Fernandez-Sanchez, M. T., & Novelli, A. (1993). Basic fibroblast growth factor protects cerebellar neurons in primary culture from NMDA and non-NMDA receptor mediated neurotoxicity. *FEBS Letters, 335*, 124–131.

Fujita, T., Tanaka, T., Yonemasu, Y., Cendes, F., Cashman, N. R., & Andermann, F. (1996). Electroclinical and pathological studies after parenteral administration of domoic acid in freely moving nonanesthetized rats: An animal model of excitotoxicity. *Journal of Epilepsy, 9*, 87–93.

Gill, S. S., Hou, Y., Ghane, T., & Pulido, O. M. (2008). Regional susceptibility to domoic acid in primary astrocyte cells cultured from the brain stem and hippocampus. *Marine Drugs, 6*, 25–38.

Gill, D. A., Bastlund, J. F., Anderson, N. J., & Tasker, R. A. (2009). Reductions in paradoxical sleep time in adult rats treated neonatally with low dose domoic acid. *Behavioural Brain Research, 205*, 564–567.

Gill, D. A., Bastlund, J. F., Watson, W. P., Ryan, C. L., Reynolds, D. S., & Tasker, R. A. (2010a). Neonatal exposure to low-dose domoic acid lowers seizure threshold in adult rats. *Neuroscience, 169*, 1789–1799.

Gill, D. A., Ramsay, S. L., & Tasker, R. A. (2010b). Selective reductions in subpopulations of GABAergic neurons in a developmental rat model of epilepsy. *Brain Research, 1331*, 114–123.

Gill, D. A., Perry, M. A., McGuire, E. P., Pérez-Gómez, A., & Tasker, R. A. (2012). Low-dose neonatal domoic acid causes persistent changes in behavioural and molecular indicators of stress response in rats. *Behavioural Brain Research, 230*, 409–417.

Giordano, G., White, C. C., McConnachie, L. A., Fernandez, C., Kavanagh, T. J., & Costa, L. G. (2006). Neurotoxicity of domoic acid in cerebellar granule neurons in a genetic model of glutathione deficiency. *Molecular Pharmacology, 70*, 2116–2126.

Giordano, G., White, C. C., Mohar, I., Kavanagh, T. J., & Costa, L. G. (2007). Glutathione levels modulate domoic acid induced apoptosis in mouse cerebellar granule cells. *Toxicological Sciences, 100*, 433–444.

Giordano, G., Klintworth, H. M., Kavanaugh, T. J., & Costa, L. G. (2008). Apoptosis induced by domoic acid in mouse cerebellar granule neurons involves activation of p38 and JNK MAP kinases. *Neurochemistry International, 52*, 1100–1105.

Giordano, G., Li, L., White, C. C., Farin, F. M., Wilkerson, H. W., Kavanagh, T. J., & Costa, L. G. (2009). Muscarinic receptors prevent oxidative stress-mediated apoptosis induced by domoic acid in mouse cerebellar granule cells. *Journal of Neurochemistry, 109*, 525–538.

Gjedde, A., & Evens, A. C. (1990). PET studies of domoic acid poisoning in humans: Excitotoxic destruction of brain glutamatergic pathways, revealed in measurements of glucose metabolism by positron emission tomography. *Canada Diseases Weekly Report, 16*(Suppl. 1E), 105–109.

Glavin, G., Pinsky, C., & Bose, R. (1989). Toxicology of mussels contaminated by neuroexcitant domoic acid. *Lancet, 1*(8636), 506–507.

Glavin, G. B., Pinsky, C., & Bose, R. (1990). Domoic acid-induced neurovisceral toxic syndrome: Characterization of an animal model and putative antidotes. *Brain Research Bulletin, 24*, 701–703.

Goldstein, T., Mazet, J. A., Zabka, T. S., Langlois, G., Colegrove, K. M., Silver, M., Bargu, S., Van Dolah, F., Leighfield, T., Conrad, P. A., Barakos, J., Williams, D. C., Dennison, S., Haulena, M., & Gulland, F. M. (2008). Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (Zalophus californianus): An increasing risk to marine mammal health. *Proceeding of the Biological Society, 275*, 267–276.

Gressens, P., Spedding, M., Gigler, G., Kertesz, S., Villa, P., Medja, F., Williamson, T., Kapus, G., Levay, G., Szenasi, G., Barkoczy, J., & Harsing, L. G., Jr. (2005). The effects of AMPA receptor antagonists in models of stroke and neurodegeneration. *European Journal of Pharmacology, 519*, 58–67.

Grimmelt, B., Nijjar, M. S., Brown, J., MacNair, N., Wagner, S., Johnson, G. R., & Amend, J. F. (1990). Relationship between domoic acid levels in the blue mussel (*Mytilus edulis*) and toxicity in mice. *Toxicon, 28*, 501–508.

Gruol, D. L., Parsons, K. L., & DiJulio, N. (1997). Acute ethanol alters calcium signals elicited by glutamate receptor agonists and K^+ depolarization in cultured cerebellar Purkinje neurons. *Brain Research, 773*, 82–89.

Gulland, F. M. D., Haulena, M., Fauquier, D., Langlois, G., Lander, M. E., Zabka, T., & Duerr, R. (2002). Domoic acid toxicity in Californian sea lions (*Zalophus californianus*): Clinical signs, treatment and survival. *The Veterinary Record, 150*, 475–480.

Hampson, D. R., & Manalo, J. L. (1998). The activation of glutamate receptors by kainic acid and domoic acid. *Natural Toxins, 6*, 153–158.

Hampson, D. R., Huang, X., Wells, J. W., Walter, J. A., & Wright, J. L. C. (1992). Interaction of domoic acid and several derivatives with kainic acid and AMPA binding sites in rat brain. *European Journal of Pharmacology, 218*, 1–8.

Hogberg, H. T., & Bal-Price, A. K. (2011). Domoic acid-induced neurotoxicity Is mainly mediated by the AMPA/KA receptor: Comparison between immature and mature primary cultures of neurons and glial cells from rat cerebellum. *Journal of Toxicology, 2011*, 543512.

Hogberg, H. T., Sobanski, T., Novellino, A., Whelan, M., Weiss, D. G., & Bal-Price, A. K. (2011). Application of micro-electrode arrays (MEAs) as an emerging technology for developmental neurotoxicity: Evaluation of domoic acid-induced effects in primary cultures of rat cortical neurons. *Neurotoxicology, 32*, 158–168.

Iverson, F., Truelove, J., Nera, E., Tryphonas, L., Campbell, J., & Lok, E. (1989). Domoic acid poisoning and mussel-associated intoxication: Preliminary investigations into the response of mice and rats to toxic mussel extract. *Food and Chemical Toxicology, 27*, 377–384.

Iverson, F., Truelove, J., Tryphonas, L., & Nera, E. A. (1990). The toxicology of domoic acid administered systematically to rodents and primates. *Canada Diseases Weekly Report, 16* (Suppl. 1E), 15–19.

Jakobsen, B., Tasker, A., & Zimmer, J. (2002). Domoic acid neurotoxicity in hippocampal slice cultures. *Amino Acids, 23*, 37–44.

Jeffery, B., Barlow, T., Moizer, K., Paul, S., & Boyle, C. (2004). Amnesic shellfish poison. *Food and Chemical Toxicology, 42*, 545–557.

Jensen, J. B., Schousboe, A., & Pickering, D. S. (1999). Role of desensitization and subunit expression for kainate receptor-mediated neurotoxicity in murine neocortical cultures. *Journal of Neuroscience Research, 55*, 208–217.

Joseph, D. J., Williams, D. J., & MacDermott, A. B. (2011). Modulation of neurite outgrowth by activation of calcium-permeable kainate receptors expressed by rat nociceptive-like dorsal root ganglion neurons. *Developmental Neurobiology, 71*, 818–835.

Konig, N., Serrano, P., & Drian, M. J. (1994). AMPA elicits long-lasting, partly hypothermia-sensitive calcium responses in acutely dissociated or cultured embryonic brainstem cells. *Neurochemistry International, 24*, 459–471.

Larm, J. A., Beart, P. M., & Cheung, N. S. (1997). Neurotoxin domoic acid produces cytotoxicity via kainate- and AMPA-sensitive receptors in cultured cortical neurones. *Neurochemistry International, 31*, 677–682.

Lefebvre, K. A., & Robertson, A. (2010). Domoic acid and human exposure risks: A review. *Toxicon, 56*, 218–230.

Lefebvre, K. A., Powell, C. L., Busman, M., Doucette, G. J., Moeller, P. D. R., Silver, J. B., Miller, P. E., Hughes, M. P., Singaram, S., Silver, M. W., & Tjeerdema, R. S. (1999). Detection of domoic acid in Northern anchovies and California sea lions associated with an unusual mortality event. *Natural Toxins, 7*(3), 85–92.

Lefebvre, K., Powell, C., Doucette, G., Silver, J., Miller, P., Hughes, P., Silver, M., & Tjeerdema, R. (2000). Domoic acid-producing diatoms: Probable cause of neuroexcitotoxicity in California sea lions. *Marine Environmental Research, 50*, 485–488.

Lefebvre, K. A., Dovel, S. L., & Silver, M. W. (2001). Tissue distribution and neurotoxic effects of domoic acid in a prominent vector species, the Northern anchovy *Engraulis mordax*. *Marine Biology, 138*, 693–700.

Lefebvre, K. A., Bargu, S., Kieckhefer, T., & Silver, M. S. (2002). From sandabs to blue whales: The pervasiveness of domoic acid. *Toxicon, 40*, 971–977.

Lester, D. S., Lyon, R. C., McGregor, G. N., Engelhardt, R. T., Schmued, L. C., Johnson, G. A., & Johannessen, J. N. (1999). 3-dimensional visualization of lesions in rat brain using magnetic resonance imaging microscopy. *Neuroreport, 10*, 737–741.

Liu, L. L., Li, L., Chen, D., & Liu, Y. S. (2008). Effects of domoic acid on membrane function of primary cultured rat glial cells. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi, 26*, 725–728.

Maeda, M., Kodama, T., Tanaka, T., Yoshizumi, H., Takemoto, T., Nomoto, K., & Fujita, T. (1986). Structures of isodomoic acids A, B and C, novel insecticidal amino acids from the red alga *Chondria armata*. *Chemical and Pharmaceutical Bulletin, 34*, 4892–4895.

Marriott, A. L., Ryan, C. L., & Doucette, T. A. (2012). Neonatal domoic acid treatment produces alterations to prepulse inhibition and latent inhibition in adult rats. *Pharmacology Biochemistry and Behavior, 103*, 338–344.

Maucher, J. M., & Ramsdell, J. S. (2007). Maternal-fetal transfer of domoic acid in rats at two gestational time points. *Environmental Health Perspectives, 115*, 1743–1746.

Maucher-Fuquay, J., Muha, N., Wang, Z., & Ramsdell, J. S. (2012). Toxicokinetics of domoic acid in the fetal rat. *Toxicology, 294*, 36–41.

Mayer, A. M. (2000). The marine toxin domoic acid may affect the developing brain by activation of neonatal brain microglia and subsequent neurotoxic mediator generation. *Medical Hypotheses, 54*, 837–841.

Mayer, A. M., Hall, M., Fay, M. J., Lamar, P., Pearson, C., Prozialeck, W. C., Lehmann, V. K., Jacobson, P. B., Romanic, A. M., Uz, T. & Manev, H. (2001). Effect of a short-term in vitro exposure to the marine toxin domoic acid on viability, tumor necrosis factor-alpha, matrix metalloproteinase-9 and superoxide anion release by rat neonatal microglia. *BMC Pharmacology*, *1*, 7.

Mayer, A. M., Guzman, M., Peksa, R., Hall, M., Fay, M. J., Jacobson, P. B., Romanic, A. M., & Gunasekera, S. P. (2007). Differential effects of domoic acid and *E. coli* lipopolysaccharide on tumor necrosis factor-alpha, transforming growth factor-beta1 and matrix metalloproteinase-9 release by rat neonatal microglia: Evaluation of the direct activation hypothesis. *Marine Drugs, 5*, 113–135.

Munday, R., Holland, P. T., McNabb, P., Selwood, A. I., & Rhodes, L. L. (2008). Comparative toxicity to mice of domoic acid and isodomoic acids A, B and C. *Toxicon, 52*, 954–956.

Nakajima, S., & Potvin, J. L. (1992). Neural and behavioural effects of domoic acid, an amnesic shellfish toxin, in the rat. *Canadian Journal of Psychology, 46*, 569–581.

Nduaka, C. I., Taylor, R. E., Green, S., Flynn, T., Sathyamoorthy, V. V., Sprando, R. L., & Johannessen, J. N. (1999). Glutamate release from chick retina explants in response to domoic acid. *In Vitro & Molecular Toxicology, 12*, 173–182.

Novelli, A., Kispert, J., Fernandez-Sanchez, M. T., Torreblanca, A., & Zitko, V. (1992). Domoic acid-containing toxic mussels produce neurotoxicity in neuronal cultures through a synergism between excitatory amino acids. *Brain Research, 577*, 41–48.

Peng, Y. G., Clayton, E. C., & Ramsdell, J. S. (1997). Repeated independent exposures to domoic acid do not enhance symptomatic toxicity in outbred or seizure-sensitive inbred mice. *Toxicological Sciences, 40*, 63–67.

Pérez-Gómez, A., & Tasker, R. A. (2012). Enhanced neurogenesis in organotypic cultures of rat hippocampus after transient subfield-selective excitotoxic insult induced by domoic acid. *Neuroscience, 208*, 97–108.

Pérez-Gómez, A., Novelli, A., & Fernandez-Sanchez, M. T. (2010). Na^+/K^+-ATPase inhibitor palytoxin enhances vulnerability of cultured cerebellar neurons to domoic acid via sodium-dependent mechanisms. *Journal of Neurochemistry, 114*, 28–38.

Perl, T. M., Bédard, L., Kosatsky, T., Hockin, J. C., Todd, E. C. D., & Remis, R. S. (1990). An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *The New England Journal of Medicine, 322*, 1775–1780.

Petrie, B. F., Pinsky, C., Standish, N. M., Bose, R., & Glavin, G. B. (1992). Parenteral domoic acid impairs spatial learning in mice. *Pharmacology, Biochemistry, and Behavior, 41*, 211–214.

Preston, E., & Hynie, I. (1991). Transfer constants for blood–brain barrier permeation of the neuroexcitatory shellfish toxin, domoic acid. *Canadian Journal of Neurological, 18*, 39–44.

Pulido, O. M. (2008). Domoic acid toxicologic pathology: A review. *Marine Drugs, 6*, 180–219.

Qiu, S., Pak, C. W., & Curras-Collazo, M. C. (2006). Sequential involvement of distinct glutamate receptors in domoic acid-induced neurotoxicity in rat mixed cortical cultures: Effect of multiple dose/duration paradigms, chronological age, and repeated exposure. *Toxicological Sciences, 89*, 243–256.

Quilliam, M. A. (2003). Chemical methods for domoic acid, the amnesic shellfish poisoning (ASP) toxin. In G. M. Hallegraeff, D. M. Anderson, & A. D. Cembella (Eds.), *Manual on harmful marine microalgae* (Vol. 11). Paris: Intergovernmental Oceanographic Commission (UNESCO). Chpt 9.

Raman, M., Chen, W., & Cobb, M. H. (2007). Differential regulation and properties of MAPKs. *Oncogene, 26*, 3100–3112.

Ramsdell, J. S., & Zabka, T. S. (2008). In utero domoic acid toxicity: A fetal basis to adult disease in the California sea lion (Zalophus californianus). *Marine Drugs, 6*, 262–290.

Rodriquez-Moreno, A., Lopez-Garcia, J., & Lerma, J. (2000). Two populations of kainate receptors with separate signaling mechanisms in hippocampal interneurons. *Proceeding of the National Academy of Sciences, 97*, 1293–1298.

Ross, I. A., Johnson, W., Sapienza, P. P., & Kim, C. S. (2000). Effects of the seafood toxin domoic acid on glutamate uptake by rat astrocytes. *Food and Chemical Toxicology, 38*, 1005–1111.

Roy, M., & Sapolsky, R. M. (2003). The neuroprotective effects of virally-derived caspase inhibitors p35 and crmA following a necrotic insult. *Neurobiology of Disease, 14*, 1–9.

Sawant, P. M., Weare, B. A., Holland, P. T., Selwood, A. I., King, K. L., Mikulski, C. M., Doucette, G. J., Mountfort, D. O., & Kerr, D. S. (2007). Isodomoic acids A and C exhibit low KA receptor affinity and reduced in vitro potency relative to domoic acid in region CA1 of rat hippocampus. *Toxicon, 50*, 627–638.

Sawant, P. M., Holland, P. T., Mountfort, D. O., & Kerr, D. S. (2008). In vivo seizure induction and pharmacological preconditioning by domoic acid and isodomoic acids A, B and C. *Neuropharmacology, 55*, 1412–1418.

Sawant, P. M., Tyndall, J. D. A., Holland, P. T., Peake, B. M., Mountfort, D. O., & Kerr, D. S. (2010). In vivo seizure induction and affinity studies of domoic acid and isodomoic acids D, E and F. *Neuropharmacology, 59*, 129–138.

Scallet, A. C., Binienda, Z., Caputo, F. A., Hall, S., Paule, M. G., Roundtree, R. L., Schmued, L., Sobotka, T., & Slikker, W., Jr. (1993). Domoic acid-treated cynomolgus monkeys (*M. fascicularis*): Effects of dose on hippocampal neuronal and terminal degeneration. *Brain Research, 627*, 307–313.

Scallet, A. C., Kowalke, P. K., Rountree, R. L., Thorn, B. T., & Binienda, Z. K. (2004). Electroencephalographic, behavioral, and c-fos responses to acute domoic acid exposure. *Neurotoxicology and Teratology, 26*, 331–342.

Schmued, L. C., Scallet, A. C., & Slikker, W., Jr. (1995). Domoic acid-induced neuronal degeneration in the primate forebrain revealed by degeneration specific histochemistry. *Brain Research, 695*, 64–70.

Scholin, C. A., Gulland, F., Doucette, G. J., Benson, S., Busman, M., Chavez, F. P., Cordaro, J., DeLong, R., De Vogelaere, A., Harvey, J., Haulena, M., Lefebvre, K., Lipscomb, T., Loscutoff, S., Lowenstine, L. J., Marin, R., Miller, P. E., McLellan, W. A., Moeller, P. D. R., Powell,

C. L., Rowles, T., Silvagni, P., Silver, M., Spraker, T., Trainer, V., & Van Dolah, F. M. (2000). Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature, 403*, 80–84.

Silvagni, P. A., Lowenstine, L. J., Spraker, T., Lipscomb, T. P., & Gulland, F. M. (2005). Pathology of domoic acid toxicity in California sea lions (*Zalophus californianus*). *Veterinary Pathology, 42*, 184–191.

Slikker, W., Jr., Scallet, A. C., & Gaylor, D. W. (1998). Biologically-based dose–response model for neurotoxicity risk assessment. *Toxicology Letters, 102*/*103*, 429–433.

Sobotka, T. J., Brown, R., Quander, D. Y., Jackson, R., Smith, M., Long, S. A., Barton, C. N., Rountree, R. L., Hall, S., Eilers, P., Johannessen, J. N., & Scallet, A. C. (1996). Domoic acid: Neurobehavioral and neurohistological effects of low-dose exposure in adult rats. *Neurotoxicology and Teratology, 18*, 659–670.

Stewart, I. (2010). Environmental risk factors for temporal lobe epilepsy – is prenatal exposure to the marine algal neurotoxin domoic acid a potentially preventable cause? *Medical Hypotheses, 74*, 466–481.

Stewart, G. R., Zorumski, C. F., Price, M. T., & Olney, J. W. (1990). Domoic acid: A dementia-inducing excitotoxic food poison with kainic acid receptor specificity. *Experimental Neurology, 110*, 127–138.

Strain, S. M., & Tasker, R. A. R. (1991). Hippocampal damage produced by systemic injections of domoic acid in mice. *Neuroscience, 44*, 343–352.

Sutherland, R. J., Hoesing, J. M., & Whishaw, I. Q. (1990). Domoic acid, an environmental toxin, produces hippocampal damage and severe memory loss. *Neuroscience Letters, 120*, 221–223.

Suzukin, C. A. M., & Hierlihy, S. L. (1993). Renal clearance of domoic acid in the rat. *Food and Chemical Toxicology, 31*, 701–706.

Takemoto, T., & Daigo, K. (1960). Constituents of Chondria armata and their pharmacological effects. *Archives of Pharmacology, 293*, 627–633.

Tanemura, K., Igarashi, K., Matsugami, T. R., Aisaki, K., Kitajima, S., & Kanno, J. (2009). Intrauterine environment-genome interaction and children's development (2): Brain structure impairment and behavioral disturbance induced in male mice offspring by a single intraperitoneal administration of domoic acid (DA) to their dams. *The Journal of Toxicological Sciences, 34*(Suppl 2), SP279–SP286.

Tasker, R. A. R., & Strain, S. M. (1998). Synergism between NMDA and domoic acid in a murine model of behavioural neurotoxicity. *Neurotoxicology, 19*, 593–598.

Tasker, R. A. R., Connell, B. J., & Strain, S. M. (1991). Pharmacology of systemically administered domoic acid in mice. *Canadian Journal of Physiology and Pharmacology, 69*, 378–382.

Tasker, R. A., Strain, S. M., & Drejer, J. (1996). Selective reduction in domoic acid toxicity in vivo by a novel non-N-methyl-D-aspartate antagonist. *Canadian Journal of Physiology and Pharmacology, 74*, 1047–1054.

Tasker, R. A. R., Perry, M. A., Doucette, T. A., & Ryan, C. L. (2005). NMDA receptor involvement in the effects of low dose domoic acid in neonatal rats. *Amino Acids, 28*, 193–196.

Tasker, R. A., Kuiper-Goodman, T., Oulido, O., & Lawrence, J. F. (2011). Domoic acid. In J. Lawrence, H. Loreal, H. Toyyofuku, P. Hess, K. Iddya, & L. Ababouch (Eds.), *Assessment and management of biotoxin risks in bivalve molluscs* (pp. 111–162). Rome: Food and Agriculture Organization of the United Nations.

Teitelbaum, J. S., Zatorre, R. J., Carpenter, S., Gendron, D., Evans, A. C., Gjedde, A., & Cashman, N. R. (1990). Neurologic sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. *The New England Journal of Medicine, 322*, 1781–1787.

Todd, E. C. D. (1990). Amnesic shellfish poisoning – a new toxin syndrome. In E. Graneli, B. Sundstrom, L. Edler, & D. M. Anderson (Eds.), *Toxic marine phytoplankton* (pp. 504–508). New York: Elsevier Science Publishing.

Todd, E. C. D. (1993). Domoic acid and amnesic shellfish poisoning – a review. *Journal of Food Protection, 56*, 69–83.

Truelove, J., & Iverson, F. (1994). Serum domoic acid clearance and clinical observations in the Cynomolgus monkey and Sprague–Dawley rat following a single IV dose. *Bulletin of Environmental Contamination and Toxicology, 52*, 479–486.

Truelove, J., Mueller, R., Pulido, O., & Iverson, F. (1996). Subchronic toxicity study of domoic acid in the rat. *Food and Chemical Toxicology, 34*, 525–529.

Tryphonas, L., & Iverson, F. (1990). Neuropathology of excitatory neurotoxins: The domoic acid model. *Toxicologic Pathology, 18*, 165–169.

Tryphonas, L., Truelove, J., & Iverson, F. (1990a). Acute parenteral neurotoxicity of domoic acid in cynomolgus monkeys (M. fascicularis). *Toxicologic Pathology, 18*, 297–303.

Tryphonas, L., Truelove, J., Nera, E., & Iverson, F. (1990b). Acute neurotoxicity of domoic acid in the rat. *Toxicologic Pathology, 18*, 1–9.

Vale-Gonzalez, C., Alfonso, A., Sunol, C., Vieytes, M. R., & Botana, L. M. (2006). Role of the plasma membrane calcium adenosine triphosphatase on domoate-induced intracellular acidification in primary cultures of cerebellar granule cells. *Journal of Neuroscience Research, 84*, 326–337.

Verdoorn, T. A., Johansen, T. H., Drejer, J., & Neilsen, E. O. (1994). Selective block of recombinant glur6 receptors by NS-102; a novel non-NMDA receptor antagonist. *European Journal of Pharmacology, 269*, 43–49.

Vranyac-Tramoundanas, A., Harrison, J. C., Sawant, P. M., Kerr, D. S., & Sammut, I. A. (2011). Ischemic cardiomyopathy following seizure induction by domoic acid. *American Journal of Pathology, 179*, 141–154.

Walter, J. A., Falk, M., & Wright, J. L. C. (1994). Chemistry of the shellfish toxin domoic acid: Characterization of related compounds. *Canadian Journal of Chemistry, 72*, 430.

Wright, J. L. C., Bird, C. J., deFreitas, A. S. W., Hampson, D., McDonald, J., & Quilliam, M. A. (1990). Chemistry, biology, and toxicology of domoic acid and its isomers. *Canada Disease Weekly Report, 16*(Suppl. 1E), 15–18.

Xi, D., & Ramsdell, J. S. (1996). Glutamate receptors and calcium entry mechanisms for domoic acid in hippocampal neurons. *Neuroreport, 7*, 1115–1120.

Xi, D., Peng, Y. G., & Ramsdell, J. S. (1997). Domoic acid is a potent neurotoxin to neonatal rats. *Natural Toxins, 5*, 74–79.

Xu, R., Tao, Y., Wu, C., Yi, J., Yang, Y., Yang, R., & Hong, D. (2008). Domoic acid induced spinal cord lesions in adult mice: Evidence for the possible molecular pathways of excitatory amino acids in spinal cord lesions. *Neurotoxicology, 29*, 700–707.

Zaman, L., Arakawa, O., Shimosu, A., Onoue, Y., Nishio, S., Shida, Y., & Noguchi, T. (1997). Two new isomers of domoic acid from a red alga, Chondria armata. *Toxicon, 35*, 205–212.

Endogenous Kynurenic Acid and Neurotoxicity

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Abstract

Tryptophan metabolism along kynurenine pathway yields a number of compounds affecting brain function. Among kynurenine derivatives, neuroprotective kynurenic acid (KYNA) and neurotoxic quinolinic acid and 3-hydroxykynurenine have stimulated the greatest scientific interest. KYNA, initially considered merely a side-product of tryptophan degradation, was discovered in 1982 to act as excitatory amino acid receptor antagonist. Since then, a number of novel KYNA targets emerged. KYNA was suggested to play a role as antagonist of α7 nicotinic receptors and ligand of G protein-coupled GPR35 and human aryl hydrocarbon (AHR) receptors. In here, research data is reviewed supporting the idea that produced by astrocytes KYNA serves as an endogenous neuroprotectant. Mechanisms controlling brain levels of KYNA are discussed in the context of neurodegenerative disorders, brain ischemia, and seizures. Available data concerning changes of brain KYNA in respective animal models and in human diseases, together with an overview of effects following the application of KYNA, KYNA analogues or compounds influencing the activity of enzymes along kynurenine pathway are presented. Emerging therapies designed to increase the level of neuroprotective KYNA may become an important avenue in the treatment of brain disorders accompanied by neuronal loss.

1 Introduction

The history of kynurenic acid (KYNA; see Fig. 1) dates to year 1853, when German chemist Baron Justus von Liebig determined its presence in canine urine (Liebig 1853). Half a century later, the discovery of tryptophan was followed by the observation that KYNA is one of its metabolites (Hopkins and Cole 1901; Ellinger 1904). For almost eight decades, the compound was considered merely a side-product of *tryptophan* degradation. In early 1980s, KYNA was reported to antagonize amino acid-induced excitation of central neurons (Perkins and Stone 1982) and to block neurotoxicity and seizures evoked by another tryptophan metabolite, quinolinic acid (Foster et al. 1984). Soon, the presence of KYNA within the brain of mammals and rodents was confirmed (Moroni et al. 1988a, b; Turski et al. 1988). Subsequently, numerous basic and clinical investigations addressed the role of KYNA in brain function, and the potential ways to modulate KYNA central levels were elucidated.

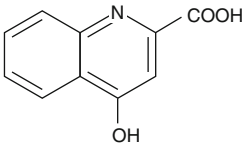

Fig. 1 Structure of kynurenic acid

2 KYNA Synthesis and Brain Levels

Tryptophan, after entering the brain in a free form, is utilized along various metabolic pathways yielding structural and enzymatic proteins or centrally active substances such as serotonin and kynurenines. In mammals, approx. 95–99 % of dietary tryptophan not bound into proteins is converted to kynurenine along the so-called *kynurenine* pathway (Stone and Darlington 2002); see Fig. 2.

Brain kynurenine derives from production in situ (40–50 %) and from peripheral pool. Kynurenine enters the brain via large neutral amino acid transporter (Fukui et al. 1991; Németh et al. 2006). KYNA itself penetrates across the blood-brain barrier to a lesser degree (Scharfman and Goodman 1998; Salvati et al. 1999). However, peripheral application of KYNA prior to ischemia may dramatically increase brain concentrations of compound (Salvati et al. 1999). Transport occurs mostly via passive diffusion (Fukui et al. 1991). Notably, human organic anion transporter hOT3 might play a role in the uptake of KYNA from the brain into brain capillary endothelial cells (Uwai et al. 2012).

Within the brain, kynurenine can be either transaminated to KYNA or hydroxylated to *3-hydroxykynurenine*, which is further converted to *quinolinic acid*, and finally to nicotinamide adenine dinucleotide (NAD) (Moroni 1999; Németh et al. 2005, 2006). 3-Hydroxykynurenine generates large quantities of free radicals, whereas quinolinic acid is an *N*-methyl-D-aspartate receptor agonist with potent excitotoxic properties (Stone 2001). Of many kynurenine derivatives, neuroinhibitory KYNA and neurotoxic quinolinic acid and 3-hydroxykynurenine have stimulated the greatest scientific interest.

The brain level of KYNA depends directly on the activity of kynurenine aminotransferases (KATs), but, indirectly, it is also strongly associated with the action of enzymes contributing to the production and degradation of its precursor, kynurenine. The synthesis of kynurenine is regulated by two separate enzymes, tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). TDO and IDO are expressed in the brain but their activities are rather low. Therefore, as mentioned above, more than 50 % of brain kynurenine is regarded to originate from peripheral sources.

Increasing scientific interest has focused recently on *kynurenine 3-monooxygenase* (KMO), which converts kynurenine to 3-hydroxykynurenine. As KMO displays lower K_m for kynurenine than KATs (Bender and McCreanor 1985), it is considered a key enzyme controlling the availability of kynurenine for KYNA synthesis. The process of irreversible transamination of kynurenine to

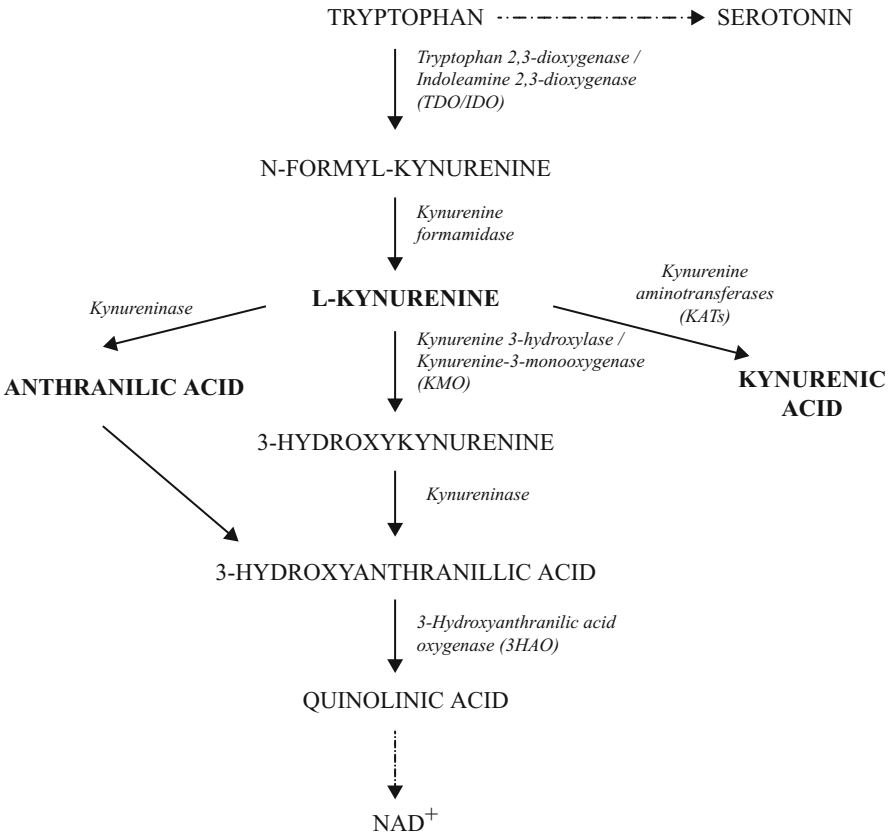

Fig. 2 Scheme of kynurenine pathway

KYNA is catalyzed by four distinct kynurenine aminotransferases I–IV (KATs) (Okuno et al. 1991a; Guidetti et al. 2007; Bellocchi et al. 2009) considered nowadays as potential targets for regulating brain KYNA levels. KAT I [glutamine transaminase K (GTK)/cysteine conjugate beta-lyase (CCBL) 1], KAT II [aminoadipate aminotransferase (AADAT)], KAT III (CCBL2), and KAT IV [glutamic-oxaloacetic transaminase (GOT) 2/mitochondrial aspartate aminotransferase (mASPAT)] have been identified in mouse, rat, and human brains (Han et al. 2010). KATs are expressed primarily in the *astrocytes* (Roberts et al. 1992; Guidetti et al. 1997), but neurons are also able to produce KYNA (Du et al. 1992; Roberts et al. 1992; Rzeski et al. 2005). KATs display diverse optimal pH range and different levels of specific activity in specific brain regions and, although sharing a number of amino acid and α-ketoacid substrates, have distinct substrate profile (Okuno et al. 1991b; Guidetti et al. 2007; Bellocchi et al. 2009).

Concentrations of KYNA in the central nervous system (CNS) vary depending on the analyzed species, age, and CNS area. In brain structures, KYNA levels range

from 0.014 to 3.38 pmol/mg of tissue (Carlá et al. 1988; Turski et al. 1988; Swartz et al. 1990; Beal et al. 1992; Jauch et al. 1995). The lowest concentrations of KYNA in the human brain were detected in cerebellum and medulla (approx. 0.1–0.3 pmol/mg). In cortical areas and substantia nigra, KYNA reaches approx. 0.2–0.6 pmol/mg, whereas the highest concentrations occur in globus pallidus and putamen (0.7–1.4 pmol/mg) (Turski et al. 1988; Swartz et al. 1990; Jauch et al. 1995; Baran et al. 1996). The content of KYNA in *cerebrospinal fluid* (CSF) ranges from 1 to 5 nM (Swartz et al. 1990; Erhardt et al. 2001; Rejdak et al. 2002; Iłżecka et al. 2003).

The level of KYNA in the CNS increases with age. In humans aged <50 years, KYNA content in CSF is lower in comparison with elderly individuals (2.8 vs. 4.1 fmol/μl) (Kepplinger et al. 2005). In rats between 1st week and 18th month of age, KYNA increases over 50-fold, from approx. 15 to 750 pmol/g of protein (Moroni et al. 1988a). Others reported rise of brain KYNA by 80 % up to 350 %, between 3rd and 24th month of age, from approx. 20 to approx. 70 pmol/g of tissue (Gramsbergen et al. 1992; Braidy et al. 2011).

In periphery, the presence of KYNA was demonstrated in various animal and human tissues such as kidneys, liver, or heart (Carlá et al. 1988). Large quantities of KYNA are released by vascular *endothelium*, as demonstrated in rat aortic rings and endothelial cell cultures (Stazka et al. 2002; 2005). In humans, serum levels of KYNA range between 4 and 30 nM (Swan et al. 1983; Urbanska et al. 2006; Hartai et al. 2007; Baran et al. 2010). In rats, the concentrations are slightly higher and reach 20–90 nM (Stazka et al. 2005; Fukushima et al. 2009; Baran et al. 2010).

Interestingly, KYNA is not only synthesized de novo by mammalian tissues but also produced by saprophytic bacteria in the intestinal tract and exogenously delivered with food (Kuc et al. 2008; Turski et al. 2009). High concentration of KYNA was found in food, e.g., in herbs, bee products, broccoli, and potatoes (Turski et al. 2009).

3 Regulation of KYNA Synthesis

Brain level of KYNA may be regulated in various ways, including the availability of direct precursor and the activity of biosynthetic enzymes. Accumulating data identified also a number of other modulatory factors, of either endo- or exogenous origin.

Extracellular milieu, including ionic composition, pH, glucose, and oxygen availability, can affect KYNA production, as shown in vitro in brain cortical slices (Turski et al. 1989; Gramsbergen et al. 1991, 1997). Low concentrations of Cl^- and K^+ as well as depolarizing concentrations of K^+ (50 mM) reduce, whereas high Na^+ increases KYNA production. Hypoxia, anoxia, and hypoglycemia inhibit KYNA synthesis, while hyperglycemia remains without effect (Turski et al. 1989; Chmiel-Perzyńska et al. 2007). It has also been shown that β-hydroxybutyrate, one of the ketone bodies rising in the serum during diabetic ketoacidosis or *ketogenic diet*, stimulates brain KYNA synthesis in cortical slices and glial cultures (Chmiel-Perzyńska et al. 2011). *β-hydroxybutyrate* did not affect directly the activity of semi-purified preparation of KYNA biosynthetic enzymes in vitro. However, in

glial cultures β-hydroxybutyrate stimulated the de novo expression of KAT I and II. These data suggest that during fasting or ketogenic diet, high level of β-hydroxybutyrate may not only protect brain from the reduction of KYNA elicited by hypoglycemia and/or acidosis but actually enhance its synthesis above control values (Chmiel-Perzyńska et al. 2011).

Endogenous agonists of ionotropic glutamate receptors, such as *L-cysteine sulfinate*, L-aspartate, L-glutamate, L-cysteate, L-homocysteine sulfinate, and L-homocysteate, reduce the production of KYNA in cortical slices with IC_{50} values of 0.08, 0.3, 0.79, 1.77, 2.9, and 3.93 mM, respectively (Urbanska et al. 1997, 2000; Kocki et al. 2003). Their action is mediated most probably via inhibition of KAT I/KAT II activity. Among studied agonists, L-cysteine sulfinate appears one of the most potent KAT II inhibitors, displaying the IC_{50}=2 μM (Kocki et al. 2003).

Synthetic *metabotropic receptor agonists* were also shown to reduce KYNA synthesis in cortical slices. Among them, quisqualate was unequivocally the most potent inhibitor, acting in the micromolar range of concentrations, while agonists of group I (DHPG), group I/II (t-ACPD), group II (L-CCG-I), and group III (L-AP4) acted weaker (Urbanska et al. 1997). Interestingly, L-AP4 and t-ACPD were ineffective in spinal cord slices (Urbanska et al. 2000).

The effect of *dopaminergic* ligands on striatal KYNA production was studied in vivo in developing and adult rats (Rassoulpour et al. 1998). D_1 receptor antagonist SCH 23390, but not the D_2 receptor antagonists, raclopride and haloperidol, increased striatal KYNA levels, whereas D_1 and D_2 receptor agonists reduced KYNA levels approximately to the same extent in young animals. D-Amphetamine impaired striatal synthesis of KYNA irrespectively of animals' age (Rassoulpour et al. 1998). Similarly, peripheral application of L-DOPA, precursor of dopamine, decreased extracellular KYNA in rat striatum (Wu et al. 2002). In humans, however, L-DOPA treatment does not seem to affect central KYNA levels (Ogawa et al. 1992; Samadi et al. 2005).

Selective and mixed β_1- and β_2-adrenergic agonists, such as xamoterol, denopamine, albuterol, clenbuterol, or epinephrine, as well as cAMP analogues, enhanced KYNA synthesis in vitro and in vivo (Luchowska et al. 2008, 2009). KT5720, a selective inhibitor of *protein kinase A* (PKA), strongly reduced KYNA formation in cortical slices and in glial cultures. Notably, the effective concentrations were extremely low, ranging between 0.1 and 10 μM. β-adrenergic agonists, cAMP analogues, or KT5720 did not affect directly the activity of KATs measured in vitro, in semi-purified homogenate. However, the exposure of cultured glial cells to cAMP analogues and β-adrenergic agonists stimulated the enzymatic conversion of kynurenine, most probably due to an enhancement of KATs synthesis. The cAMP/PKA-related pathway seems an important factor regulating the endogenous levels of KYNA via expression of KATs (Luchowska et al. 2008, 2009).

Nitric oxide (NO) donors, S-nitroso-N-acetylpenicillamine (SNAP), and 3-morpholinosydnonimine (SIN-1) enhanced KYNA production in cortical slices yet did not affect KATs activity (Luchowski and Urbanska 2007). SNAP is the source of NO and S-nitrosylating agent, whereas SIN-1 is the NO/peroxynitrite (ONOO–) generator with a very weak S-nitrosylating activity. Their stimulatory effects were abolished in the presence of free radicals scavenger, L-ascorbate,

indicating possible involvement of the excessive release of reactive oxygen species in the observed phenomenon (Luchowski and Urbanska 2007).

Mitochondrial toxins compromising the status of mitochondrial respiration, such as aminooxyacetic acid (AOAA), *3-nitropropionic acid (3-NPA)*, or *1-methyl-4-phenylpyridinium (MPP$^+$)*, inhibit KYNA synthesis in vitro and in vivo (Turski et al 1989; Urbanska et al. 1997; Luchowski et al. 2002; Kocki et al. 2003). AOAA disrupts the function of aspartate-malate shuttle across the mitochondrial membrane; 3-NPA is an irreversible inhibitor of succinate dehydrogenase and mitochondrial complex II, whereas MPP$^+$ blocks the activity of complex I and IV (Urbanska 2005). 3-NPA and AOAA inhibit the activity of KAT I and KAT II, whereas MPP$^+$ blocks only KAT II action (Luchowski et al. 2002). Interestingly, mitochondrial toxins exert indirect excitotoxic effects, modeling Parkinson's (MPP$^+$) and Huntington's (AOAA and 3-NPA) diseases upon local or intracerebral application (Urbanska 2005).

D,L-homocysteine, which increased levels are considered a risk factor in atherosclerosis, biphasically affects KYNA production in the endothelium. In micromolar concentrations (approx. 40–100 μM), D,L-homocysteine stimulates, whereas in higher concentrations, it inhibits KYNA formation (Stazka et al. 2005). Similarly, low concentrations of compound stimulated KYNA synthesis, while millimolar concentrations impaired KYNA formation (IC_{50}= approx. 5–6 mM) in the brain (Luchowska et al. 2005; Chmiel-Perzyńska et al. 2007). Metabolic derivative of D,L-homocysteine, *S*-adenosylhomocysteine, displayed similar but more potent activity than parent compound, augmenting KYNA production at 0.03–0.08 mM and reducing it at $\geq$0.5 mM. The stimulatory effect of *S*-adenosylhomocysteine was abolished in the presence of the kynurenine uptake inhibitors, L-leucine and L-phenyloalanine (Luchowska et al. 2005).

Number of clinically effective compounds may exert their therapeutic effects, at least in part, via increase of central KYNA synthesis. *Memantine*, a well-tolerated derivative of amantadine acting predominantly as an open-channel blocker of *N*-methyl-D-aspartate (NMDA) receptors, protects neurons against excitotoxic insults under different experimental paradigms in vitro and in vivo (Danysz and Parsons 2003). The compound is used clinically to ameliorate symptoms of Parkinson's and Alzheimer's diseases. Recently, memantine was found to stimulate KYNA production at low, micromolar concentrations, possibly via PKA-dependent mechanism (Kloc et al. 2008).

Antiepileptic drugs, carbamazepine, felbamate, phenobarbital, phenytoin, and lamotrigine (at 0.5–3.0 mM), were found to increase KYNA synthesis in rat cortical slices and to enhance KAT I activity (Kocki et al. 2004, 2006). On the contrary, vigabatrin, gabapentin, and tiagabine decreased KYNA production, possibly due to the inhibition of KAT I and KAT II. This aspect of their action may be partially involved in the paradoxical exacerbation of absence or myoclonic seizures, as sometimes observed clinically after administration of vigabatrin, gabapentin, and tiagabine (Kocki et al. 2006).

Recently, *antidepressants* fluoxetine, citalopram, amitriptyline, and imipramine (1–10 μM) were demonstrated to increase de novo production of KYNA and

to diminish 3-hydroxykynurenie synthesis (at 24 and 48, but not 2-h incubation). RT-PCR studies revealed that antidepressants did not alter KAT1, KAT2 and kynurenine-3-monooxygenase (Kmo) gene expressions after 2-h exposure. After 48 h, the expression of KAT1 and KAT2 was strongly upregulated, and Kmo expression was downregulated by all antidepressants. These findings indicate that antidepressants may act in part via restoring the beneficial ratio between KYNA and 3-hydroxykynurenine (Kocki et al. 2012).

Notably, also the environmental factors may affect central KYNA production. Pyrethroid *pesticides*, such as deltamethrin and fenpropathrin, were shown to inhibit KYNA production in cortical slices (Zielińska et al. 2005). Tobacco smoking may either increase or decrease central KYNA levels, depending on the exposure time. In rats, short, 4- to 6-day administration of *nicotine* decreased brain KYNA. Withdrawal of nicotine resulted in the return of KYNA concentration to control values (Rassoulpour et al. 2005). In contrast, 10-day exposure to nicotine increased KYNA level in hippocampus, striatum, and cortex, but not in the serum (Rassoulpour et al. 2005). Others confirmed the stimulatory effect of 10-day nicotine application. However, chronic, 30-day exposure to nicotine was reported to increase serum KYNA level and to decrease cortical KYNA content, despite the enhanced KATs activity (Zielińska et al. 2009). Only central, but not the plasma changes of KYNA, were reversed by the use of an antagonist of nicotinic receptors, mecamylamine (Zielińska et al. 2009). Possibly, nicotine application induces distinct mechanisms involved in the regulation of KYNA synthesis, distribution, and elimination (Zielińska et al. 2009).

4 Mechanisms of KYNA Action

Since the initial discovery identifying KYNA as en endogenous antagonist of ionotropic glutamate receptors (Perkins and Stone 1982), a number of novel KYNA targets emerged. Currently, KYNA is also suggested to act as an antagonist of nicotinic receptors and as ligand of G protein-coupled GPR35 and human aryl hydrocarbon (AHR) receptors.

In electrophysiological studies, KYNA was shown to block competitively the activation of ionotropic glutamate receptors of *NMDA*, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (*AMPA*) and kainate type, at relatively high μM concentrations (Perkins and Stone 1982, 1983; Stone 1993). At much lower concentrations (10–30 μM; IC_{50}=8 μM), KYNA acts as an antagonist of the strychnine-insensitive glycine site of NMDA receptor complex (Kessler et al. 1989; Stone 1993). Bearing in mind that extracellular concentration of KYNA is within nM range, initially endogenous KYNA levels were considered too low to modulate excitatory neurotransmission. However, accumulated data leave no doubt that even modest increase of KYNA may alter brain function, e.g., change the neuronal firing in locus coeruleus, reduce the locomotor activity, impair cognition, and prevent seizures or impair excitotoxic neuronal loss (Vécsei and Beal 1991; Russi et al. 1992; Carpenedo et al. 1994, 2002; Pocivavsek et al. 2011).

Moreover, locally applied low nanomolar concentrations of KYNA were shown to reduce the release of glutamate in caudate nucleus and to inhibit the neurotransmitter release in striatal preparations (Carpenedo et al. 2001). Experimental studies confirmed that fluctuations of KYNA level may alter glutamine, acetylcholine, and dopamine release (Wonodi and Schwarcz 2010).

KYNA was also reported to antagonize the α_7 *nicotinic receptors* (nAChRs) in hippocampus, already at low 100 nM concentration, reaching $IC_{50}\sim7$ μM. However, KYNA effect in slices was much less robust than that seen in cultured neurons (Hilmas et al. 2001; Stone 2007). Hippocampal α_7 nAChRs are located on some GABAergic neuronal cell bodies and on glutamatergic dendrites and axon terminals and seem to modulate neurotransmitter release rather than to mediate neurotransmission (Vizi and Lendvai 1999; Dajas-Bailador et al. 2000). Axonal α_7 nAChRs are involved in the modulation of presynaptic NMDAR expression and structural plasticity of glutamatergic presynaptic boutons during early synaptic development (Lin et al. 2010). The role of KYNA in the modulation of α_7 nAChRs in vivo is yet not clear, because three groups failed to detect any effect of KYNA on α_7 nAChRs-mediated events using electrophysiological methods and direct patch-clamp recording in hippocampal cultures and rodent acute brain slices (Arnaiz-Cot et al. 2008; Mok et al. 2009; Dobelis et al. 2012).

In 2006, KYNA was discovered to act as a ligand of *G protein-bound receptor, GPR35* (Wang et al. 2006). The possible role of altered GPR35 function in the development of hypertension, immune modulation, and gastric cancer has been suggested (Okumura et al. 2004; Min et al. 2010). GPR35 is highly expressed in human monocytes, T cells, neutrophils, dendritic cells, and within the gastrointestinal tract (Wang et al. 2006). The potency of KYNA toward GPR35 varies depending on experimental paradigm and effector system. In Chinese hamster ovary cells transfected with GPR35, KYNA elevated intracellular Ca^{2+} with the EC_{50} of approx. 7 μM (rat) and 40 μM (human) (Wang et al. 2006). Using GPR35-β-arrestin-2 interaction assay, KYNA was demonstrated to promote the interaction of GPR35 with β-arrestin-2 and cause internalization of the receptor systems with low potency, at >100 μM (Jenkins et al. 2011). Others, however, have shown that the arrest of human monocytes on human umbilical vein endothelial cells expressing *ICAM-1 (intercellular adhesion molecule 1)*, in the process mediated by β_2-integrins, occurs at low, 300 nM concentration of KYNA (Barth et al. 2009). Thus, stimulation of GPR35 with KYNA may contribute to the development of inflammatory changes. GPR35 presence was also confirmed within CNS, in the spinal cord and dorsal root ganglia, cerebellum, and brain (Wang et al. 2006; Guo et al. 2008; Ohshiro et al. 2008). In mouse glial cell cultures, the expression of GPR35 receptors was confined to the cytosol and the membranes (Cosi et al. 2011). In these cells, KYNA significantly reduced the forskolin-induced cAMP accumulation with EC_{50} of approx. 1 μM (Cosi et al. 2011). Other effects assigned to the activation of GPR35 include voltage-dependent Ca^{2+}-current inhibition (10 μM–1 mM), antinociception, and regulation of neuronal firing (Guo et al. 2008; Zhao et al. 2010). Thus, to date the majority of reports suggest that the interaction of KYNA with GPR35 receptors occurs at concentrations exceeding its

extracellular levels. Further research is needed to clarify whether KYNA indeed plays a physiologically important role as a ligand of GPR35.

The latest discovery indicates that KYNA and kynurenine acts as the agonists of *human aryl hydrocarbon receptor (AHR)* (DiNatale et al. 2010; Opitz et al. 2011). AHR is essential for the toxicity of dioxins and related compounds. Additionally, it plays a role in adaptive metabolism and development, hormonal signaling, hematopoiesis, insulin release, and tumor development. Hyperactivation of AHR may lead to a variety of transcriptional responses (Bradshaw and Bell 2009; Bock and Köhle 2009; Stevens et al. 2009; Esser 2012). Stimulation of AHR by exogenous ligands coupled with inflammatory signals can evoke synergistic induction of *interleukin-6* (IL-6) expressions in tumor cells (DiNatale et al. 2010). KYNA was shown to enhance the expression of IL-6 at low 100 nM concentration and was suggested as a factor involved in the escape of tumors, via IL-6-dependent pathway, from the immune surveillance (DiNatale et al. 2010).

Initially considered to act primarily through the inhibition of glutamate-mediated neurotransmission, KYNA, with new reports emerging, is not regarded an inert metabolite anymore. Accumulating data support the view that this product of kynurenine pathway may be essentially involved in human physiology and pathology.

5 Experimental KYNA Deficiency

In the search for selective tools able to decrease KYNA formation, scientific attention was drawn to *aminooxyacetic acid* (AOAA) (Urbanska et al. 1998). AOAA, originally recognized as a nonselective inhibitor of aminotransferases (Wood and Peesker 1973), was identified as one of the most potent inhibitors of KYNA synthesis in vitro and in vivo (Turski et al. 1989; Speciale et al. 1990). It blocks KATs activity displaying IC_{50} values in very low micromolar range and is only slightly weaker than L-cysteine-sulfinate (Turski et al. 1989; Okuno et al. 1991b; Chmiel-Perzyńska et al. 2007). Using in vivo approach, it was demonstrated that intrastriatally injected AOAA evokes selective excitotoxic quinolinate-like neuronal loss in rats (Urbanska et al. 1989, 1991), what was confirmed by others (Beal et al. 1991). Neurochemical, histological, and behavioral features of striatal neuronal loss closely resembled changes observed in the course of Huntington's disease (HD) (Urbanska et al. 1989, 1991; Beal et al. 1991). Lesions blocked with the use of KYNA and NMDA antagonists did not occur following ablation of *corticostriatal glutamatergic input* and were age dependent (Urbanska et al. 1989, 1991). Bilateral lesions of caudate with AOAA markedly affected cataleptic response of animals, increasing the reaction to arecoline and morphine and reducing the response to haloperidol (Urbanska et al. 1989, 1991), mimicking the behavioral changes characteristic for other HD models (Sanberg 1980; Sanberg et al. 1981). Bearing in mind that AOAA is a nonspecific inhibitor of aminotransferases, AOAA-induced effects could be attributed to the inhibition of enzymes other than KATs. However, neither application of GABA aminotransferase inhibitor nor

L-aspartate aminotransferase inhibitor evoked neuronal degeneration in striatum (Urbanska et al. 1989, 1991). It was also proposed that AOAA-induced indirect excitotoxicity results from mitochondrial dysfunction, as AOAA is known to disrupt the malate-aspartate shuttle across mitochondrial membrane (Beal et al. 1991). However, AOAA affects KYNA synthesis at much lower concentration than required to alter mitochondrial respiration (Hotta 1968). Thus, the diminished formation of KYNA cannot be ruled out as one of the mechanism involved in the AOAA-evoked neuronal loss.

Apart from *excitotoxicity*, AOAA displays potent convulsant properties. The compound evokes clonic seizures in rodents when applied systemically, intracerebroventricularly, or intrahippocampally (McMaster et al. 1991; Turski et al. 1991b, 1992). Diazepam, phenobarbital, and valproate, but not ethosuximide, trimethadione, acetazolamide, diphenylhydantoin, or carbamazepine, prevent convulsions induced by systemic administration of AOAA. Seizures evoked by intracerebroventricular injection of AOAA are blocked with KYNA and selective NMDA antagonists (Turski et al. 1991b, 1992).

Additionally, AOAA was demonstrated to induce seizures when injected into the rat entorhinal cortex (Du and Schwarcz 1992; Eid et al. 1995). In this paradigm, AOAA application resulted in selective neuronal loss within layer III of *entorhinal cortex* (Du and Schwarcz 1992). Notably, in contrast to striatum, the sequel of changes following application of quinolinic acid into the entorhinal cortex was qualitatively different from AOAA-evoked changes. Animals subjected to quinolinate did not display behavioral seizures; however, neuronal loss in layer V of the entorhinal cortex, often accompanied by neurodegeneration in the superficial layers of the dorsal perirhinal cortex, was a constant finding (Eid et al. 1995).

Novel data indicate that AOAA inhibits KYNA synthesis more potently in the milieu mimicking diabetic hyperglycemia. High level of glucose (30 mM) reduced the IC_{50} value of AOAA by approx. 50 % (from 11.6 to 7.1 μM), suggesting that severe hyperglycemia may render the brain more susceptible to the excitotoxic events (Chmiel-Perzyńska et al. 2007).

The above data suggest that AOAA is a valuable tool allowing detailed studies of excitotoxicity associated, at least in part, with deficient synthesis of brain KYNA.

6 KYNA and Brain Disorders

6.1 Parkinson's Disease

Parkinson's disease (PD) is one of the most common progressive neurodegenerative disorders, affecting approx. six million people worldwide. Although clinically heterogenous, PD is primarily characterized by tremor, bradykinesia, and muscle rigidity associated with dopaminergic denervation, primarily due to neuronal loss within substantia nigra pars compacta. Later on, other motor signs such as postural instability and impairment of gait and speech develop due to alterations in

non-dopaminergic transmission. Moreover, autonomic dysfunction and cognitive impairment arise in the course of disease (Lees et al. 2009). Familial forms of PD account for 10–15 % of diagnoses. Pathogenesis of remaining 85–90 % cases of PD is still unclear, and only some of the underlying mechanisms have been clarified so far. Disturbances of *ubiquitin-proteasome system*, dysfunction of mitochondria, and oxidative stress are among the recognized factors (Ali et al. 2011). Accumulating data indicate possible role of altered KYNA metabolism in the pathogenesis of PD (Szabó et al. 2011).

Consistently with neuroprotective as well as antiparkinsonian effect of glutamate receptors antagonists (Klockgether and Turski 1990; Klockgether et al. 1991; Turski et al. 1991a), application of KYNA or approaches aimed to increase its central level were shown to ameliorate neuronal loss and behavioral symptoms in experimental models of PD.

KYNA, injected intracerebrally, reduced akinesia, tremor, and rigidity in a severely parkinsonian monkeys subjected to *1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP*), mitochondrial toxin commonly used to replicate PD-like symptoms in experimental animals (Graham et al. 1990). Parkinsonian symptoms are thought to result partially from the overactivation of neurons in the output areas of basal ganglia, i.e., substantia nigra pars reticulata and the medial segment of globus pallidus. In rat and primate models of PD, the direct injection of KYNA into the medial segment of the globus pallidus reduced behavioral changes (Brotchie et al. 1991). Similarly, in monkeys with hemiparkinsonism evoked by unilateral, intra-arterial MPTP application, the infusion of KYNA into contralateral globus pallidus internus partially alleviated symptoms of disease (Butler et al. 1997).

In rats, intraventricular administration of KMO inhibitor, *nicotinylalanine*, together with intraperitoneal kynurenine and probenecid infusion, evoked up to threefold increase of brain KYNA concentration (Miranda et al. 1997). Immunohistochemical assessment revealed that this rise of KYNA level was sufficient to protect nigrostriatal dopaminergic neurons from neurodegeneration caused by intranigral administration of quinolinic acid and to reverse some behavioral alterations (Miranda et al. 1997, 1999). Intraperitoneal treatment with kynurenine and probenecid prevented also neuronal damage and behavioral alterations induced by intrastriatal injections of *6-hydroxydopamine* (Silva-Adaya et al. 2011).

In primate model of MPTP-induced parkinsonism, orally administered *KMO inhibitor, Ro 61-8048*, increased central kynurenine and KYNA levels. This treatment was shown to improve L-DOPA-induced dyskinesias without alteration of drug antiparkinsonian efficacy (Samadi et al. 2005). Indeed, accumulating data suggest that glutamatergic manipulation of basal ganglia output is a promising approach to treat L-DOPA-induced dyskinesia in PD (Brotchie and Jenner 2011).

KYNA deficiency seems to play an important role in the development and progress of parkinsonian symptoms. An active metabolite of *MPTP*, MPP^+ reduced cortical KYNA synthesis and inhibits KAT activity in vitro (Luchowski et al. 2002; Urbanska 2005). These results were confirmed in vivo, in a study demonstrating

that MPTP treatment decreases the number and optical density of KAT I immunoreactive neurons in substantia nigra of mice (Knyihár-Csillik et al. 2004). Similarly, application of 6-hydroxydopamine induced dose-dependent loss of KAT I immunoreactivity in the middle and lateral areas of substantia nigra pars compacta (Knyihár-Csillik et al. 2006). In human neuroblastoma cell line, pretreatment with KYNA attenuated MPP^+-induced neuronal apoptotic cell death, decreased level of proapoptotic *Bax* protein, reduced MPP^+-evoked mitochondrial dysfunction, and prevented activation of *caspase* 3 and 9 (Lee et al. 2008).

Human studies revealed decline of KYNA and kynurenine in frontal cortex, putamen, and substantia nigra of PD victims (Ogawa et al. 1992). Tryptophan/kynurenine and kynurenine/KYNA ratios remained unchanged, and the therapy with L-DOPA did not seem to influence brain KYNA (Ogawa et al. 1992). In contrast, the level of KYNA in caudate and precentral cortical gyrus did not differ from control values, as shown by another study from the same group aimed to analyze brain KYNA content in Huntington's, Parkinson's, and Alzheimer's diseases (Beal et al. 1992).

In periphery, KYNA content within erythrocytes obtained from PD patients, as well as the activity of KAT II but not of KAT I, are increased (Hartai et al. 2005). Interestingly, the authors have also detected the activity of KAT I and KAT II in the serum of both patients and controls. In PD, the activity of both enzymes was decreased; however, there was no concomitant change in serum KYNA level (Hartai et al. 2005).

In the view of available data, deficient KYNA synthesis seems to be one of the underlying factors contributing to the pathogenesis of PD.

Surprisingly, there is no clinical data assessing potential efficacy of KYNA derivatives or NMDA antagonists in the treatment of dyskinesia in PD. Future research should be aimed to develop compounds acting selectively within basal ganglia. Targeting KYNA synthesis within specific brain areas seems to be one of the attractive therapeutic options.

6.2 Huntington's Disease

Huntington's disease (HD) is an inherited autosomal dominant neurodegenerative disorder associated with progressive loss of neurons, primarily in caudate-putamen. During later stages of disease, the cerebral cortex (particularly layers III, V, and VI), globus pallidus, thalamus, subthalamic nucleus, substantia nigra, white matter, and the cerebellum can also be markedly affected (Zuccato et al. 2010). Clinically, HD is manifested by progressive emotional, motor, and cognitive impairments. The development of HD results from *CAG trinucleotide* repeat expansion in the exon-1 of the gene encoding for huntingtin protein on chromosome 4. The length of CAG repeats correlates with age at onset of motor symptoms; however, HD patients may differ dramatically in disease manifestations, despite similar genetic changes. It is established now that multiple genes distinct from the HD locus itself may modify the course of disease (Zuccato et al 2010; Bano et al. 2011).

As with other human pathologies, proper animal models are invaluable tools that allow detailed studies of pathophysiological mechanisms underlying disease development. Initially, HD animal models were based on the intrastriatal injections of selective *excitotoxins*, such as kainate and quinolinic acid, indirect excitotoxin, AOAA, and peripheral application of mitochondrial toxin, 3-NPA (Coyle and Schwarcz 1976; Schwarcz et al. 1983; Urbanska et al. 1991; Brouillet et al. 1993). In rats, intrastriatal injection of AOAA leads to neuropathological and behavioral sequel mimicking HD (Urbanska et al. 1989, 1991; Beal et al. 1991) (see paragraph 5). Chronic, intraperitoneal administration of low doses of 3-NPA was demonstrated to induce relatively selective neuronal loss within striatum, accompanied by behavioral changes replicating symptoms of HD (Gould and Gustine 1982; Hamilton and Gould 1987; Brouillet et al. 1993).

It seems of interest that both AOAA and 3-NPA inhibit KATs activity, and, notably, AOAA is one of the most potent inhibitors of KATs (Luchowski et al. 2002; Urbanska 2005). 3-NPA was demonstrated to reduce the number of KAT I immunopositive glial cells in rat striatum and temporal cortex (Csillik et al. 2002). Susceptibility to 3-NPA action increases with age of animals (Csillik et al. 2002). Thus, results from chemical models of HD suggest that striatal deficiency of KYNA might be one of the factors contributing to the development of HD.

Following the discovery of genetic base of HD, novel animal models emerged. Disturbed function of kynurenine pathway was reported in heterozygous, symptomatic FVB/N mice transgenic for mutant *huntingtin* (Guidetti et al. 2000). Interestingly, transgenic animals showed large, over tenfold elevations in 3-hydroxykynurenine content in both striatum and cortex accompanied by smaller increases of KYNA levels, resulting in a prominent increase of the 3-hydroxykynurenine/KYNA ratio (Guidetti et al. 2000).

Human studies performed in later stages of disease revealed decline of CSF KYNA levels in HD (Beal et al. 1990; Heyes et al. 1994). In the brain tissue, twofold increase of KYNA in the motor cortex in Brodmann's area 4, but not within caudate, globus pallidus, and prefrontal cortex, was reported (Connick et al. 1988, 1989). Further studies demonstrated, however, that KYNA concentration in *putamen* and cortex of HD patients is decreased (Beal et al. 1990, 1992). Others have also reported approx. 50 % reduction of KYNA content in caudate, but not in hippocampus or cortical areas, in specimens obtained from late stage of HD (Jauch et al. 1995). In putamen, KYNA content reached approx. 67 % of control, however, at the border of statistical significance. Enzymatic analyses revealed decreased activity of KAT I and II (in putamen ~48–55 % of control) (Jauch et al. 1995). Notably, deficiency of KYNA in putamen seems to be associated with an increase of kynurenine/KYNA ratio (Beal et al. 1990), indicating possible activation of the kynurenine pathway arm leading to neurotoxic metabolites.

Indeed, an increase of *3-hydroxykynurenine* in the brain was demonstrated by different groups (Reynolds and Pearson 1989; Pearson and Reynolds 1992; Guidetti et al. 2000, 2004). In specimens of putamen and temporal and frontal cortices, the concentration of 3-hydroxykynurenine was increased by nearly twofold

(Reynolds and Pearson 1989; Pearson and Reynolds 1992). Others reported that neostriatal and neocortical 3-hydroxykynurenine levels are increased by five- to tenfold, whereas KYNA was only slightly elevated in cortical samples obtained from early-stage HD patients (Guidetti et al. 2000). Further analysis of 3-hydroxykynurenine and quinolinic acid levels in the brains of patients with early or late HD has revealed an increase of these toxic kynurenine metabolites in 0/1 stage of disorder, but not during later phases (Guidetti et al. 2004).

Overall, an increased 3-hydroxykynurenine/KYNA ratio in HD patients and transgenic mice modeling the disease indicates the presence of abnormally high level of neurotoxic kynurenine metabolite accompanied by comparatively deficient formation of neuroprotective KYNA (Pearson and Reynolds 1992; Jauch et al. 1995; Guidetti et al. 2000, 2004). Based on accumulated data, it seems that changes in brain KYNA content and kynurenine pathway function are hallmarks of HD. In early stages of disease, both KYNA and 3-hydroxykynurenine levels may be increased. Later on, KYNA production seems to be severely compromised and 3-hydroxykynurenine/KYNA ratio is consistently higher (Pearson and Reynolds 1992; Jauch et al. 1995; Guidetti et al. 2000, 2004). Whether these changes reveal causative association or merely reflect consequences of progressive neuropathology remains to be established. However, accumulated data favor the opinion that disturbed kynurenine pathway function could be a component of pathological sequel contributing to the development of HD (Schwarcz et al. 2010).

Consequently, a number of studies were undertaken to evaluate the potential therapeutic benefits of increasing KYNA and/or decreasing 3-hydroxykynurenine level, via inhibition of KMO. The depletion of *NADPH-diaphorase* positive neurons caused by intrastriatal infusion of quinolinic acid was prevented by the intracerebral injections of nicotylalanine, an inhibitor of kynureninase and KMO, combined with intraperitoneal administration of kynurenine and probenecid, an inhibitor of organic acid transport (Harris et al. 1998). Chronic oral administration of *2-(3,4-dimethoxybenzenesulfonylamino)-4-(3-nitrophenyl)-5-(piperidin-1-yl) methylthiazole (JM6)*, small-molecule prodrug, acting as inhibitor of KMO, extended life span, prevented synaptic loss, and decreased microglial activation in the mouse model of HD (Zwilling et al. 2011). Similarly, in a fruit fly model of HD, KMO inhibition with *UPF 648 [(1S,2S)-2-(3,4-dichlorobenzoyl)-cyclopropane-1-carboxylic acid]* provided neuroprotection against degeneration of photoreceptor neurons (Campesan et al. 2011). In transgenic mkat-2-/- mice, characterized by higher susceptibility to quinolinate-induced excitotoxic neuronal loss, application of UPF 648, KMO inhibitor, reduced quinolinic acid toxicity (Sapko et al. 2006).

Recently, a novel KYNA analogue, *N-(2-N,N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide hydrochloride*, administered intraperitoneally, completely prevented the atrophy of the striatal neurons, increased survival time by about 30 %, and ameliorated hypolocomotion in transgenic *N171-82Q mice* (Zádori et al. 2011).

Thus, available data suggest that shift of kynurenine pathway toward KYNA synthesis may be neuroprotective in HD, whereas an excess of

3-hydroxykynurenine can be pathogenic. Moreover, changes in the levels of kynurenine pathway metabolites resulting in decrease of 3-hydroxykynurenine/KYNA ratio seem to be of potential therapeutic value. It would be of great interest to evaluate the efficacy of KMO inhibitors, as well as modulators of other kynurenine pathway enzymes, in early-stage and advanced HD.

6.3 Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia characterized by the progressive memory loss and deterioration of higher cognitive functions. Neuropathological findings include the presence of extracellular senile (neuritic) plaques composed from various proteins, such as β-amyloid, α-synuclein, or presenilins. Another hallmark of AD is the occurrence of intracellular neurofibrillary tangles containing hyperphosphorylated-tau protein and apolipoprotein E (Chintamaneni and Bhaskar 2012). The pathogenic process seems to start earlier than the onset of clinical symptoms, and it is estimated nowadays that neurodegeneration in AD begins decades before episodes of memory loss (Chintamaneni and Bhaskar 2012).

KYNA metabolism in the course of AD has been researched; however, the data are not conclusive. Initially, no change in the concentration of L-kynurenine and KYNA in the brains of AD victims was reported (Beal et al. 1992). In contrast, CSF KYNA was significantly lower in patients suffering from AD (Heyes et al. 1992). Others have demonstrated a significant rise of KYNA level in putamen (192 %) and caudate nucleus (177 %), but not in cortical specimens obtained from 11 patients with advanced form of AD (Baran et al. 1999). A trend for a decrease of kynurenine and 3-hydroxykynurenine in frontal cortex, caudate nucleus, putamen, hippocampus, and cerebellum of AD brain was also noted (Baran et al. 1999). In putamen and caudate, elevated KYNA correlated with an enhanced KAT I activity. A minor increase of KAT II activity was detected only in the caudate nucleus of AD subjects (Baran et al. 1999).

Peripheral KYNA status in AD was subject of two recent studies. Serum and erythrocytic KYNA were decreased by approx. 35 % in patients with AD, whereas kynurenine level and the activities of KAT I and KAT II remained unchanged (Hartai et al. 2007). The peripheral decline of KYNA accompanied by unaltered kynurenine levels was confirmed in study analyzing plasma samples of AD patients. Notably, quinolinic acid concentration was increased, suggesting an activation of kynurenine pathway arm leading to neurotoxic metabolites (Gulaj et al. 2010).

Only few reports analyzed the concentration of another neurotoxic kynurenine derivative, *3-hydroxykynurenine*, in the course of AD. Studies postmortem revealed an increase of 3-hydroxykynurenine content in temporal cortex of AD victims (Pearson and Reynolds 1992). In contrast, patients with AD exhibited decreased 3-hydroxykynurenine levels in CSF (Tohgi et al. 1992). Others found an increased

3-hydroxykynurenine/tryptophan ratio in the serum of AD patients, compared to age-matched and younger control (Widner et al. 2000).

In vitro studies have shown that fragments of amyloid peptide can stimulate IDO, thus activating an early step in kynurenine pathway what leads to an enhanced production of QUIN by human *macrophages* and *microglia* (Guillemin et al. 2003). Thus, existing data implicate the peripheral deficiency of KYNA in earlier stages of AD accompanied by shift of kynurenine pathway toward synthesis of neurotoxic 3-hydroxykynurenine. Considering the fact that most of the current data are based on the analysis performed during late stage of disease, when cerebral neurodegeneration is advanced, it seems that the central changes in kynurenine metabolism require further studies.

So far, potential usefulness of increasing brain KYNA as a possible therapy in early AD has been the subject of one study. Indeed, 4-month oral application of KMO inhibitor, *JM6*, blocked the development of spatial memory deficits in a transgenic mouse model of AD (Zwilling et al. 2011). JM6 also reduced anxiety-related behavior and prevented synaptophysin loss indicating its protective efficacy toward synapses; however, it did not alter the occurrence of Aβ plaques (Zwilling et al. 2011).

On the other hand, reduction of NMDA-mediated transmission by excessive KYNA levels may contribute to the deterioration of cognitive functions and memory in patients with AD (Baran et al. 1999). In fact, plasma levels of KYNA correlate positively with cognitive functions assessment in Mini Mental State Examination and with the result of Hachinski Ischaemic Score (HIS) in AD patients (Gulaj et al. 2010).

In the view of above findings, disturbances of kynurenine pathway seem to be evident in AD; however, the nature of central KYNA changes and their role in AD-related structural and behavioral pathologies require further studies.

6.4 Ischemia

It is estimated that cerebral stroke affects approx. 15 million of people every year and is the second leading cause of mortality worldwide. Rapid occlusion of cerebral artery (ischemic stroke) or rupture of cerebral blood vessel (hemorrhagic stroke) evokes inadequate local supply of oxygen and glucose followed by an increase of extracellular glutamate levels, overactivation of glutamate receptors, and subsequent influx of excessive Ca^{2+} into neurons. Increased *intracellular* Ca^{2+} combined with oxidative stress finally lead to neuronal death (Mattson 2008).

Various experimental data indicated that blockade of NMDA receptors may reduce consequences of ischemia and injury of brain tissue; however, the therapeutic window seems to be very narrow and limited to several minutes post-insult (Ginsberg 2008; McIntosh et al. 1990; Muir and Lees 2003). Consequently, different manipulations increasing the brain level of KYNA have become an interesting alternative and were studied in various models of ischemia/anoxia.

In vitro, KYNA derivative, *7-Cl-KYNA*, reduced neuronal damage in CA1 area of organotypic hippocampal slice cultures deprived of oxygen and glucose

(Newell et al. 1995). Other attempts demonstrated the efficacy of *KMO inhibitors* acting via reduction of 3-hydroxkynurenine and quinolinate synthesis, thus shifting kynurenine metabolism to the formation of KYNA. In organotypic hippocampal slice cultures, KMO inhibitors decreased hypoxia-/hypoglycemia-induced neuronal death in the CA1 region (Carpenedo et al. 2002).

In vivo experiments demonstrated that KYNA attenuates brain edema associated with anoxic-ischemic brain damage in neonatal rats (Simon et al. 1986). In 1-week-old rats subjected to hypoxia combined with ligation of the left carotid artery, peripheral administration of KYNA reduced brain damage (Andiné et al. 1988). Pretreatment with KYNA reduced the size of infarction and improved neurological outcome in the rat model of *focal ischemia* following middle cerebral artery occlusion (Germano et al. 1987). Peripheral administration of high doses of KYNA (1,000 mg/kg) markedly attenuated the hippocampal neurodegeneration in the gerbil model of global ischemia (Salvati et al. 1999). In the model of transient ischemia in gerbils, application of KYNA via microdialysis probe attenuated lactate accumulation and subsequent hippocampal damage (Katayama et al. 1992). KYNA, given systemically (500 mg kg^{-1}, IP) or administered locally through the dialysis probe (10 mM), significantly reduced the forebrain ischemia-evoked release of glutamate and aspartate within rat striatum (Ghribi et al. 1994). In contrast, topical administration of KYNA onto the cortex in a four-vessel occlusion rat model did not reveal any changes in ischemia-evoked release of aspartate or glutamate (Phillis et al. 1999).

KYNA precursor, kynurenine, reduced infarct size in mice subjected to permanent focal cerebral ischemia produced by electrocoagulation of the distal middle cerebral artery and prevented the pyramidal cell loss in the CA1 area of the *hippocampus* in gerbils subjected to transient bilateral carotid artery occlusion (Gigler et al. 2007). Moreover, kynurenine markedly impaired the ischemia-evoked changes of spatial memory (Gigler et al. 2007). In the model of global brain ischemia, kynurenine, applied either pre- or early post-insult, reduced cortical neuronal loss (Robotka et al. 2008).

Neuroprotective properties of KYNA were also confirmed in an animal model of *heatstroke*. Treatment with KYNA (30–100 mg/kg, IV), 4 h prior to the heat stress, attenuated hypothalamic neuronal degeneration and apoptosis in rats. Authors suggest that systemic delivery of KYNA may attenuate not only neuronal degeneration but also multiorgan dysfunction in rats after heatstroke (Hsieh et al. 2011).

Increase of endogenous KYNA in the brain achieved through pharmacological blockade of KMO effectively protects against *excitotoxicity* (Miranda et al. 1997; Harris et al. 1998; Schwarcz and Pellicciari 2002). Administration of KMO inhibitors, causing 200–300 % rise of brain KYNA level, significantly reduced postischemic brain damage induced by bilateral carotid occlusion in gerbils or by middle cerebral artery occlusion in rats (Cozzi et al. 1999).

Alterations in endogenously produced KYNA were assessed in some models of ischemia. In cortical slices, KYNA synthesis was inhibited by hypoxia and glucose deprivation (Turski et al. 1989). Similarly, in *organotypic hippocampal slice cultures*, the lack of oxygen and glucose decreased the amount of KYNA

released into the incubation medium (Carpenedo et al. 2002). Only limited data come from in vivo animal models. In gerbils subjected to transient global ischemia, the endogenous levels of KYNA and the activity of its biosynthetic enzymes, kynurenine aminotransferases I (KAT I) and II (KAT II) in the hippocampus, were not altered 24 and 72 h after ischemic episode (Luchowska et al. 2003).

Interestingly, in patients at early (up to 24 h after infarct) stage of stroke, serum KYNA correlated positively with *D,L-homocysteine* which is considered an independent risk factor of atherosclerosis and an emerging risk factor in cognitive dysfunction and stroke (Urbanska et al. 2006). Following acute ischemic episode, an elevated kynurenine/tryptophan ratio suggested activation of kynurenine pathway (Darlington et al. 2007). Patients who died within first 3 weeks after stroke demonstrated increased serum KYNA level in comparison to subjects who survived (Darlington et al. 2007). Recent clinical study revealed that the kynurenine/tryptophan ratio, measured up to 7 days poststroke, correlated with infarct size and severity of clinical symptoms assessed with NIHSS (Brouns et al. 2010).

Disturbances of kynurenine pathway in the course of acute brain ischemia seem to have potentially important implications. It is not clear yet whether observed alterations reflect reactive, inflammation-linked activation of kynurenine pathway or develop prior to the inflammatory changes. Further studies, evaluating the ratio between neurotoxic kynurenine metabolite 3-hydroxykynurenine and neuroprotective KYNA during early and late phase of stroke, should clarify this issue. Nevertheless, encouraging experimental data suggest that increasing brain KYNA may be an interesting alternative in the therapy of early stroke.

6.5 Seizures and Epilepsy

Among kynurenine pathway metabolites, *quinolinic acid* is a well-described, neurotoxic agent able to evoke seizures in rodents, similarly to kynurenine itself (Lapin 1978, 1983). In contrast, KYNA displays clear anticonvulsant properties, as demonstrated by various experimental approaches in vitro and in vivo.

Pretreatment of brain slices with KYNA precursor, kynurenine, inhibited spontaneous epileptiform activity induced by the lack of Mg^{2+} (Scharfman and Ofer 1997). KYNA suppressed spontaneous epileptiform burst discharges in the *CA3* region of rat hippocampal slices induced by Mg^{2+}-free medium or picrotoxin (Stone 1988) and inhibited penicillin-induced synchronized afterdischarges in hippocampal slices from immature rats (Brady and Swann 1988). KYNA also depressed the amplitude and duration of Mg^{2+}-free bursts and reduced the frequency of penicillin-evoked discharges (Schneiderman and MacDonald 1989).

In vivo, KYNA efficiently blocks convulsions evoked by quinolinate, produced along second arm of kynurenine pathway (Foster et al. 1984; Lapin et al. 1986). An increase of central KYNA levels, due to systemic administration of kynurenine, prevented the development of pentylenetetrazol-evoked seizures (Vécsei et al. 1992; Németh et al. 2004) and convulsions induced by the extract of poisonous

Atlantic coast mussels (Pinsky et al. 1989). Systemic or intracerebroventricular injection of KYNA inhibited seizures caused by the administration of bicuculline (Turski et al. 1990). Infusion of KYNA into the nucleus of the solitary tract reduced the severity of seizures induced by bicuculline or pentylenetetrazol (Walker et al. 1999). KYNA was also demonstrated to block the NMDA-evoked convulsions (Chiamulera et al. 1990). In WAG/Rij rats, genetic model of *absence epilepsy*, spontaneous spike-wave discharges were inhibited by KYNA administration. KYNA blocked also NMDA-induced potentiation of spike-wave discharges in this model (Peeters et al. 1994a, b).

Intracerebroventricularly applied KYNA displays protective effects in electrically kindled seizures (Thompson et al. 1988). Treatment with KYNA prior to the electrical *kindling* stimulus significantly reduced the rate of kindling in prepubescent and adult rats (Thompson et al. 1988). However, systemic administration of kynurenine or tryptophan did not protect rats against seizures induced by hyperbaric oxygen (Dale et al. 2000). KYNA had no effect on nicotine-induced seizures in mice (Kiś et al. 2000).

Interestingly, *antiepileptic drugs* may affect the synthesis of KYNA. Phenobarbital, felbamate, phenytoin, and lamotrigine increased KYNA production and activity of KAT I in vitro (Kocki et al. 2006). Moreover, high-fat and low-protein and low-carbohydrate *ketogenic diet*, leading to depletion of glucose and stimulation of ketone bodies synthesis, may also increase KYNA formation (Chmiel-Perzyńska et al. 2011). Exposure to profound ketosis (15 mM β-hydroxybutyrate) (BHB) with concomitant low glucose level (0.5 mM) increased synthesis of KYNA both in cortical slices and glial cultures. An increased activity of KAT I and II was detected in *glial cultures* but not in cortical slices (Chmiel-Perzyńska et al. 2011). Similar results were observed under in vivo conditions. In rats maintained on ketogenic diet, concentration of KYNA in hippocampus and striatum, but not in cortex, increased about two- to fourfold (Zarnowski et al. 2012).

Brain synthesis of KYNA is altered by a seizure episode, possibly due to the activation of glial cells (Wu et al. 2005). Reactive *astrogliosis* is characteristic for seizure models that are accompanied by neuronal loss in susceptible brain regions. Considering the fact that astrocytes are the main source of KYNA within the brain, increased KYNA level in the areas with prominent gliosis is not unexpected and may constitute defense mechanism.

Indeed, in the model of kainate-induced limbic seizures associated with widespread neuronal brain damage, the extracellular KYNA concentration in piriform cortex was not changed during the first 4 h of status epilepticus, but increased 1 month later, when astrogliosis was prominent (Wu et al. 1991). Various chemically induced seizures seem to increase KYNA level in dorsal hippocampus, starting in the first few hours after seizure development (Wu and Schwarcz 1996). Others have not confirmed hippocampal increase of KYNA during kainate-evoked seizures (Baran et al. 1995). However, 3 h after kainate injection, KYNA levels were raised in other brain areas, e.g., in piriform cortex, frontal cortex, amygdala, caudate, and putamen. The most prominent KYNA increases were observed in animals that developed status epilepticus (Baran et al. 1995).

Contrasting data were obtained in models of kindled seizures, developing over long period and assumed to mimic *epileptogenesis* more adequately. In amygdala-kindled seizures, KYNA level was reported to increase only in nucleus accumbens, but not in hippocampus, cerebral cortex, olfactory bulb, striatum, thalamus, tectum, cerebellum, or pons/medulla, as assessed 50 days after the last kindled seizure (Löscher et al. 1996). Moreover, the development of pentylenetetrazol-induced kindling was shown to be associated with the progressive decrease of KYNA in caudate-putamen, entorhinal cortex, piriform cortex, amygdala, and hippocampus (Maciejak et al. 2009). Changes in KYNA levels correlated with the stage of seizures (Maciejak et al. 2009). Similarly, deficiency of brain KYNA was revealed in a genetic model of absence seizures (WAG/Rij rats). In these rats, the level of KYNA in frontal cortex is lower, comparing to age-matched controls, at the time point when spontaneous seizures develop (Kamiński et al. 2003).

Human studies have also revealed alterations of central KYNA levels in epilepsy. Concentration of KYNA in CSF from epileptic patients was not changed, but serum KYNA in patients with intractable complex *partial seizures* was decreased (Heyes et al. 1994). Others have shown that in children with *West syndrome* or infantile spasm, the level of KYNA in CSF is lower, whereas among patients with grand mal seizures or occipital paroxysm, the CSF KYNA remains unaltered (Yamamoto et al. 1994, 1995). In the group of children with epileptic or febrile seizures, disturbances in circadian rhythm of kynurenine synthesis were found. The production of KYNA, kynurenine, 3-hydroxykynurenine, and xanthurenic acid was elevated during nocturnal hours in comparison to controls. The increase of KYNA and xanthurenic acid was not as high as of other kynurenines during epileptic seizures, whereas during febrile convulsions all kynurenines were increased by the same degree (Muñóz-Hoyos et al. 1997).

7 Conclusion

Systematic evaluation and interpretation of data indicates in general the beneficial effects of increasing central KYNA level in models of neuronal loss and seizures. Current state of research supports the notion that the selective blockade of excessive glutamate-mediated neurotransmission with exogenous compounds may be valuable in terms of neuroprotection; on the other hand, it might hamper processes of neuronal repair.

Promising experimental data indicate that novel, devoid of side-effects methods to manipulate glutamate transmission in humans should be sought. Endogenously produced KYNA is an attractive candidate for such studies. Sophisticated research adopting broader, multifactorial approach such as the development of drugs able to discriminate between brain structures or able to modify kynurenine metabolism selectively, in different cellular and subcellular compartments, is needed. Emerging therapies aimed to increase the level of neuroprotective KYNA or to reduce the brain expression of neurotoxic 3-hydroxykynurenine may become an important avenue in the treatment of neurodegeneration, ischemia, and seizures.

References

Ali, S. F., Binienda, Z. K., & Imam, S. Z. (2011). Molecular aspects of dopaminergic neurodegeneration: Gene-environment interaction in parkin dysfunction. *International Journal of Environmental Research and Public Health, 8*, 4702–4713.

Andiné, P., Lehmann, A., Ellren, K., Wennberg, E., Kjellmer, I., Nielsen, T., & Hagberg, H. (1988). The excitatory amino acid antagonist kynurenic acid administered after hypoxic-ischemia in neonatal rats offers neuroprotection. *Neuroscience Letters, 90*, 208–212.

Arnaiz-Cot, J. J., González, J. C., Sobrado, M., Baldelli, P., Carbone, E., Gandía, L., García, A. G., & Hernández-Guijo, J. M. (2008). Allosteric modulation of alpha 7 nicotinic receptors selectively depolarizes hippocampal interneurons, enhancing spontaneous GABAergic transmission. *European Journal of Neuroscience, 27*, 1097–1110.

Bano, D., Zanetti, F., Mende, Y., & Nicotera, P. (2011). Neurodegenerative processes in Huntington's disease. *Cell Death and Disease, 2*, e228. 10.

Baran, H., Gramer, M., Hönack, D., & Löscher, W. (1995). Systemic administration of kainate induces marked increases of endogenous kynurenic acid in various brain regions and plasma of rats. *European Journal of Pharmacology, 286*, 167–175.

Baran, H., Cairns, N., Lubec, B., & Lubec, G. (1996). Increased kynurenic acid levels and decreased brain kynurenine aminotransferase I in patients with down syndrome. *Life Sciences, 58*, 1891–1899.

Baran, H., Jellinger, K., & Deecke, L. (1999). Kynurenine metabolism in Alzheimer's disease. *Journal of Neural Transmission, 106*, 165–181.

Baran, H., Kepplinger, B., & Draxler, M. (2010). Endogenous kynurenine aminotransferases inhibitor is proposed to act as "Glia depressing factor" (GDF). *International Journal of Tryptophan Research, 3*, 13–22.

Barth, M. C., Ahluwalia, N., Anderson, T. J., Hardy, G. J., Sinha, S., Alvarez-Cardona, J. A., Pruitt, I. E., Rhee, E. P., Colvin, R. A., & Gerszten, R. E. (2009). Kynurenic acid triggers firm arrest of leukocytes to vascular endothelium under flow conditions. *Journal of Biological Chemistry, 17*(284), 19189–19195.

Beal, M. F., Matson, W. R., Swartz, K. J., Gamache, P. H., & Bird, E. D. (1990). Kynurenine pathway measurements in Huntington's disease striatum: Evidence for reduced formation of kynurenic acid. *Journal of Neurochemistry, 55*, 1327–1339.

Beal, M. F., Swartz, K. J., Hyman, B. T., Storey, E., Finn, S. F., & Koroshetz, W. (1991). Aminooxyacetic acid results in excitotoxin lesions by a novel indirect mechanism. *Journal of Neurochemistry, 57*, 1068–1073.

Beal, M. F., Matson, W. R., Storey, E., Milbury, P., Ryan, E. A., Ogawa, T., & Bird, E. D. (1992). Kynurenic acid concentrations are reduced in Huntington's disease cerebral cortex. *Journal of Neurological Sciences, 108*, 80–87.

Bellocchi, D., Macchiarulo, A., Carotti, A., & Pellicciari, R. (2009). Quantum mechanics/molecular mechanics (QM/MM) modeling of the irreversible transamination of L-kynurenine to kynurenic acid: The round dance of kynurenine aminotransferase II. *Biochimica et Biophysica Acta, 1794*, 1802–1812.

Bender, D. A., & McCreanor, G. M. (1985). Kynurenine hydroxylase: A potential rate-limiting enzyme in tryptophan metabolism. *Biochemical Society Transactions, 13*, 441–443.

Bock, K. W., & Köhle, C. (2009). The mammalian aryl hydrocarbon (Ah) receptor: From mediator of dioxin toxicity toward physiological functions in skin and liver. *Biological Chemistry, 390*, 1225–1235.

Bradshaw, T. D., & Bell, D. R. (2009). Relevance of the aryl hydrocarbon receptor (AhR) for clinical toxicology. *Clinical Toxicology (Philadelphia, Pa.), 47*, 632–642.

Brady, R. J., & Swann, J. W. (1988). Suppression of ictal-like activity by kynurenic acid does not correlate with its efficacy as an NMDA receptor antagonist. *Epilepsy Research, 2*, 232–238.

Braidy, N., Guillemin, G. J., Mansour, H., Chan-Ling, T., & Grant, R. (2011). Changes in kynurenine pathway metabolism in the brain, liver and kidney of aged female Wistar rats. *FEBS Journal, 278*, 4425–4434.

Brotchie, J., & Jenner, P. (2011). New approaches to therapy. *International Review of Neurobiology, 98*, 123–150.

Brotchie, J. M., Mitchell, I. J., Sambrook, M. A., & Crossman, A. R. (1991). Alleviation of parkinsonism by antagonism of excitatory amino acid transmission in the medial segment of the globus pallidus in rat and primate. *Movement Disorders, 6*, 133–138.

Brouillet, E., Jenkins, B. G., Hyman, B. T., Ferrante, R. J., Kowall, N. W., Srivastava, R., Roy, D. S., Rosen, B. R., & Beal, M. F. (1993). Age-dependent vulnerability of the striatum to the mitochondrial toxin 3-nitropropionic acid. *Journal of Neurochemistry, 60*, 356–359.

Brouns, R., Verkerk, R., Aerts, T., De Surgeloose, D., Wauters, A., Scharpé, S., & De Deyn, P. P. (2010). The role of tryptophan catabolism along the kynurenine pathway in acute ischemic stroke. *Neurochemical Research, 35*, 1315–1322.

Butler, E. G., Bourke, D. W., Finkelstein, D. I., & Horne, M. K. (1997). The effects of reversible inactivation of the subthalamo-pallidal pathway on the behaviour of naive and hemiparkinsonian monkeys. *Journal of Clinical Neuroscience, 4*, 218–227.

Campesan, S., Green, E. W., Breda, C., Sathyasaikumar, K. V., Muchowski, P. J., Schwarcz, R., Kyriacou, C. P., & Giorgini, F. (2011). The kynurenine pathway modulates neurodegeneration in a Drosophila model of Huntington's disease. *Current Biology, 21*, 961–966.

Carlá, V., Lombardi, G., Beni, M., Russi, P., Moneti, G., & Moroni, F. (1988). Identification and measurement of kynurenic acid in the rat brain and other organs. *Analytical Biochemistry, 169*, 89–94.

Carpenedo, R., Chiarugi, A., Russi, P., Lombardi, G., Carlà, V., Pellicciari, R., Mattoli, L., & Moroni, F. (1994). Inhibitors of kynurenine hydroxylase and kynureninase increase cerebral formation of kynurenate and have sedative and anticonvulsant activities. *Neuroscience, 61*, 237–243.

Carpenedo, R., Pittaluga, A., Cozzi, A., Attucci, S., Galli, A., Raiteri, M., & Moroni, F. (2001). Presynaptic kynurenate-sensitive receptors inhibit glutamate release. *European Journal of Neuroscience, 13*, 2141–2147.

Carpenedo, R., Meli, E., Peruginelli, F., Pellegrini-Giampietro, D. E., & Moroni, F. (2002). Kynurenine 3-mono-oxygenase inhibitors attenuate post-ischemic neuronal death in organotypic hippocampal slice cultures. *Journal of Neurochemistry, 82*, 1465–1471.

Chiamulera, C., Costa, S., & Reggiani, A. (1990). Effect of NMDA- and strychnine-insensitive glycine site antagonists on NMDA-mediated convulsions and learning. *Psychopharmacology, 102*, 551–552.

Chintamaneni, M., & Bhaskar, M. (2012). Biomarkers in Alzheimer's disease: A review. *ISRN Pharmacology*, *2012*, 984786.

Chmiel-Perzyńska, I., Perzyński, A., Wielosz, M., & Urbańska, E. M. (2007). Hyperglycemia enhances the inhibitory effect of mitochondrial toxins and D,L-homocysteine on the brain production of kynurenic acid. *Pharmacological Reports, 59*, 268–273.

Chmiel-Perzyńska, I., Kloc, R., Perzyński, A., Rudzki, S., & Urbańska, E. M. (2011). Novel aspect of ketone action: β-hydroxybutyrate increases brain synthesis of kynurenic acid in vitro. *Neurotoxicity Research, 20*, 40–50.

Connick, J. H., Stone, T. W., Carla, V., & Moroni, F. (1988). Increased kynurenic acid levels in Huntington's disease. *Lancet, 2*, 1373.

Connick, J. H., Carlà, V., Moroni, F., & Stone, T. W. (1989). Increase in kynurenic acid in Huntington's disease motor cortex. *Journal of Neurochemistry, 52*, 985–987.

Cosi, C., Mannaioni, G., Cozzi, A., Carlà, V., Sili, M., Cavone, L., Maratea, D., & Moroni, F. (2011). G-protein coupled receptor 35 (GPR35) activation and inflammatory pain: Studies on the antinociceptive effects of kynurenic acid and zaprinast. *Neuropharmacology, 60*, 1227–1231.

Coyle, J. T., & Schwarcz, R. (1976). Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. *Nature, 263*, 244–246.

Cozzi, A., Carpenedo, R., & Moroni, F. (1999). Kynurenine hydroxylase inhibitors reduce ischemic brain damage: Studies with (m-nitrobenzoyl)-alanine (mNBA) and 3,4-dimethoxy-[-N-4-(nitrophenyl)thiazol-2yl]-benzenesulfonamide (Ro 61-8048) in models of focal or global brain ischemia. *Journal of Cerebral Blood Flow and Metabolism, 19*, 771–777.

Csillik, A., Knyihár, E., Okuno, E., Krisztin-Péva, B., Csillik, B., & Vécsei, L. (2002). Effect of 3-nitropropionic acid on kynurenine aminotransferase in the rat brain. *Experimental Neurology, 177*, 233–241.

Dajas-Bailador, F. A., Lima, P. A., & Wonnacott, S. (2000). The alpha7 nicotinic acetylcholine receptor subtype mediates nicotine protection against NMDA excitotoxicity in primary hippocampal cultures through a Ca(2+) dependent mechanism. *Neuropharmacology, 39*, 2799–2807.

Dale, W. E., Dang, Y., Amiridze, N., & Brown, O. R. (2000). Evidence that kynurenine pathway metabolites mediate hyperbaric oxygen-induced convulsions. *Toxicology Letters, 117*, 37–43.

Danysz, W., & Parsons, C. G. (2003). The NMDA receptor antagonist memantine as a symptomatological and neuroprotective treatment for Alzheimer's disease: Preclinical evidence. *International Journal of Geriatric Psychiatry, 18*, S23–S32.

Darlington, L. G., Mackay, G. M., Forrest, C. M., Stoy, N., George, C., & Stone, T. W. (2007). Altered kynurenine metabolism correlates with infarct volume in stroke. *European Journal of Neuroscience, 26*, 2211–2221.

DiNatale, B. C., Murray, I. A., Schroeder, J. C., Flaveny, C. A., Lahoti, T. S., Laurenzana, E. M., Omiecinski, C. J., & Perdew, G. H. (2010). Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. *Toxicological Sciences, 115*, 89–97.

Dobelis, P., Staley, K. J., & Cooper, D. C. (2012). Lack of modulation of nicotinic acetylcholine alpha-7 receptor currents by kynurenic acid in adult hippocampal interneurons. *PLoS One, 7*(7), e41108. Epub 2012 Jul 25.

Du, F., & Schwarcz, R. (1992). Aminooxyacetic acid causes selective neuronal loss in layer III of the rat medial entorhinal cortex. *Neuroscience Letters, 147*, 185–188.

Du, F., Schmidt, W., Okuno, E., Kido, R., Köhler, C., & Schwarcz, R. (1992). Localization of kynurenine aminotransferase immunoreactivity in the rat hippocampus. *The Journal of Comparative Neurology, 321*, 477–487.

Eid, T., Du, F., & Schwarcz, R. (1995). Differential neuronal vulnerability to amino-oxyacetate and quinolinate in the rat parahippocampal region. *Neuroscience, 68*, 645–656.

Ellinger, A. (1904). Die Entstehung der Kynurensaure. *Zeitschrift für Physiologische Chemie, 43*, 325–337.

Erhardt, S., Blennow, K., Nordin, C., Skogh, E., Lindström, L. H., & Engberg, G. (2001). Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neuroscience Letters, 313*, 96–98.

Esser, C. (2012). Biology and function of the aryl hydrocarbon receptor: Report of an international and interdisciplinary conference. *Archives of Toxicology, 86*, 1323–1329.

Foster, A. C., Vezzani, A., French, E. D., & Schwarcz, R. (1984). Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. *Neuroscience Letters, 48*, 273–278.

Fukui, S., Schwarcz, R., Rapoport, S. I., Takada, Y., & Smith, Q. R. (1991). Blood-brain barrier transport of kynurenines: Implications for brain synthesis and metabolism. *Journal of Neurochemistry, 56*, 2007–2017.

Fukushima, T., Sone, Y., Mitsuhashi, S., Tomiya, M., & Toyo'oka, T. (2009). Alteration of kynurenic acid concentration in rat plasma following optically pure kynurenine administration: A comparative study between enantiomers. *Chirality, 21*, 468–472.

Germano, I. M., Pitts, L. H., Meldrum, B. S., Bartnikowski, H. M., & Simon, R. P. (1987). Kynurenate inhibition of cell excitation decreases stroke size and deficits. *Annals of Neurology, 22*, 730–734.

Ghribi, O., Callebert, J., Plotkine, M., & Boulu, R. G. (1994). Effect of kynurenic acid on the ischaemia-induced accumulation of glutamate in rat striatum. *Neuroreport, 5*, 435–437.

Gigler, G., Szénási, G., Simó, A., Lévay, G., Hársing, L. G., Jr., Sas, K., Vécsei, L., & Toldi, J. (2007). Neuroprotective effect of L-kynurenine sulfate administered before focal cerebral ischemia in mice and global cerebral ischemia in gerbils. *European Journal of Pharmacology, 564*, 116–122.

Ginsberg, M. D. (2008). Neuroprotection for ischemic stroke: Past, present and future. *Neuropharmacology, 55*, 363–389.

Gould, D. H., & Gustine, D. L. (1982). Basal ganglia degeneration, myelin alterations, and enzyme inhibition induced in mice by the plant toxin 3-nitropropanoic acid. *Neuropathology and Applied Neurobiology, 8*, 377–393.

Graham, W. C., Robertson, R. G., Sambrook, M. A., & Crossman, A. R. (1990). Injection of excitatory amino acid antagonists into the medial pallidal segment of a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated primate reverses motor symptoms of parkinsonism. *Life Sciences, 47*, PL91–PL97.

Gramsbergen, J. B., Turski, W. A., & Schwarcz, R. (1991). Brain-specific control of kynurenic acid production by depolarizing agents. *Advances in Experimental Medicine and Biology, 294*, 587–590.

Gramsbergen, J. B., Schmidt, W., Turski, W. A., & Schwarcz, R. (1992). Age-related changes in kynurenic acid production in rat brain. *Brain Research, 588*, 1–5.

Gramsbergen, J. B., Hodgkins, P. S., Rassoulpour, A., Turski, W. A., Guidetti, P., & Schwarcz, R. (1997). Brain-specific modulation of kynurenic acid synthesis in the rat. *Journal of Neurochemistry, 69*, 290–298.

Guidetti, P., Okuno, E., & Schwarcz, R. (1997). Characterization of rat brain kynurenine aminotransferases I and II. *Journal of Neuroscience Research, 50*, 457–465.

Guidetti, P., Reddy, P. H., Tagle, D. A., & Schwarcz, R. (2000). Early kynurenergic impairment in Huntington's disease and in a transgenic animal model. *Neuroscience Letters, 283*, 233–235.

Guidetti, P., Luthi-Carter, R. E., Augood, S. J., & Schwarcz, R. (2004). Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. *Neurobiology of Disease, 17*, 455–461.

Guidetti, P., Amori, L., Sapko, M. T., Okuno, E., & Schwarcz, R. (2007). Mitochondrial aspartate aminotransferase: A third kynurenate-producing enzyme in the mammalian brain. *Journal of Neurochemistry, 102*, 103–111.

Guillemin, G. J., Williams, K. R., Smith, D. G., Smythe, G. A., Croitoru-Lamoury, J., & Brew, B. J. (2003). Quinolinic acid in the pathogenesis of Alzheimer's disease. *Advances in Experimental Medicine and Biology, 527*, 167–176.

Gulaj, E., Pawlak, K., Bien, B., & Pawlak, D. (2010). Kynurenine and its metabolites in Alzheimer's disease patients. *Advances in Medical Sciences, 55*, 204–211.

Guo, J., Williams, D. J., Puhl, H. L., 3rd, & Ikeda, S. R. (2008). Inhibition of N-type calcium channels by activation of GPR35, an orphan receptor, heterologously expressed in rat sympathetic neurons. *Journal of Pharmacology and Experimental Therapeutics, 324*, 342–251.

Hamilton, B. F., & Gould, D. H. (1987). Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: A type of hypoxic (energy deficient) brain damage. *Acta Neuropathologica, 72*, 286–297.

Han, Q., Cai, T., Tagle, D. A., & Li, J. (2010). Structure, expression, and function of kynurenine aminotransferases in human and rodent brains. *Cellular and Molecular Life Sciences, 67*, 353–368.

Harris, C. A., Miranda, A. F., Tanguay, J. J., Boegman, R. J., Beninger, R. J., & Jhamandas, K. (1998). Modulation of striatal quinolinate neurotoxicity by elevation of endogenous brain kynurenic acid. *British Journal of Pharmacology, 124*, 391–399.

Hartai, Z., Klivenyi, P., Janaky, T., Penke, B., Dux, L., & Vecsei, L. (2005). Kynurenine metabolism in plasma and in red blood cells in Parkinson's disease. *Journal of Neurological Sciences, 239*, 31–35.

Hartai, Z., Juhász, A., Rimanóczy, A., Janáky, T., Donkó, T., Dux, L., Penke, B., Tóth, G. K., Janka, Z., & Kálmán, J. (2007). Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease. *Neurochemistry International, 50*, 308–313.

Heyes, M. P., Saito, K., Crowley, J. S., Davis, L. E., Demitrack, M. A., Der, M., Dilling, L. A., Elia, J., Kruesi, M. J., Lackner, A., et al. (1992). Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain, 115*, 1249–1273.

Heyes, M. P., Saito, K., Devinsky, O., & Nadi, N. S. (1994). Kynurenine pathway metabolites in cerebrospinal fluid and serum in complex partial seizures. *Epilepsia, 35*, 251–257.

Hilmas, C., Pereira, E. F., Alkondon, M., Rassoulpour, A., Schwarcz, R., & Albuquerque, E. X. (2001). The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: Physiopathological implications. *Journal of Neuroscience, 21*, 7463–7473.

Hopkins, F. G., & Cole, S. W. (1901). A contribution to the chemistry of proteids: Part I. A preliminary study of a hitherto undescribed product of tryptic digestion. *The Journal of Physiology, 27*, 418–428.

Hotta, S. S. (1968). Oxidative metabolism of isolated brain mitochondria: Changes caused by aminooxyacetate. *Archives of Biochemistry and Biophysics, 127*, 132–139.

Hsieh, Y. C., Chen, R. F., Yeh, Y. S., Lin, M. T., Hsieh, J. H., & Chen, S. H. (2011). Kynurenic acid attenuates multiorgan dysfunction in rats after heatstroke. *Acta Pharmacologica Sinica, 32*, 167–174.

Iłżecka, J., Kocki, T., Stelmasiak, Z., & Turski, W. A. (2003). Endogenous protectant kynurenic acid in amyotrophic lateral sclerosis. *Acta Neurologica Scandinavica, 107*, 412–418.

Jauch, D., Urbanska, E. M., Guidetti, P., Bird, E. D., Vonsattel, J. P., Whetsell, W. O., Jr., & Schwarcz, R. (1995). Dysfunction of brain kynurenic acid metabolism in Huntington's disease: Focus on kynurenine aminotransferases. *Journal of Neurological Sciences, 130*, 39–47.

Jenkins, L., Alvarez-Curto, E., Campbell, K., de Munnik, S., Canals, M., Schlyer, S., & Milligan, G. (2011). Agonist activation of the G protein-coupled receptor GPR35 involves transmembrane domain III and is transduced via $G\alpha_{13}$ and β-arrestin-2. *British Journal of Pharmacology, 162*, 733–748.

Kamiński, R. M., Zielińska, E., Dekundy, A., van Luijtelaar, G., & Turski, W. (2003). Deficit of endogenous kynurenic acid in the frontal cortex of rats with a genetic form of absence epilepsy. *Polish Journal of Pharmacology, 55*, 741–746.

Katayama, Y., Kawamata, T., Kano, T., & Tsubokawa, T. (1992). Excitatory amino acid antagonist administered via microdialysis attenuates lactate accumulation during cerebral ischemia and subsequent hippocampal damage. *Brain Research, 584*, 329–333.

Kepplinger, B., Baran, H., Kainz, A., Ferraz-Leite, H., Newcombe, J., & Kalina, P. (2005). Age-related increase of kynurenic acid in human cerebrospinal fluid – IgG and beta2-microglobulin changes. *Neurosignals, 14*, 126–135.

Kessler, M., Terramani, T., Lynch, G., & Baudry, M. (1989). A glycine site associated with *N*-methyl-D-aspartic acid receptors: Characterization and identification of a new class of antagonists. *Journal of Neurochemistry, 52*, 1319–1328.

Kiś, J., Czuczwar, M., Zielińska, E., Bojar, I., Czuczwar, S. J., & Turski, W. A. (2000). Kynurenic acid does not protect against nicotine-induced seizures in mice. *Polish Journal of Pharmacology, 52*, 477–480.

Kloc, R., Luchowska, E., Wielosz, M., Owe-Larsson, B., & Urbanska, E. M. (2008). Memantine increases brain production of kynurenic acid via protein kinase A-dependent mechanism. *Neuroscience Letters, 435*, 169–173.

Klockgether, T., & Turski, L. (1990). NMDA antagonists potentiate antiparkinsonian actions of L-dopa in monoamine-depleted rats. *Annals of Neurology, 28*, 539–546.

Klockgether, T., Turski, L., Honoré, T., Zhang, Z. M., Gash, D. M., Kurlan, R., & Greenamyre, J. T. (1991). The AMPA receptor antagonist NBQX has antiparkinsonian effects in monoamine-depleted rats and MPTP-treated monkeys. *Annals of Neurology, 30*, 717–723.

Knyihár-Csillik, E., Csillik, B., Pákáski, M., Krisztin-Péva, B., Dobó, E., Okuno, E., & Vécsei, L. (2004). Decreased expression of kynurenine aminotransferase-I (KAT I) in the substantia nigra of mice after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment. *Neuroscience, 126*, 899–914.

Knyihár-Csillik, E., Chadaide, Z., Mihály, A., Krisztin-Péva, B., Fenyo, R., & Vécsei, L. (2006). Effect of 6-hydroxydopamine treatment on kynurenine aminotransferase-I (KAT I) immunoreactivity of neurons and glial cells in the rat substantia nigra. *Acta Neuropathologica, 112*, 127–137.

Kocki, T., Luchowski, P., Luchowska, E., Wielosz, M., Turski, W. A., & Urbanska, E. M. (2003). L-cysteine sulphinate, endogenous sulphur-containing amino acid, inhibits rat brain kynurenic acid production via selective interference with kynurenine aminotransferase II. *Neuroscience Letters, 346*, 97–100.

Kocki, T., Kocki, J., Wielosz, M., Turski, W. A., & Urbanska, E. M. (2004). Carbamazepine enhances brain production of kynurenic acid in vitro. *European Journal of Pharmacology, 498*, 325–326.

Kocki, T., Wielosz, M., Turski, W. A., & Urbanska, E. M. (2006). Enhancement of brain kynurenic acid production by anticonvulsants–novel mechanism of antiepileptic activity? *European Journal of Pharmacology, 541*, 147–151.

Kocki, T., Wnuk, S., Kloc, R., Kocki, J., Owe-Larsson, B., & Urbanska, E. M. (2012). New insight into the antidepressants action: Modulation of kynurenine pathway by increasing the kynurenic acid/3-hydroxykynurenine ratio. *Journal of Neural Transmission, 119*, 235–243.

Kuc, D., Zgrajka, W., Parada-Turska, J., Urbanik-Sypniewska, T., & Turski, W. A. (2008). Micromolar concentration of kynurenic acid in rat small intestine. *Amino Acids, 35*, 503–505.

Lapin, I. P. (1978). Stimulant and convulsive effects of kynurenines injected into brain ventricles in mice. *Journal of Neural Transmission, 42*, 37–43.

Lapin, I. P. (1983). Antagonism of kynurenine-induced seizures by picolinic, kynurenic and xanthurenic acids. *Journal of Neural Transmission, 56*, 177–185.

Lapin, I. P., Prakhie, I. B., & Kiseleva, I. P. (1986). Antagonism of seizures induced by the administration of the endogenous convulsant quinolinic acid into rat brain ventricles. *Journal of Neural Transmission, 65*, 177–185.

Lee do, Y., Lee, K. S., Lee, H. J., Noh, Y. H., Kim do, H., Lee, J. Y., Cho, S. H., Yoon, O. J., Lee, W. B., Kim, K. Y., Chung, Y. H., & Kim, S. S. (2008). Kynurenic acid attenuates MPP^+-induced dopaminergic neuronal cell death via a Bax-mediated mitochondrial pathway. *European Journal of Cell Biology, 87*, 389–397.

Lees, A. J., Hardy, J., & Revesz, T. (2009). Parkinson's disease. *Lancet, 373*, 2055–2066.

Liebig, J. (1853). Uber Kynurensäure. *Justus Liebigs Annalen der Chemie, 86*, 125–126.

Lin, H., Vicini, S., Hsu, F. C., Doshi, S., Takano, H., Coulter, D. A., & Lynch, D. R. (2010). Axonal α_7 nicotinic ACh receptors modulate presynaptic NMDA receptor expression and structural plasticity of glutamatergic presynaptic boutons. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 16661–16666.

Löscher, W., Ebert, U., & Lehmann, H. (1996). Kindling induces a lasting, regionally selective increase of kynurenic acid in the nucleus accumbens. *Brain Research, 725*, 252–256.

Luchowska, E., Luchowski, P., Sarnowska, A., Wielosz, M., Turski, W. A., & Urbańska, E. M. (2003). Endogenous level of kynurenic acid and activities of kynurenine aminotransferases following transient global ischemia in the gerbil hippocampus. *Polish Journal of Pharmacology, 55*, 443–447.

Luchowska, E., Luchowski, P., Paczek, R., Ziembowicz, A., Kocki, T., Turski, W. A., Wielosz, M., Lazarewicz, J., & Urbanska, E. M. (2005). Dual effect of DL-homocysteine and S-adenosylhomocysteine on brain synthesis of the glutamate receptor antagonist, kynurenic acid. *Journal of Neuroscience Research, 79*, 375–382.

Luchowska, E., Kloc, R., Wnuk, S., Olajossy, B., Wielosz, M., & Urbańska, E. M. (2008). Clenbuterol enhances the production of kynurenic acid in brain cortical slices and glial cultures. *Pharmacological Reports, 60*, 574–577.

Luchowska, E., Kloc, R., Olajossy, B., Wnuk, S., Wielosz, M., Owe-Larsson, B., & Urbanska, E. M. (2009). β-adrenergic enhancement of brain kynurenic acid production mediated via cAMP-related protein kinase A signaling. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 33*, 519–529.

Luchowski, P., & Urbanska, E. M. (2007). SNAP and SIN-1 increase brain production of kynurenic acid. *European Journal of Pharmacology, 563*, 130–133.

Luchowski, P., Luchowska, E., Turski, W. A., & Urbanska, E. M. (2002). 1-Methyl-4-phenylpyridinium and 3-nitropropionic acid diminish cortical synthesis of kynurenic acid via interference with kynurenine aminotransferases in rats. *Neuroscience Letters, 330*, 49–52.

Maciejak, P., Szyndler, J., Turzyńska, D., Sobolewska, A., Taracha, E., Skórzewska, A., Lehner, M., Bidziński, A., & Płaźnik, A. (2009). Time course of changes in the concentration of kynurenic acid in the brain of pentylenetetrazol-kindled rats. *Brain Research Bulletin, 78*, 299–305.

Mattson, M. P. (2008). Glutamate and neurotrophic factors in neuronal plasticity and disease. *Annals of the New York Academy of Sciences, 1144*, 97–112.

McIntosh, T. K., Vink, R., Soares, H., Hayes, R., & Simon, R. (1990). Effect of noncompetitive blockade of *N*-methyl-D-aspartate receptors on the neurochemical sequelae of experimental brain injury. *Journal of Neurochemistry, 55*, 1170–1179.

McMaster, O. G., Du, F., French, E. D., & Schwarcz, R. (1991). Focal injection of aminooxyacetic acid produces seizures and lesions in rat hippocampus: Evidence for mediation by NMDA receptors. *Experimental Neurology, 113*, 378–385.

Min, K. D., Asakura, M., Liao, Y., Nakamaru, K., Okazaki, H., Takahashi, T., Fujimoto, K., Ito, S., Takahashi, A., Asanuma, H., Yamazaki, S., Minamino, T., Sanada, S., Seguchi, O., Nakano, A., Ando, Y., Otsuka, T., Furukawa, H., Isomura, T., Takashima, S., Mochizuki, N., & Kitakaze, M. (2010). Identification of genes related to heart failure using global gene expression profiling of human failing myocardium. *Biochemical and Biophysical Research Communications, 393*, 55–60.

Miranda, A. F., Boegman, R. J., Beninger, R. J., & Jhamandas, K. (1997). Protection against quinolinic acid-mediated excitotoxicity in nigrostriatal dopaminergic neurons by endogenous kynurenic acid. *Neuroscience, 78*, 967–975.

Miranda, A. F., Sutton, M. A., Beninger, R. J., Jhamandas, K., & Boegman, R. J. (1999). Quinolinic acid lesion of the nigrostriatal pathway: Effect on turning behaviour and protection by elevation of endogenous kynurenic acid in Rattus norvegicus. *Neuroscience Letters, 262*, 81–84.

Mok, M. H., Fricker, A. C., Weil, A., & Kew, J. N. (2009). Electrophysiological characterisation of the actions of kynurenic acid at ligand-gated ion channels. *Neuropharmacology, 57*, 242–249.

Moroni, F. (1999). Tryptophan metabolism and brain function: Focus on kynurenine and other indole metabolites. *European Journal of Pharmacology, 375*, 87–100.

Moroni, F., Russi, P., Carla, V., & Lombardi, G. (1988a). Kynurenic acid is present in the rat brain and its content increases during development and aging processes. *Neuroscience Letters, 94*, 145–150.

Moroni, F., Russi, P., Lombardi, G., Beni, M., & Carlà, V. (1988b). Presence of kynurenic acid in the mammalian brain. *Journal of Neurochemistry, 51*, 177–180.

Muir, K. W., & Lees, K. R. (2003). Excitatory amino acid antagonists for acute stroke. *Cochrane Database of Systematic Reviews, 3*, CD001244.

Muñóz-Hoyos, A., Molina-Carballo, A., Rodríguez-Cabezas, T., Uberos-Fernández, J., Ruiz-Cosano, C., & Acuña-Castroviejo, D. (1997). Relationships between methoxyindole and kynurenine pathway metabolites in plasma and urine in children suffering from febrile and epileptic seizures. *Clinical Endocrinology (Oxf), 47*, 667–677.

Németh, H., Robotka, H., Kis, Z., Rózsa, E., Janáky, T., Somlai, C., Marosi, M., Farkas, T., Toldi, J., & Vécsei, L. (2004). Kynurenine administered together with probenecid markedly inhibits pentylenetetrazol-induced seizures. An electrophysiological and behavioural study. *Neuropharmacology, 47*, 916–925.

Németh, H., Toldi, J., & Vecsei, L. (2005). Role of kynurenines in the central and peripheral nervous systems. *Current Neurovascular Research, 2*, 249–260.

Németh, H., Toldi, J., & Vécsei, L. (2006). Kynurenines, Parkinson's disease and other neurodegenerative disorders: Preclinical and clinical studies. *Journal of Neural Transmission. Supplementum, 70*, 285–304.

Newell, D. W., Barth, A., & Malouf, A. T. (1995). Glycine site NMDA receptor antagonists provide protection against ischemia-induced neuronal damage in hippocampal slice cultures. *Brain Research, 675*, 38–44.

Ogawa, T., Matson, W. R., Beal, M. F., Myers, R. H., Bird, E. D., Milbury, P., & Saso, S. (1992). Kynurenine pathway abnormalities in Parkinson's disease. *Neurology, 42*, 1702–1706.

Ohshiro, H., Tonai-Kachi, H., & Ichikawa, K. (2008). GPR35 is a functional receptor in rat dorsal root ganglion neurons. *Biochemical and Biophysical Research Communications, 365*, 344–348.

Okumura, S., Baba, H., Kumada, T., Nanmoku, K., Nakajima, H., Nakane, Y., Hioki, K., & Ikenaka, K. (2004). Cloning of a G-protein-coupled receptor that shows an activity to transform NIH3T3 cells and is expressed in gastric cancer cells. *Cancer Science, 95*, 131–135.

Okuno, E., Nakamura, M., & Schwarcz, R. (1991a). Two kynurenine aminotransferases in human brain. *Brain Research, 542*, 307–312.

Okuno, E., Schmidt, W., Parks, D. A., Nakamura, M., & Schwarcz, R. (1991b). Measurement of rat brain kynurenine aminotransferase at physiological kynurenine concentrations. *Journal of Neurochemistry, 57*, 533–540.

Opitz, C. A., Litzenburger, U. M., Sahm, F., Ott, M., Tritschler, I., Trump, S., Schumacher, T., Jestaedt, L., Schrenk, D., Weller, M., Jugold, M., Guillemin, G. J., Miller, C. L., Lutz, C., Radlwimmer, B., Lehmann, I., von Deimling, A., Wick, W., & Platten, M. (2011). An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature, 478*, 197–203.

Pearson, S. J., & Reynolds, G. P. (1992). Increased brain concentrations of a neurotoxin, 3-hydroxykynurenine, in Huntington's disease. *Neuroscience Letters, 144*, 199–201.

Peeters, B. W., Ramakers, G. M., Ellenbroek, B. A., Vossen, J. M., & Coenen, A. M. (1994a). Interactions between NMDA and nonNMDA receptors in nonconvulsive epilepsy in the WAG/Rij inbred strain. *Brain Research Bulletin, 33*, 715–718.

Peeters, B. W., Ramakers, G. M., Vossen, J. M., & Coenen, A. M. (1994b). The WAG/Rij rat model for nonconvulsive absence epilepsy: Involvement of nonNMDA receptors. *Brain Research Bulletin, 33*, 709–713.

Perkins, M. N., & Stone, T. W. (1982). An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Research, 247*, 184–187.

Perkins, M. N., & Stone, T. W. (1983). In vivo release of [3H]-purines by quinolinic acid and related compounds. *British Journal of Pharmacology, 80*, 263–267.

Phillis, J. W., Song, D., Guyot, L. L., & O'Regan, M. H. (1999). Failure of kynurenic acid to inhibit amino acid release from the ischemic rat cerebral cortex. *Neuroscience Letters, 273*, 21–24.

Pinsky, C., Glavin, G. B., & Bose, R. (1989). Kynurenic acid protects against neurotoxicity and lethality of toxic extracts from contaminated Atlantic coast mussels. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 13*, 595–598.

Pocivavsek, A., Wu, H. Q., Potter, M. C., Elmer, G. I., Pellicciari, R., & Schwarcz, R. (2011). Fluctuations in endogenous kynurenic acid control hippocampal glutamate and memory. *Neuropsychopharmacology, 36*, 2357–2367.

Rassoulpour, A., Wu, H. Q., Poeggeler, B., & Schwarcz, R. (1998). Systemic d-amphetamine administration causes a reduction of kynurenic acid levels in rat brain. *Brain Research, 802*, 111–118.

Rassoulpour, A., Wu, H. Q., Albuquerque, E. X., & Schwarcz, R. (2005). Prolonged nicotine administration results in biphasic, brain-specific changes in kynurenate levels in the rat. *Neuropsychopharmacology, 30*, 697–704.

Rejdak, K., Bartosik-Psujek, H., Dobosz, B., Kocki, T., Grieb, P., Giovannoni, G., Turski, W. A., & Stelmasiak, Z. (2002). Decreased level of kynurenic acid in cerebrospinal fluid of relapsing-onset multiple sclerosis patients. *Neuroscience Letters, 331*, 63–65.

Reynolds, G. P., & Pearson, S. J. (1989). Increased brain 3-hydroxykynurenine in Huntington's disease. *Lancet, 2*, 979–980.

Roberts, R. C., Du, F., McCarthy, K. E., Okuno, E., & Schwarcz, R. (1992). Immunocytochemical localization of kynurenine aminotransferase in the rat striatum: A light and electron microscopic study. *The Journal of Comparative Neurology, 326*, 82–90.

Robotka, H., Sas, K., Agoston, M., Rózsa, E., Szénási, G., Gigler, G., Vécsei, L., & Toldi, J. (2008). Neuroprotection achieved in the ischaemic rat cortex with L-kynurenine sulphate. *Life Sciences, 82*, 915–919.

Russi, P., Alesiani, M., Lombardi, G., Davolio, P., Pellicciari, R., & Moroni, F. (1992). Nicotinylalanine increases the formation of kynurenic acid in the brain and antagonizes convulsions. *Journal of Neurochemistry, 59*, 2076–2080.

Rzeski, W., Kocki, T., Dybel, A., Wejksza, K., Zdzisińska, B., Kandefer-Szerszeń, M., Turski, W. A., Okuno, E., & Albrecht, J. (2005). Demonstration of kynurenine aminotransferases I and II and characterization of kynurenic acid synthesis in cultured cerebral cortical neurons. *Journal of Neuroscience Research, 80*, 677–682.

Salvati, P., Ukmar, G., Dho, L., Rosa, B., Cini, M., Marconi, M., Molinari, A., & Post, C. (1999). Brain concentrations of kynurenic acid after a systemic neuroprotective dose in the gerbil model of global ischemia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 23*, 741–752.

Samadi, P., Grégoire, L., Rassoulpour, A., Guidetti, P., Izzo, E., Schwarcz, R., & Bédard, P. J. (2005). Effect of kynurenine 3-hydroxylase inhibition on the dyskinetic and antiparkinsonian responses to levodopa in Parkinsonian monkeys. *Movement Disorders, 20*, 792–802.

Sanberg, P. R. (1980). Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. *Nature, 284*, 472–473.

Sanberg, P. R., Pisa, M., & Fibiger, H. C. (1981). Kainic acid injections in the striatum alter the cataleptic and locomotor effects of drugs influencing dopaminergic and cholinergic systems. *European Journal of Pharmacology, 74*, 347–357.

Sapko, M. T., Guidetti, P., Yu, P., Tagle, D. A., Pellicciari, R., & Schwarcz, R. (2006). Endogenous kynurenate controls the vulnerability of striatal neurons to quinolinate: Implications for Huntington's disease. *Experimental Neurology, 197*, 31–40.

Scharfman, H. E., & Goodman, J. H. (1998). Effects of central and peripheral administration of kynurenic acid on hippocampal evoked responses in vivo and in vitro. *Neuroscience, 86*, 751–764.

Scharfman, H. E., & Ofer, A. (1997). Pretreatment with L-kynurenine, the precursor to the excitatory amino acid antagonist kynurenic acid, suppresses epileptiform activity in combined entorhinal/hippocampal slices. *Neuroscience Letters, 224*, 115–118.

Schneiderman, J. H., & MacDonald, J. F. (1989). Excitatory amino acid blockers differentially affect bursting of in vitro hippocampal neurons in two pharmacological models of epilepsy. *Neuroscience, 31*, 593–603.

Schwarcz, R., & Pellicciari, R. (2002). Manipulation of brain kynurenines: Glial targets, neuronal effects, and clinical opportunities. *Journal of Pharmacology and Experimental Therapeutics, 303*, 1–10.

Schwarcz, R., Whetsell, W. O., Jr., & Mangano, R. M. (1983). Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. *Science, 219*, 316–318.

Schwarcz, R., Guidetti, P., Sathyasaikumar, K. V., & Muchowski, P. J. (2010). Of mice, rats and men: Revisiting the quinolinic acid hypothesis of Huntington's disease. *Progress in Neurobiology, 90*, 230–245.

Silva-Adaya, D., Pérez-De La Cruz, V., Villeda-Hernández, J., Carrillo-Mora, P., González-Herrera, I. G., García, E., Colín-Barenque, L., Pedraza-Chaverrí, J., & Santamaría, A. (2011). Protective effect of L-kynurenine and probenecid on 6-hydroxydopamine-induced

striatal toxicity in rats: Implications of modulating kynurenate as a protective strategy. *Neurotoxicology and Teratology, 33*, 303–312.

Simon, R. P., Young, R. S., Stout, S., & Cheng, J. (1986). Inhibition of excitatory neurotransmission with kynurenate reduces brain edema in neonatal anoxia. *Neuroscience Letters, 71*, 361–364.

Speciale, C., Wu, H. Q., Gramsbergen, J. B., Turski, W. A., Ungerstedt, U., & Schwarcz, R. (1990). Determination of extracellular kynurenic acid in the striatum of unanesthetized rats: Effect of aminooxyacetic acid. *Neuroscience Letters, 116*, 198–203.

Stazka, J., Luchowski, P., Wielosz, M., Kleinrok, Z., & Urbanska, E. M. (2002). Endothelium-dependent production and liberation of kynurenic acid by rat aortic rings exposed to L-kynurenine. *European Journal of Pharmacology, 448*, 133–137.

Stazka, J., Luchowski, P., & Urbanska, E. M. (2005). Homocysteine, a risk factor for atherosclerosis, biphasically changes the endothelial production of kynurenic acid. *European Journal of Pharmacology, 517*, 217–223.

Stevens, E. A., Mezrich, J. D., & Bradfield, C. A. (2009). The aryl hydrocarbon receptor: A perspective on potential roles in the immune system. *Immunology, 127*, 299–311.

Stone, T. W. (1988). Comparison of kynurenic acid and 2-APV suppression of epileptiform activity in rat hippocampal slices. *Neuroscience Letters, 84*, 234–238.

Stone, T. W. (1993). Neuropharmacology of quinolinic and kynurenic acids. *Pharmacological Reviews, 45*, 309–379.

Stone, T. W. (2001). Endogenous neurotoxins from tryptophan. *Toxicon, 39*, 61–73.

Stone, T. W. (2007). Kynurenic acid blocks nicotinic synaptic transmission to hippocampal interneurons in young rats. *European Journal of Neuroscience, 25*, 2656–2665.

Stone, T. W., & Darlington, L. G. (2002). Endogenous kynurenines as targets for drug discovery and development. *Nature Reviews. Drug Discovery, 1*, 609–620.

Swan, J. S., Kragten, E. Y., & Veening, H. (1983). Liquid-chromatographic study of fluorescent materials in uremic fluids. *Clinical Chemistry, 29*, 1082–1084.

Swartz, K. J., Matson, W. R., MacGarvey, U., Ryan, E. A., & Beal, M. F. (1990). Measurement of kynurenic acid in mammalian brain extracts and cerebrospinal fluid by high-performance liquid chromatography with fluorometric and coulometric electrode array detection. *Analytical Biochemistry, 185*, 363–376.

Szabó, N., Kincses, Z. T., Toldi, J., & Vécsei, L. (2011). Altered tryptophan metabolism in Parkinson's disease: A possible novel therapeutic approach. *Journal of Neurological Sciences, 310*, 256–260.

Thompson, J. L., Holmes, G. L., Taylor, G. W., & Feldman, D. R. (1988). Effects of kynurenic acid on amygdaloid kindling in the rat. *Epilepsy Research, 2*, 302–308.

Tohgi, H., Abe, T., Takahashi, S., Kimura, M., Takahashi, J., & Kikuchi, T. (1992). Concentrations of serotonin and its related substances in the cerebrospinal fluid in patients with Alzheimer type dementia. *Neuroscience Letters, 141*, 9–12.

Turski, W. A., Nakamura, M., Todd, W. P., Carpenter, B. K., Whetsell, W. O., Jr., & Schwarcz, R. (1988). Identification and quantification of kynurenic acid in human brain tissue. *Brain Research, 454*, 164–169.

Turski, W. A., Gramsbergen, J. B., Traitler, H., & Schwarcz, R. (1989). Rat brain slices produce and liberate kynurenic acid upon exposure to L-kynurenine. *Journal of Neurochemistry, 52*, 1629–1636.

Turski, W. A., Urbanska, E., Dziki, M., Parada-Turska, J., & Ikonomidou, C. (1990). Excitatory amino acid antagonists protect mice against seizures induced by bicuculline. *Brain Research, 514*, 131–134.

Turski, L., Bressler, K., Rettig, K. J., Löschmann, P. A., & Wachtel, H. (1991a). Protection of substantia nigra from MPP^+ neurotoxicity by *N*-methyl-D-aspartate antagonists. *Nature, 349*, 414–418.

Turski, W. A., Dziki, M., Urbanska, E., Calderazzo-Filho, L. S., & Cavalheiro, E. A. (1991b). Seizures induced by aminooxyacetic acid in mice: Pharmacological characteristics. *Synapse, 7*, 173–180.

Turski, W., Dziki, M., Parada, J., Kleinrok, Z., & Cavalheiro, E. A. (1992). Age dependency of the susceptibility of rats to aminooxyacetic acid seizures. *Brain Research. Developmental Brain Research, 67*, 137–144.

Turski, M. P., Turska, M., Zgrajka, W., Kuc, D., & Turski, W. A. (2009). Presence of kynurenic acid in food and honeybee products. *Amino Acids, 36*, 75–80.

Urbańska, E. M. (2005). Mitochondrial toxins with potent neurodegenerative and convulsive properties – effects on kynurenine metabolism in the brain. In L. Vecsei (Ed.), *Kynurenines in the brain: From experiments to clinics* (pp. 115–131). New York: Nova Science.

Urbańska, E., Ikonomidou, C., Sieklucka, M., & Turski, W. A. (1989). Aminooxyacetic acid produces excitotoxic lesions in the rat striatum. *Society for Neuroscience Abstract, 15*, 764.

Urbańska, E., Ikonomidou, C., Sieklucka, M., & Turski, W. A. (1991). Aminooxyacetic acid produces excitotoxic lesions in the rat striatum. *Synapse, 9*, 129–135.

Urbańska, E. M., Kocki, T., Saran, T., Kleinrok, Z., & Turski, W. A. (1997). Impairment of brain kynurenic acid production by glutamate metabotropic receptor agonists. *Neuroreport, 8*, 3501–3505.

Urbańska, E. M., Dekundy, A., Kleinrok, Z., Turski, W. A., & Czuczwar, S. J. (1998). Glutamatergic receptor agonists and brain pathology. In R. M. Kostrzewa (Ed.), *Highly selective neurotoxins: Basic and clinical applications* (pp. 329–354). Totowa: Humana Press.

Urbańska, E. M., Chmielewski, M., Kocki, T., & Turski, W. A. (2000). Formation of endogenous glutamatergic receptors antagonist kynurenic acid–differences between cortical and spinal cord slices. *Brain Research, 878*, 210–212.

Urbańska, E. M., Luchowski, P., Luchowska, E., Pniewski, J., Woźniak, R., Chodakowska-Zebrowska, M., & Lazarewicz, J. (2006). Serum kynurenic acid positively correlates with cardiovascular disease risk factor, homocysteine: A study in stroke patients. *Pharmacological Reports, 58*, 507–511.

Uwai, Y., Honjo, H., & Iwamoto, K. (2012). Interaction and transport of kynurenic acid via human organic anion transporters hOAT1 and hOAT3. *Pharmacological Research, 65*, 254–260.

Vécsei, L., & Beal, M. F. (1991). Comparative behavioral and pharmacological studies with centrally administered kynurenine and kynurenic acid in rats. *European Journal of Pharmacology, 196*, 239–246.

Vécsei, L., Miller, J., MacGarvey, U., & Beal, M. F. (1992). Kynurenine and probenecid inhibit pentylenetetrazol- and NMDLA-induced seizures and increase kynurenic acid concentrations in the brain. *Brain Research Bulletin, 28*, 233–238.

Vizi, E. S., & Lendvai, B. (1999). Modulatory role of presynaptic nicotinic receptors in synaptic and non-synaptic chemical communication in the central nervous system. *Brain Research. Brain Research Reviews, 30*, 219–235.

Walker, B. R., Easton, A., & Gale, K. (1999). Regulation of limbic motor seizures by GABA and glutamate transmission in nucleus tractus solitarius. *Epilepsia, 40*, 1051–1057.

Wang, J., Simonavicius, N., Wu, X., Swaminath, G., Reagan, J., Tian, H., & Ling, L. (2006). Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *Journal of Biological Chemistry, 281*, 22021–22028.

Widner, B., Leblhuber, F., Walli, J., Tilz, G. P., Demel, U., & Fuchs, D. (2000). Tryptophan degradation and immune activation in Alzheimer's disease. *Journal of Neural Transmission, 107*, 343–353.

Wonodi, I., & Schwarcz, R. (2010). Cortical kynurenine pathway metabolism: A novel target for cognitive enhancement in Schizophrenia. *Schizophrenia Bulletin, 36*, 211–218.

Wood, J. D., & Peesker, S. J. (1973). The role of GABA metabolism in the convulsant and anticonvulsant actions of aminooxyacetic acid. *Journal of Neurochemistry, 20*, 379–387.

Wu, H. Q., & Schwarcz, R. (1996). Seizure activity causes elevation of endogenous extracellular kynurenic acid in the rat brain. *Brain Research Bulletin, 39*, 155–162.

Wu, H. Q., Turski, W. A., Ungerstedt, U., & Schwarcz, R. (1991). Systemic kainic acid administration in rats: Effects on kynurenic acid production in vitro and in vivo. *Experimental Neurology, 113*, 47–52.

Wu, H. Q., Rassoulpour, A., & Schwarcz, R. (2002). Effect of systemic L-DOPA administration on extracellular kynurenate levels in the rat striatum. *Journal of Neural Transmission, 109*, 239–249.

Wu, H. Q., Rassoulpour, A., Goodman, J. H., Scharfman, H. E., Bertram, E. H., & Schwarcz, R. (2005). Kynurenate and 7-chlorokynurenate formation in chronically epileptic rats. *Epilepsia, 46*, 1010–1016.

Yamamoto, H., Shindo, I., Egawa, B., & Horiguchi, K. (1994). Kynurenic acid is decreased in cerebrospinal fluid of patients with infantile spasms. *Pediatric Neurology, 10*, 9–12.

Yamamoto, H., Murakami, H., Horiguchi, K., & Egawa, B. (1995). Studies on cerebrospinal fluid kynurenic acid concentrations in epileptic children. *Brain & Development, 17*, 327–329.

Zádori, D., Nyiri, G., Szonyi, A., Szatmári, I., Fülöp, F., Toldi, J., Freund, T. F., Vécsei, L., & Klivényi, P. (2011). Neuroprotective effects of a novel kynurenic acid analogue in a transgenic mouse model of Huntington's disease. *Journal of Neural Transmission, 118*, 865–875.

Zarnowski, T., Chorągiewicz, T., Tulidowicz-Bielak, M., Thaler, S., Rejdak, R., Żarnowski, I., Turski, W. A., & Gasior, M. (2012). Ketogenic diet increases concentrations of kynurenic acid in discrete brain structures of young and adult rats. *Journal of Neural Transmission, 119*, 679–684.

Zhao, P., Sharir, H., Kapur, A., Cowan, A., Geller, E. B., Adler, M. W., Seltzman, H. H., Reggio, P. H., Heynen-Genel, S., Sauer, M., Chung, T. D., Bai, Y., Chen, W., Caron, M. G., Barak, L. S., & Abood, M. E. (2010). Targeting of the orphan receptor GPR35 by pamoic acid: A potent activator of extracellular signal-regulated kinase and β-arrestin2 with antinociceptive activity. *Molecular Pharmacology, 78*, 560–568.

Zielińska, E., Kocki, T., Saran, T., Borbely, S., Kuc, D., Vilagi, I., Urbańska, E. M., & Turski, W. A. (2005). Effect of pesticides on kynurenic acid production in rat brain slices. *Annals of Agricultural and Environmental Medicine, 12*, 177–179.

Zielińska, E., Kuc, D., Zgrajka, W., Turski, W. A., & Dekundy, A. (2009). Long-term exposure to nicotine markedly reduces kynurenic acid in rat brain – in vitro and ex vivo evidence. *Toxicology and Applied Pharmacology, 240*, 174–179.

Zuccato, C., Valenza, M., & Cattaneo, E. (2010). Molecular mechanisms and potential therapeutical targets in Huntington's disease. *Physiological Reviews, 90*, 905–981.

Zwilling, D., Huang, S. Y., Sathyasaikumar, K. V., Notarangelo, F. M., Guidetti, P., Wu, H. Q., Lee, J., Truong, J., Andrews-Zwilling, Y., Hsieh, E. W., Louie, J. Y., Wu, T., Scearce-Levie, K., Patrick, C., Adame, A., Giorgini, F., Moussaoui, S., Laue, G., Rassoulpour, A., Flik, G., Huang, Y., Muchowski, J. M., Masliah, E., Schwarcz, R., & Muchowski, P. J. (2011). Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. *Cell, 145*, 863–874.

Neurotoxicity in Psychostimulant and Opiate Addiction

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Contents

Abstract

Psychostimulants and opioids evoke toxicity within the central nervous system (CNS), primarily linked to action of the substance (e.g., enhancement of synaptic concentrations of dopamine) or by indirect effects (increases in extrasynaptic glutamate levels, toxic metabolites). Associated with the development of drug addiction, the above mechanisms lead to the several pathological processes within the complex network of brain cell-to-cell

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interactions, such as alteration in neurotransmitter systems, dysregulation of energy metabolism, changes in structure of cellular membrane, apoptosis, generation of reactive species (RS), glial/microglial cell overactivation, excitotoxicity and prominent deficits in brain morphology (neuronal loss, axonal damage), alterations of transcription factors, immediate early genes and epigenetic mechanisms, as well as neurocognitive and neuropsychological impairments.

Keywords

Abuse • Addiction • Amphetamine • Animal studies • Cocaine • Dependence • Human studies • Neurotoxicity • Opiate

List of Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	Serotonin
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	Brain-derived neurotrophic factor
CART	Cocaine- and amphetamine-regulated transcript
CAT	Catalase
CNS	Central nervous system
CpG	Cytosine–guanine dinucleotide
CREB	cAMP response element binding
CYP450	Cytochrome P450
DNMT	DNA methyltransferase
FADD	Fas-associated death domain
FasL	Fas ligand
fMRI	Functional magnetic resonance imaging
GABA	ɣ-aminobutyric acid
GPx	Glutathione peroxidase
GSH	Glutathione
HDAC	Histone deacetylase
HMT	Histone methyltransferase
HSP72	72-kDa heat shock protein
HVA	Homovanillic acid
MAO	Monoamine oxidase
MDA	Malondialdehyde
MDMA	3,4-methylenedioxymethamphetamine ecstasy
miRNA	microRNA
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NMDA	N-methyl-D-aspartic acid
OPRD	Delta opioid receptor gene
OPRK	kappa opioid receptor gene
OPRM	mu opioid receptor gene
PET	Positron emission tomography
rCBF	Cerebral blood flow

rCMR	Regional cerebral metabolic rate
ROS	Reactive oxygen species
RS	Reactive species
SNP	Single-nucleotide polymorphism
SOD	Superoxide dismutase
SPECT	Single photon emission computed tomography
TH	Tyrosine hydroxylase
TNF	Tumor necrosis factor
VMAT	Vesicular monoamine transporter

1 Introduction

Neurotoxicity can result from exposure to several exogenous factors and drugs of abuse are of such example. Psychostimulant and opiate intake is widespread, and their repeated exposure leads to either physical damage or disruption in the activity of the nervous system.

2 Psychostimulants and Opiates

Psychostimulants (e.g., cocaine and amphetamines, like methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA=ecstasy)) are drugs that temporarily enhance the activity of the CNS and can induce the feeling of euphoria in humans, while in laboratory animals locomotor overstimulation, reinforcement/reward, as well as subjective effects in a drug discrimination task are produced. The most prominent pharmacological mechanism of psychostimulants is the facilitation of dopaminergic neurotransmission; however, these drugs also enhance noradrenaline and/or serotonin (5-HT) activity (for a review, see Fleckenstein et al. 2007; Sulzer 2011). Psychostimulants can be characterized as "releasers" (amphetamines) and "uptake blockers" (cocaine) based on the mechanism of their acute effect on neurotransmitter flux through the monoamine transporter.

The direct actions of amphetamine and methamphetamine include the reversal of transport across the dopamine transporter (in lower extracellular concentrations; Sitte et al. 1998), diffusion into the cell due to its lipophilicity at higher extracellular concentrations (Sulzer et al. 1995; Kahlig et al. 2005), inhibition of the dopamine transporter, and inhibition of the vesicular monoamine transporter (VMAT)2 function; together, these actions induce stimulation-independent dopamine release (Sitte et al. 1998; Riddle et al. 2002). Amphetamine also enhances dopamine synthesis (Kuczenski 1975) and impairs dopamine metabolism by inhibiting monoamine oxidase (MAO) (Ramsay and Hunter 2002). In high concentrations or with repeated treatment, amphetamine inhibits tyrosine hydroxylase (TH) and/or TH protein levels (Ellison et al. 1978; Bowyer et al. 1998).

In contrast to amphetamine and methamphetamine, MDMA's mechanism depends on the animal species (Green et al. 2003). In nonhuman primates MDMA acts as a 5-HT neurotoxin; in rats, MDMA depletes 5-HT terminal markers due to enhanced 5-HT release (reviewed by Green et al. 2003); in mice MDMA releases dopamine (Rothman and Baumann 2003).

The primary mechanism of cocaine is linked to its ability to bind to the dopamine transporter, inhibit dopamine reuptake, and elevate synaptic and extrasynaptic dopamine levels (Brown et al. 2001), whereas it was more recently established that cocaine binding sites also contain both high- and low-affinity binding components (i.e., dopamine D_2 receptors) (Marcellino et al. 2010), indicating a novel allosteric agonist action of cocaine in low (1–10 nM) concentrations on dopamine receptors (Ferraro et al. 2010). Apart from being a dopamine transporter blocker, cocaine also inhibits the noradrenaline and 5-HT transporters (Gatley et al. 1998). Of less significance is cocaine's action on the VMAT-2; cocaine enhances the storage of cytoplasmic catecholamines into synaptic vesicles, acting on the VMAT-2 which further allows for massive synaptic neurotransmitter release in response to depolarization (Brown et al. 2001).

It should be noted that metabolism of psychostimulants generates active substances that have their own toxic effects on nerves, including the brain. Thus, metabolites of amphetamine and methamphetamine include norephedrine, hydroxyamphetamine, hydroxynorephedrine N-hydroxymethylamphetamine, and possibly N-formylamphetamine (Jenkins and Cone 1998; Soloway and Patil 2011), by virtue of an action of cytochrome P450 (CYP450) on the parent drug. Metabolism of MDMA starts with O- or N-demethylation resulting in α-methyldopamine, N-methyl-α-methyldopamine, or 6-hydroxy-α-methyldopamine, while during further biotransformation, conjugation with thiols or glutathione (GSH) leads to thioether derivatives or 5-(glutathion-S-yl)-α-methyldopamine; all the above metabolites are toxic to neurons (Milhazes et al. 2006; Capela et al. 2006, 2007; Cunha-Oliveira et al. 2008; Barbosa et al. 2012; Martinez et al. 2012).

Neurotoxic cocaine metabolites are benzoylecgonine, norcocaine, and cocaethylene (Nassogne et al. 1998; Bunney et al. 2001).

Opiates are any of the narcotics derived from the opium poppy plant. Together with other psychoactive drugs called "opioids," they share mechanisms of action, i.e., they bind to opioid receptors (see below). Opiates (e.g., morphine, heroin) and opioids (oxycodone, methadone, pentanyl, tramadol, pethidine, dextropropoxyphene, etc.) induce a feeling of well-being and a relief of pain, while their long-term repeated use leads to addiction and tolerance. This group of addictive drugs is characterized by a strong withdrawal syndrome that includes both somatic and affective components. In laboratory animals, opioids produce a biphasic locomotor response, consisting of an initial reduction followed by an increase in locomotor activity, discriminative stimulus, and reinforcement, whereas withdrawal syndrome includes a constellation of jumping, wet-dog shakes, teeth chattering, forepaw treading, forepaw tremors, and diarrhea.

Opiates/opioids exert their actions by binding to three opioid receptors, μ, δ, and κ, and the former two are involved in drug pleasurable effects due to the stimulation of dopaminergic neurons. Following opiates/opioids, enhanced dopamine extrasynaptic levels result from two points: at the level of the dopamine synapse and at the level of the afferents to the dopamine cell bodies (reviewed in Wise 1987; Sulzer 2011). The enhancement of dopamine release in the striatum may result from an indirect effect of opioids leading to the disinhibition of tegmental dopaminergic neurons by inhibiting γ-aminobutyric acid (GABA) interneurons or GABA terminals containing opioid receptors in the ventral tegmental area or by the direct activation of opioid receptors on accumbal cholinergic or GABAergic neurons (Sulzer 2011).

Opiates undergo several metabolic changes leading to assorted derivatives possessing toxic potential. Morphine is converted mainly by glucuronidation into morphine-3-glucoronide and morphine-6-glucoronide. However, oxidative demethylation and further oxidation via CYT450-dependent processes (principally via CYP3A4 and CYP2D6) may lead to N-hydroxymethylnormorphine, normorphine, and hydromorphone (Coller et al. 2009). Of note, morphine-3-glucoronide shows rewarding efficacy in mice (Vindenes et al. 2006).

Heroin (diacetylmorphine) undergoes rapid deacetylation to 6-monoacetylmorphine, which is approximately six times more potent than morphine, and further deacetylation to morphine (Sawynok 1986).

Pethidine is converted via CYT450 to a neurotoxic norpethidine, while dextropropoxyphene undergoes biotransformation into nordextropropoxyphene, a cardiotoxic agent.

A hallmark of psychostimulants and opioids (as well as other abused drugs), even having different mechanisms of action, is that they all impinge and increase dopamine neurotransmission within the mesolimbic circuitry of the brain (the so-called reward system); these effects are seen after acute (above) or chronic drug exposure (Filip et al. 2010; Sulzer 2011). The reward pathway is composed of dopaminergic neurons that project from the ventral tegmental area to several cortical and subcortical structures. The key structure that executes reward processes and is implicated in the addictive effects of the drugs is the nucleus accumbens, the terminus of the mesolimbic dopaminergic pathway. The nucleus accumbens is a regulator of motivation and learning processes (Di Chiara et al. 1999). It should be underlined that although impairment evoked by abuse of addictive drugs starts in the brain areas processing reward, the long-term drug intake disrupts the whole brain and leads to dysfunctions of emotions, motivations, learning, memory, executive control, and cognitive awareness. These functions are executed by the amygdala and hippocampus (memory and learning), the insula (conscious emotions), the dorsal striatum (habit forming learning), the orbitofrontal cortex (motivation/drive, salience attribution, motivation, and compulsive behaviors), the prefrontal cortex (executive control), and the cingulate gyrus (inhibitory control, attention, and impulsivity) (Volkow et al. 2003). Interestingly, in drug addiction there are recognized strong feedback mechanisms within the above brain structures with a special significance for the prefrontal cortex. In fact, in drug addicts, dysregulation of

prefrontal cortex activity impairs its inhibitory coordination of other brain circuits, fixing the maintenance of drug habit. The above impairment behaviors are under control of glutamatergic neurotransmission (reviewed by Tzschentke and Schmidt 2003; Volkow et al. 2003; Kalivas 2004). Glutamategic neurotransmission is a cellular mediator of synaptic plasticity as well as learning and memory processes, and the current notion of addiction indicates that aberrant forms of drug-induced synaptic plasticity and learning in the brain drive compulsive and relapsing behaviors (Dacher and Nugent 2011).

3 In Vivo Evidences of Neurotoxicity

3.1 Human Studies

3.1.1 Psychostimulants

Neurological and Psychiatric Impairments

As reported in vivo, chronic use with amphetamines or cocaine has been implicated in cognitive deficits and psychological and anatomical impairments in human addicts. Among chronic drug users, methamphetamine abuse is associated with impairment of episodic memory, executive functions (e.g., novel problem solving), complex information processing speed, and decision-making abilities (impulsivity and risky decision-making) (Bechara and Martin 2004; Scott et al. 2007). Smaller impairments are present in motor skills (e.g., coordination) and language abilities (Caligiuri and Buitenhuys 2005; Scott et al. 2007). Other studies report a high level of psychiatric symptoms such as auditory/visual hallucinations, suspiciousness, and delusional thoughts in methamphetamine users (McKetin et al. 2006); such symptoms typically last only hours or diminish within a week of withdrawal, but prolonged symptom episodes have also been reported (McKetin et al. 2006). Abstinent methamphetamine abusers suffer also from mood disturbances like depression and anxiety (London et al. 2004) that may occur after the use episode, but may last for many months thereafter (Zweben et al. 2004).

Literature findings about influence of chronic MDMA use on neurocognition are equivocal. Some studies point out that only a "heavy" MDMA use can cause primary memory dysfunction (Gouzoulis-Mayfrank and Daumann 2006), while a low dose of MDMA (one or two tablets containing the usual amount of MDMA) has no relevant effects on human cognitive brain function (de Win et al. 2006; Jager et al. 2007). Moreover, there is a concern that memory deficits in MDMA users, although subtle, might be a risk factor for earlier onset and/or more severe decline of age-related memory deficits in later years, possibly due to hippocampal dysfunction (Gouzoulis-Mayfrank and Daumann 2009). Moreover, chronic MDMA users reported significantly increased levels of emotional instability, depressive mood, anxiety, aggression, impulsive features, and paranoid ideation (O'Leary et al. 2001; Gouzoulis-Mayfrank and Daumann 2009). It should be added that several factors like polydrug use, genetic factors (Gouzoulis-Mayfrank and Daumann 2009), or positive family history of psychiatric problems

(O'Leary et al. 2001) have been recognized within MDMA abusers, making a linkage between MDMA abuse and the drug-induced psychological problems to be established in further studies. Disturbances in working memory were reported also for cocaine-dependent subjects in which functional magnetic resonance imaging (fMRI) analyses during a working memory task showed impaired activation in the frontal, striatal, and thalamic brain regions (Cowan 2007; Moeller et al. 2010).

Chronic cocaine users suffer from diminished decision-making capacity, disrupted perception of motivational drive, and impairments in self-control (Goldstein et al. 2007a). Some researchers indicate that cocaine use by mothers during pregnancy increases the risk of significant cognitive (e.g., lower IQ, poorer language development, impaired attention), emotional (impulsivity), and motor deficits in the offspring (Arendt et al. 1999; Smith et al. 2001; Harvey 2004; Jones et al. 2004). Of note, some researchers do not report noticeable neurological or psychiatric abnormalities in children born of mothers who abused cocaine during pregnancy (Eyler et al. 2001; Rose-Jacobs et al. 2002).

Morphological Changes

Amphetamine users, apart from functional manifestation of drug-induced neurotoxicity (above), show prominent morphological brain changes. In fact, a loss of gray matter in the cingulate, paralimbic, and limbic cortices; noticeable smaller hippocampus volume; and hypertrophy of white matter were found in chronic methamphetamine users (Thompson et al. 2004). Additionally, MDMA users reveal reduction of multiple gray matter regions in the neocortical, bilateral cerebellum, and midline brain stem brain regions, potentially involved in some previously reported neuropsychiatric impairments (Cowan et al. 2003).

There are also morphological abnormalities in cocaine abusers seen as decreases in gray matter in the orbitofrontal cortex, age-related hyperintense areas of white matter regions in the cerebral and insular subcortex in fMRI scans (Franklin et al. 2002). Apart from the above brain regions, magnetic resonance imaging (MRI) tests performed on cocaine-dependent subjects ($N = 40$) showed also lower gray matter volumes in cerebellar hemispheres and thalamus (Sim et al. 2007). The authors suggest that cerebellar deficits may contribute to neuropsychological deficits and motor dysfunction frequently observed in cocaine-dependent subjects. Long-term consumption of cocaine may also influence white matter connections in the brain (Lane et al. 2010); deficits in white matter integrity have an inverse relationship with the length of abstinence from cocaine abuse in cocaine-dependent patients (Xu et al. 2010). Cocaine was also found to upregulate heat shock protein expression, leading to blood–brain barrier breakdown and brain edema formation (Sharma et al. 2009).

Recently reviewed by Derauf et al. 2009, infants, children, and young adults display some morphological abnormalities on MR-based imaging scans as cortical infarcts, pachygyria, schizencephaly, paraventricular hemorrhage, subependymal and periventricular cysts, as well as decreases in the volume of the corpus callosum, caudate–putamen or thalamus, and in cortical gray matter in both cocaine and methamphetamine abusers or their offspring.

Microglia Changes

Chronic methamphetamine users showed an increased level of glial activation marker myoinositol in the frontal gray and left frontal white matter (Ernst et al. 2000; Chang et al. 2007; Sung et al. 2007; Krasnova and Cadet 2009). With use of positron emission tomography (PET), Sekine et al. (2008) indicated that methamphetamine-dependent individuals present a remarkable level of microgliosis in several brain regions (the midbrain, thalamus, striatum, insular, and orbitofrontal cortices); these changes were correlated inversely with the duration of the drug abstinence. Because the above observation contrasts with the findings in autopsied methamphetamine users' brains (see 4.1.1.2), further correlation analyses between the time needed for induction and progression of microglia activation are required.

Metabolic Changes

Human amphetamine addicts have serious brain metabolism impairments that are significantly altered in cortical and striatal areas (Volkow et al. 2001a; Wang et al. 2004). Recent papers reported reduced concentrations of N-acetylaspartate, being a marker of neuronal activity linked with attention control (Ernst et al. 2000; Sung et al. 2007; Licata and Renshaw 2010), and of creatine that serves as a metabolic integrity marker (Sekine et al. 2002) in the basal ganglia of methamphetamine abusers. Moreover, chronic methamphetamine users display raised striatal volumes and altered brain glucose metabolism in the orbitofrontal and limbic regions in the frontal gray and left frontal white matter (Ernst et al. 2000; Sung et al. 2007; Chang et al. 2007; Krasnova and Cadet 2009) as well as a higher glucose activity in the parietal cortex and lower activity in the thalamus and striatum (Volkow et al. 2001a).

Similarly to chronic methamphetamine, MDMA users showed decreased ratios of N-acetylaspartate to creatine which were associated with memory deficits (Reneman et al. 2001); however, other authors have not reported such differences (Daumann et al. 2004).

In vivo imaging findings on human cocaine users showed acute cocaine-evoked activation of the anterior cingulate (Daglish and Nutt 2003; Romero et al. 2010), while presentation of cocaine-associated cues resulted in the significant activation of metabolic activity of the anterior cingulate cortex region (Goldstein et al. 2007b). Local perfusion deficits were confirmed by PET and single photon emission computed tomography (SPECT) imaging in active cocaine abusers in the orbitofrontal cortex where the glucose metabolism was elevated and linked closely to changes in cerebral metabolism (Holman et al. 1991, 1993; Levin and Kaufman 1995; Levin et al. 1995; Volkow et al. 1991, 2004; Martinez et al. 2009), and this hypermetabolic response correlated with intense craving in cocaine abusers (Volkow and Fowler 2000) and persisted after months of detoxification (Volkow et al. 1988). PET studies confirmed MRI analyses about decreased metabolic activity in the orbitofrontal cortex and cingulate gyrus in the detoxified cocaine abusers (Volkow et al. 2004); such decreases were associated with reductions in striatal dopamine D_2 receptors (see below). In another study in active cocaine

abusers in both genders, PET and 2-deoxy-2[^{18}F]fluoro-D-glucose indicated a reduction in whole brain glucose metabolism in female and a trend to increase in male subjects during cocaine-cues video (Volkow et al. 2011). Children with prenatal cocaine exposure showed increased striatal creatine levels in magnetic resonance spectroscopy (MRS) study what suggested an impairment of energy metabolism in the brain of children exposed to this drug in utero (Fowler et al. 2007). The findings observed in stimulant abusers document brain metabolic changes in areas involved in reward circuitry and dopamine systems.

Changes in Neurotransmission

PET and MRI imaging studies have revealed that amphetamines can lead to neurodegenerative changes in the brains of human addicts; these abnormalities include persistent decreases in the levels of dopamine transporters in the orbitofrontal cortex, dorsolateral prefrontal cortex, and caudate–putamen (McCann et al. 1998a; Volkow et al. 2001b, c; Sekine et al. 2003; McCann et al. 2008) and the lower density of 5-HT transporters in global brain regions (Sekine et al. 2006) in methamphetamine addicts. PET imaging identified also partial recovery of dopamine transporter binding in methamphetamine abusers during abstinence (Volkow et al. 2001c). The dopamine transporter reduction paralleled slower motor function and impaired episodic memory functioning, enhanced severity of psychiatric symptoms, and longer duration of methamphetamine use, while the reduction of 5-HT transporter may have a connection with increased aggressive behavior in methamphetamine abusers (Scott et al. 2007). It should be added that deleterious effects of methamphetamine such as N-acetylaspartate, creatine decrease, and microglial overactivity are enhanced in HIV-positive methamphetamine abusers (Chang et al. 2005), which indicate additive detrimental effect of coexisting HIV infection and methamphetamine abuse on human brain function.

Studies in MDMA users have reported enduring decrements in global brain 5-HT transporter binding (Ricaurte et al. 2000); however, neither a significant correlation between reductions in 5-HT transporter availability and extent of MDMA abuse nor improvements in markers for transporter during periods of drug abstinence have been demonstrated (Buchert et al. 2006; Thomasius et al. 2006). Of note, a separate SPECT imaging study in MDMA recreational users reported reduced striatal dopamine transporter binding (Reneman et al. 2002).

A growing number of evidence indicate enhanced glutamate transmission in many limbic brain areas in the development of amphetamine neurotoxicity (Abekawa et al. 1994; Stephans and Yamamoto 1994; Rocher and Gardier 2001; Cunha-Oliveira et al. 2008). Thus, reduction in glutamate and glutamine transporter systems has been described in the frontal gray matter of abstinent chronic methamphetamine abusers but not in active drug addicts (Ernst and Chang 2008) as an adaptation leading possibly due to reduced methamphetamine excitotoxicity (Ramonet et al. 2004). Since downregulation of glutamate and/or glutamine transporter levels is lowest at the beginning of abstinence ($\leq$1 month), they may serve as a factor involved in methamphetamine craving (cf. Ernst and Chang 2008).

Another amphetamine-derived psychostimulant, MDMA, evokes reduction in the metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), levels in cerebrospinal fluid in human abusers (Ricaurte et al. 1990; McCann et al. 1994, 1999; Bolla et al. 1998), and such change is regarded as a marker of the drug-induced toxicity. Confirmed by PET studies showing significant and dose-dependent depletion of 5-HT transporter levels in most cortical and subcortical brain regions of chronic MDMA users (McCann et al. 1998b; Semple et al. 1999; Reneman et al. 2001), the latter depletion was negatively correlated with the extent of abuse (McCann et al. 1998b; Ricaurte et al. 2000) and paralleled memory and learning impairment (Cowan et al. 2003).

PET studies have been also performed to reveal cocaine-induced abnormalities in brain dopamine neurotransmission, including long-lasting decreases in D_2 receptor density in cocaine abusers (Volkow and Fowler 2000). These observations concerning D_2 receptor level could be linked to reduced sensitivity of reward circuits to stimulation by natural rewards and increased risk for drug taking (Volkow et al. 2004). Moreover, detoxified cocaine abusers had still a marked decrease in dopamine release as measured by methylphenidate-induced decreases in striatal [^{11}C] raclopride binding (Volkow et al. 1997), while the dopamine transporter density – elevated shortly after cocaine abstinence – was normalized with long-term detoxification (Malison et al. 1998). Interestingly, no difference in D_1 receptor density was observed between cocaine-dependent subjects and matched controls (Martinez et al. 2009). Another PET scan report showed the low VMAT-2 availability in the striatum of cocaine abusers which the authors link to a compensatory downregulation of the dopamine storage vesicles in response to chronic drug abuse and/or a loss of dopamine terminals (Narendran et al. 2012).

Manifestations of Genomic Mechanisms

The areas of the genome underlying amphetamine-type (cocaine and methamphetamine) substance use disorders are coming into light. In an American Indian community at high risk for substance dependence, analyses of multipoint variance component LOD scores failed to yield evidence of linkage for stimulant dependence for a panel of 791 microsatellite polymorphisms. However, the importance of "craving" as an important addiction phenotype was associated with regions on chromosomes 12, 15, and 18 in this user population. In more detail, the "craving" phenotype and "heavy stimulant use" were associated with a locus found on chromosomes 15q25.3–26.1 near the nicotinic receptor gene cluster while a loci for stimulant "craving" on chromosomes 12p13.33–13.32 and 18q22.3 (Ehlers et al. 2011). The single-nucleotide polymorphism (SNP) 118A>G in the mu opioid receptor gene (OPRM1) coding sequence was associated with methamphetamine psychosis, having latency less than 3 years from first drug intake in Japanese subjects (Ide et al. 2004). In cocaine addicts, an association with drug intake was shown with three promoter SNPs (−1793T>A, −1699insT, and −2044C>A) of OPRM1 gene (Hoehe et al. 2000), while a very recent large study identified association between the 17TT genotype and a cocaine addiction in HIV-positive and HIV-negative African addicts (Crystal et al. 2012). In a study of

methamphetamine dependence/psychosis, an association was found with SNP rs2075572 (IVS2+691G>C) of the latter gene in Japanese subjects (Ide et al. 2004, 2006), but not with the SNP rs2234918 (921T>C) in exon 3 of the delta opioid receptor gene (OPRD1) (Kobayashi et al. 2006).

3.1.2 Opiates

Neurological and Psychiatric Impairments

It is well-established that chronic exposure to heroin often results in cognitive deficits such as poorer performance in learning, cognition, and memory in human addicts (Cipolli and Galliani 1987; Guerra et al. 1987; Spain and Newsom 1991; Eisch et al. 2000). Other findings showed that methadone itself may contribute to cognitive impairment. In fact, greater impairment on verbal fluency, processing speed, response inhibition, visuospatial attention, cognitive flexibility, and set shifting was found in methadone-maintained patients than in abstinent groups. Also, in direct comparison of the methadone-maintained patients to former heroin users, similar impairment in executive function, visual memory, and impulsivity was found (cf. Martin et al. 2007). Methadone-maintained subjects rated the subjective value of a monetary reinforcement lower than the healthy comparison group, too (Martin-Soelch et al. 2001). Acquired brain injury (e.g., head trauma, nonfatal overdose) following long-term heroin use may contribute to variability in cognitive impairment. Interestingly, buprenorphine, another opioid used for maintenance treatment of former heroin abusers, was found to cause less psychomotor and cognitive impairment than methadone (Curran et al. 2001).

Morphological Changes

In heroin addicts neuroimaging studies indicate morphological changes within the posterior limb of the internal capsule and sparing of subcortical U fibers (cf. Martin et al. 2007). Another study (Lyoo et al. 2006) reported gray matter reductions in prefrontal, insular, and temporal cortices in patients on methadone maintenance compared with age- and sex-matched healthy subjects.

Metabolic Changes

Two studies have reported disturbances in frontal gray matter of patients maintained on methadone, buprenorphine, or heroin as the patients had lower concentration of N-acetylaspartate, as measured by MRS (Haselhorst et al. 2002; Yücel et al. 2007). MRI scans revealed diffuse white matter changes which paralleled widespread confluent vacuolar degeneration of the deep white matter, sparing of the brain stem and cerebellar white matter and extensive axonal injury in two patients with fatal drug-associated leukoencephalopathy (Ryan et al. 2005). Another in vivo functional assay indicates acute left-sided sensorimotor hemiplegia occurring acutely after a first inhaled dose of heroin by a 16-year-old male (Vella et al. 2003). The latter authors detected disturbances predominantly in parieto-occipital subcortical white matter and both ventral globus pallidus in MRS data and postulate defects in mitochondrial function as well as hypoxic injury in specific and limited areas of white matter due to acute heroin.

In SPECT scans conducted on opiate-dependent (methadone-maintained or heroin-using) subjects, a decrease in regional (the frontal and temporal cortex) cerebral blood flow (rCBF) was shown (Danos et al. 1998). A similar pattern of rCBF reduction has also been reported during the first week (Rose et al. 1996; Danos et al. 1998) or 10 weeks (Botelho et al. 2006) of detoxification, while Rose et al. (1996) have reported improved rCBF in the frontal cortex in patients rescanned at 3 weeks of withdrawal. Long-lasting neurobiological abnormalities in the regional cerebral metabolic rate (rCMR) in the anterior cingulate cortex were detected following a 6-month abstinence period in opiate addicts using fluorodeoxyglucose PET (FDG-PET) (Galynker et al. 2000).

Manifestation of Oxidative Stress

In heroin addicts ($N = 34$) chronic opioid use evokes significant growth of oxidative stress (Kovatsi et al. 2010).

Manifestations of Genomic and Epigenetic Mechanisms

There is a strong association between major genes of the opioid system and addiction to opiates. The most widely studied are mu, kappa, and delta receptors encoded by OPRM1, OPRK1, and OPRD1 genes (cf. Levran et al. 2012). Within two important SNPs (i.e., 17C>T (Ala6Val) and 118A>G (Asp40Asn)) out of the variants described in dbSNP in the OPRM1 gene coding sequence, only the SNP 118A>G has been linked with opioid addiction, and such observations come from Swedish (Bart et al. 2005), Chinese, European American (Drakenberg et al. 2006), and Indian patients (Deb et al. 2010). Other studies did not detect the above association (Franke et al. 2001; Glatt et al. 2007; Levran et al. 2009) nor association between the17T allele and opioid dependence in Indian (Kapur et al. 2007) and African (Crowley et al. 2003) addicts. Similarly, no association with opioid addiction has been reported in African and European American subjects analyzing SNPs −1793T>A, −1699insT, −1320A>G, 17C>T, 118A>G, as well as SNPs 540825 and 562859 (Crowley et al. 2003; Smith et al. 2005).

There are some evidences for association of OPRK1 SNPs 1051660 (Yuferov et al. 2004; Gerra et al. 2007) and of OPRK1 SNP rs6473797 (Levran et al. 2008) with opioid addiction. Recently, some papers indicated other polymorphism SNPs (e.g., rs2236861, rs2236857, rs3766951, rs204055, rs2236857, and rs2298896) for the OPRD1 gene in human addicts; associations with heroin addiction strongly depend on ethnic groups (cf. Levran et al. 2012).

Being a multifactorial and polygenic disorder, drug addiction does not conform to a simple Mendelian transmission pattern. Thus, epidemiological studies conducted on family, twin, and adoption suggest involvement in both genetic variance and environmental factors to increase susceptibility to addiction that neither act in isolation (Dick and Foroud 2003). Recent studies from the last 10 years demonstrated that posttranscriptional regulation of gene expression plays a key role in neurogenesis and synaptic plasticity (Puckett and Lubin 2011) and supports the role of epigenetic processes to control drug addiction (Robison and

Nestler 2011; Wong et al. 2011). A separate study indicated also the occurrence of epigenetic mechanisms (i.e., an increase in OPRM1 DNA methylation) in lymphocytes of methadone-maintained former heroin addicts (Nielsen et al. 2009). Another recent study demonstrates that overall methylation in human blood DNA from opioid addicts was increased and cytosine–guanine dinucleotide (CpG) islands located in *OPRM1* promoter were hypermethylated (Chorbov et al. 2011). Additionally, in sperm-derived DNA the methylation was increased at CpG sites, but global methylation was not affected (Chorbov et al. 2011). Hypermethylation of *OPRM1* promoter can cause gene silencing, while methylation in sperm may suggest heritability of dependence phenotypes.

3.2 Animal Studies

3.2.1 Psychostimulants

Behavioral Manifestation of Neurotoxicity

Methamphetamine was shown to induce only subtle to moderate functional manifestation of neurotoxic-like alterations in behavior of laboratory animals (cf. Gouzoulis-Mayfrank and Daumann 2009). Thus, only up to 3 days after a neurotoxic methamphetamine administration (4 times × 10 mg/kg, every 2 h) is there a decrease in spontaneous locomotor activity in rodents (Timar et al. 2003). In more subtle motor tests, i.e., active avoidance performance and balance beam performance, residual deficiency was reported in rats after methamphetamine neurotoxic treatment (Walsh and Wagner 1992). The most affected by methamphetamine intake were learning and memory behaviors. Neurotoxic methamphetamine exposure, under regimens of 4 × 10 mg/kg, every 2 h, or 24 × 1.67 mg/kg once every 15 min, impaired novel object recognition and impaired learning in a multiple T water maze test, with no differences on spatial navigation or reference memory in the Morris water maze in rats (Herring et al. 2008). Disturbances in consolidation of learned place preference and impairments on a radial maze sequential learning task and on a novelty preference object recognition were reported after neurotoxic methamphetamine doses in rodents (Chat-Mendes et al. 2007; Gouzoulis-Mayfrank and Daumann 2009). Interestingly, in nonhuman primates, gradual rise in methamphetamine doses in an escalating dose regimen diminished social behavior on "injection" days, with aggression declining during the study; such effects disappeared after 3 weeks of drug abstinence (Melega et al. 2008).

More conflicting findings concern functional manifestation of MDMA neurotoxicity. Some studies reported enhanced anxiety or weak memory performance in rodents and monkeys exposed to MDMA (Winsauer et al. 2002; Melega et al. 2008) but normal or return-to-normal task execution after 2–3 weeks abstinence even with previous 33-week MDMA escalated doses (Melega et al. 2008). Other findings indicate no assessment of functional impairments like risk-taking behavior or anxiety after MDMA administration (Gouzoulis-Mayfrank and Daumann 2009);

according to the latter studies, MDMA-treated animals do not present functional abnormalities despite persistent neurotoxicity reported postmortem.

Prolonged exposure to cocaine (sometimes even an acute drug injection) resulted in cognitive impairment and/or a decrease in social contacts in animals (Daza-Losada et al. 2009; Murnane and Howell 2011). Monkeys exposed to self-administered cocaine showed attentional impairments with a more specific cognitive control impairment, implicating orbitofrontal cortex dysfunction (Porter et al. 2011).

Changes in Neurotransmission

Several neuroimaging and microdialysis findings reveal abnormalities within brain neurotransmission due to chronic psychostimulant exposure. Thus, imaging studies (PET, SPECT, or fMRI) indicate neurotoxic effects regarding dopamine and 5-HT brain systems due to long-term psychostimulant exposure to animals (for a review, see Howell and Murnane 2011; Murnane and Howell 2011). Neurotoxic effects toward dopamine system alterations include decreases in dopamine synthesis (lower DOPA level) (Melega et al. 1997) and a drop in dopamine transporter densities (Villemagne et al. 1998; Melega et al. 2000; Hashimoto et al. 2007) in the striatum of monkeys after two i.m. injections with methamphetamine/amphetamine or following 3–4 i.m. injections with methamphetamine to nonhuman primate (Villemagne et al. 1998; Hashimoto et al. 2007).

Neuroimaging reports showed neurotoxic results of MDMA action on 5-HT brain system during noncontingent drug administration. In fact, an early PET imaging study in a baboon characterized the effects of MDMA on in vivo 5-HT transporter availability using MDMA (5 mg/kg s.c., once or twice a day for 4 days) that caused diminished 5-HT transporter density in the caudate–putamen and cortical regions (Scheffel et al. 1998; Szabo et al. 2002). Such decreases in 5-HT transporter availability, however, were not evidenced during contingent MDMA administration in rhesus monkeys (self-administration model) even during higher MDMA dosage (100–140 mg/kg/day) and repeated drug treatment (12 months) (Banks et al. 2008). Long-term (18 months) self-administered MDMA to rhesus monkeys evoked also no significant change in the VMAT availability following at least 2 months of drug abstinence, as assessed with PET neuroimaging, despite an evidence of attenuation of reinforcing properties of MDMA perhaps through neurotoxic effects of MDMA (Fantegrossi et al. 2004).

PET imaging studies in monkeys showed that chronic exposure to self-administered cocaine resulted in a significant reduction in dopamine D_2 receptor levels (Czoty et al. 2004; Nader et al. 2006) that persisted for up to 1 year of abstinence in some monkeys (Nader et al. 2006). These studies demonstrate that monkeys with long-term histories of cocaine use reliably display lower dopamine D_2 receptor densities in ways that correlate with cocaine dose and duration of exposure (Moore et al. 1998; Nader et al. 2002).

Data from in vivo microdialysis in rodents indicated long-lasting reductions in dopamine and 5-HT brain levels following amphetamine derivatives, including

methamphetamine (Herring et al. 2008) and MDMA (Gudelsky and Yamamoto 2008). Apart from enhanced release of monoamines in the striatum, nucleus accumbens, and prefrontal cortex, MDMA also evoked the release of acetylcholine in the striatum, hippocampus, and prefrontal cortex and increased the extracellular concentration of energy substrates, e.g., glucose and lactate, in the rat brain. In contrast to the acute stimulatory actions of MDMA on the release of monoamines and acetylcholine, repeated MDMA administration in high doses resulted in a neurotoxicity to 5-HT axon terminals in the rat (for a review, see Gudelsky and Yamamoto 2008).

Repeated cocaine self-administration evokes significant alterations in extracellular monoamine and amino acid levels in rodents' brain. Many in vivo microdialysis studies (Pettit and Justice 1989; Pontieri et al. 1996; Lecca et al. 2007; Saddoris et al. 2011) including a very recent paper by Wydra et al. (2013) indicated that self-administered cocaine evoked extracellular accumbal dopamine and that its level was much higher in comparison to yoked rats given passive *i.v.* cocaine injections. Ferris et al. (2011), using a combination of cocaine self-administration and microdialysis in freely moving rats, showed a decrease in dopamine transporter sensitivity to cocaine in the nucleus accumbens core. Such a significant decrease in the ability of cocaine to inhibit the dopamine transporter was apparent after a single day of self-administration, lasting for several days following cessation of cocaine intake (Ferris et al. 2011); the latter finding confirms PET imaging studies showing that acute cocaine administration decreases the availability of the plasma membrane transporter for binding, even after cocaine is no longer blocking dopamine uptake as evidenced by a return of dopamine to its basal levels (Kimmel et al. 2012).

A few other reports demonstrated increases in extracellular GABA derived from neuronal sources after 3 weeks of withdrawal from repeated passive cocaine administration (Xi et al. 2003), but no changes in GABA levels during cocaine self-administration (Sizemore et al. 2000). Moreover, in animals extinguished from cocaine self-administration, a reduction in basal extracellular GABA levels was observed in the ventral pallidum, while the reinstatement of cocaine seeking was associated with an augmentation of such effects (Tang et al. 2005). Changes in dopamine and GABA neurotransmission during the maintenance phase mirror the motivational aspects of cocaine intake. Apart from dopamine and GABA, cocaine also regulates glutamate extracellular levels with small increases at a few isolated time points during the self-administration sessions (Sizemore et al. 2000; McFarland et al. 2003; Madayag et al. 2007; Miguéns et al. 2008) as well as decreases in basal extracellular levels of nucleus accumbens glutamate (Wydra et al. 2013). Such glutamatergic inputs are believed to be critical for the learning and maintenance of goal-directed responding, including drug-seeking behavior (cf. Koob and Swerdlow 1988).

Metabolic Changes

In vivo studies on animals confirm human PET imaging data (see above) that amphetamines evoke serious functional alterations in glucose metabolism.

Thus, methamphetamine repeated treatment (1 mg/kg, every other day for 5 days) decreased 18-FDG uptake in the hippocampus and in the insular cortex in rats conditioned to receive methamphetamine in a distinct environment. Hypoactivation of glucose metabolism in the hippocampus, amygdala, and auditory cortex was also seen after acute MDMA (20 mg/kg or 40 mg/kg) to rats (Soto-Montenegro et al. 2007). On the other hand, another PET study indicated that rats showing strong drug-associated place preference displayed large increases in 18-FDG uptake in the dorsal and ventral striatum, cingulate cortex, and retrosplenial cortex which proves their involvement in the conditioned effects of amphetamines (Carrion et al. 2009).

Chronic use of cocaine is also associated with lasting alterations in brain metabolism. A recent study documents cocaine-induced changes in brain metabolic activity as a function of history of drug voluntary intake (Henry et al. 2010). The latter authors showed that acute *i.v.* cocaine treatment resulted in increases in glucose metabolism in the anterior cingulate and medial prefrontal cortex. Similar metabolic decrements were found by Lyons et al. (1996) who reported that an *i.v.* infusion of cocaine (1 mg/kg) markedly decreased glucose utilization in the orbitofrontal cortex, entorhinal cortex, nucleus accumbens, and parts of the hippocampal formation in monkeys; smaller decrements were observed in the caudate and anterior putamen, too. The progressive involvement of cortical and striatal areas as a function of extensive cocaine exposure has also been demonstrated in monkeys with [^{14}C]2-deoxyglucose and PET method (Lyons et al. 1996; Porrino et al. 2004). Initial exposures to cocaine resulted in metabolic effects of cocaine contained primarily in the ventromedial regions of the prefrontal cortex, while following chronic exposure to cocaine self-administration, glucose metabolism expanded mainly within the striatum.

Interestingly, changes in brain metabolism lasted even after long-term drug abstinence, as evidenced in the study with 28-day cocaine withdrawal in rats trained to self-administer cocaine where significant increases in the cerebral blood volume in the medial prefrontal cortex, caudate–putamen, motor cortex, and the pedunculopontine tegmental nucleus were detected (Chen et al. 2011).

Manifestation of Oxidative Stress

Supporting the role of oxidative stress in toxicity of amphetamines, several antioxidants (e.g., N-acetylcysteine, vitamin E) or overexpression of antioxidant enzymes (e.g., superoxide dismutase (SOD)) attenuates the neurotoxic action of amphetamines (Colado et al. 1997; Imam et al. 2001, 2000; Wan et al. 2006; Wu et al. 2007).

Manifestations of Epigenetic Mechanisms

Separate studies conducted on transgenic animals indicated that overexpression of histone deacetylase (HDAC)4 or HDAC5 decreases behavioral responses to cocaine (Kumar et al. 2005; Renthal et al. 2007), whereas genetic deletion of *Hdac5* enhanced mouse responses to the chronic effects of cocaine (Renthal et al. 2007).

3.2.2 Opiates

Behavioral Manifestation of Neurotoxicity

As evidenced in many papers, chronic exposure to opioids can lead to disturbances in cognitive functions and evoke anxiety in rodents. Impairments in learning and memory processes were detected in long-term memory consolidation in mice when heroin was administered after the learning sessions (Castellano 1975, 1980; Florian and Roullet 2004). Similar results were shown in discriminative avoidance paradigms – radial-, Y-, and plus-mazes and in the Morris water maze after chronic administration of morphine (Spain and Newsom 1991; Pu et al. 2002) and also in the Morris water maze following a single methadone dose administered 15 min before training to rats (Hepner et al. 2002) or chronic methadone in mice (Tramullas et al. 2007). Such memory impairment effects have been linked with opioid-induced neurotoxicity through activation of apoptotic pathways (see below).

Changes in Neurotransmission

With neuromolecular imaging it was documented that rats given passive single (10 mg/kg) or repeated (10 mg/kg) doses of morphine had significantly increased extracellular dopamine and 5-HT levels in the nucleus accumbens; the increases above 90 % in monoamine levels were seen already after a second dose of morphine. Decreases in dopamine neurotransmission after acute or repeated dosing of morphine, and still significantly lower on the 5th day of morphine abstinence, were detected in SPECT scans in monkeys (Xiao et al. 2006). Another effective toxic target related to morphine abuse is glutamatergic neurotransmission. Thus, in rodents chronic morphine use modifies extracellular concentrations of glutamate in the nucleus accumbens (Sepúlveda et al. 2004), prefrontal cortex, and hippocampus (Guo et al. 2005). Recently, spectroscopic analysis in rats showed that morphine administration (10 mg/kg) twice a day for one, six, or ten consecutive days resulted in a reduction in glutamate, with no statistically proven changes in glutamine, myoinositol, taurine, or N-acetylaspartate in the prefrontal cortex (i.e., 10 days of consecutive morphine treatment), while glutamate and myoinositol levels were elevated in the hippocampus (Gao et al. 2007). Other in vivo findings demonstrate short-lasting adaptations in neuronal glutamate relief (Nicol et al. 1996), synaptic glutamate uptake (Xu et al. 2003), expression of glutamate transporters (Mao et al. 2002), and neuronal activities of glutamate dehydrogenase and GABA transaminase (Kaur and Kaur 2001) following consecutive morphine administration.

4 Postmortem Evidences of Neurotoxicity

4.1 Human Studies

4.1.1 Psychostimulants

Changes in Neurotransmission

In humans several evidences of pathological changes have been observed in brain monoaminergic systems following abuse of amphetamines. Thus, postmortem

analyses in chronic methamphetamine users have shown a reduction in levels of dopamine nerve terminal markers in the caudate–putamen (Wilson et al. 1996; Moszczynska et al. 2004; Kitamura 2009) and reductions in 5-HT transporter levels in the orbitofrontal and occipital cortices (Kish et al. 2009). Moreover, VMAT-2 levels were remarkably reduced in some methamphetamine users, which may indicate a neurotoxic action of the drug resulting from disturbances in striatal dopamine terminal integrity (Kitamura 2009).

Regarding MDMA neurotoxicity in human addicts, there are some proof showing that MDMA led to the severe reduction of striatal 5-HT and 5-HIAA, assessed shortly (21-h) postmortem in the brain of an MDMA user who had taken MDMA regularly for 9 years (Kish et al. 2000), while another high-dose MDMA user had diminished 5-HT transporter level and TH activity in the brain (Kish et al. 2010).

For the development of amphetamine neurotoxicity to dopamine and 5-HT terminals, the occurrence of hyperthermia seems to be important. Physical activity, like dancing in the clubs or even staying in a warm environment which prevents proper heat dissipation, contributes significantly to the general morbidity and mortality in MDMA (Milroy et al. 1996; Armenian et al. 2012; Parrott 2012) or methamphetamine (Ishigami et al. 2003; Ago et al. 2006) users. Disruption of blood–brain barrier permeability and brain edema development in autopsy cases after acute fatal intoxication of methamphetamine (Ago et al. 2006; Inoue et al. 2006) or MDMA (Milroy et al. 1996) were found. Findings from forensic autopsy cases indicate that intake of a lethal dose of methamphetamine ($N = 11$) with the occurrence of hyperthermia immediately before death resulted in the 72-kDa heat shock protein (HSP72) expression in the microvessels and ependymal cells in the hippocampus (Kitamura 2009).

Long-term cocaine exposure in human addicts leads to neurotoxic effects on dopamine neurons seen as reductions in the total number of striatal dopamine D_2 receptors (Little et al. 2009). Other necropsy findings on cocaine abusers revealed serious dysfunction of monoaminergic systems, seen as decreases in the levels of VMAT-2 (Little et al. 2003) or an elevation of α-synuclein expression in midbrain dopamine neurons together with an increase of the 5-HT transporter in the striatum and the limbic system (for a review, see Büttner 2011). Elevation in α-synuclein expression may indicate increases in synaptic vesicle trafficking or pools, or enhancement in neuronal plasticity, or even selective loss of dopamine neurons (reviewed in Trexler and Rhoades 2012).

Activation of Microglia

Some postmortem studies in active methamphetamine users point to a significant increase in the number of microglia throughout the caudate–putamen, although such an increase neither paralleled immunohistochemical evidence for reactive microgliosis (Kitamura 2009) nor proliferation of the marker of reactive microglia CR3.43 (human complement receptor 3) (Kitamura et al. 2010). Also, long-term cocaine exposure by human addicts evokes infiltration of phagocytic cells (activated macrophages and activated microglia) within the caudate–putamen (Little et al. 2009). Of note, cocaine-evoked activation of microglial cells releasing

proinflammatory cytokines in the white matter and subcortical regions seems to be related to damage of neuronal cells (cf. Büttner 2011).

Manifestation of Oxidative Stress

The formation of reactive species (RS) and the resulting oxidative and/or nitrosative stress is a common toxicological pathway of amphetamines, and this plays an important role in the neurotoxicity observed in the brain after chronic exposure to the psychostimulants in human addicts (Mirecki et al. 2004; Fitzmaurice et al. 2006; Carvalho et al. 2012). Thus, a significant increase in levels of 4-hydroxynonenal and malondialdehyde (MDA), being products generated from lipid peroxidation, was found in the caudate nucleus and to a smaller extent in the frontal cortex in autopsied brains of chronic methamphetamine users (Fitzmaurice et al. 2006). Such increases in lipid peroxidation products were linked with a depletion in GSH concentration and with an enhancement in the levels of Cu, Zn-SOD activity in the caudate of methamphetamine users (Mirecki et al. 2004). These data suggest that methamphetamine exposure in humans leads to above-normal formation of potentially toxic lipid peroxidation products in the brain, while increased Cu,Zn-SOD activity seems to reflect possible compensatory response to oxidative stress due to amphetamine administration.

Manifestations of Apoptosis

Up to now, there is no definitive proof that apoptosis accounts for psychostimulant neurotoxicity in human addicts. Thus, a necropsy analysis on the striatum of chronic METH users ($N = 11$) showed a lack of caspase-3 activation (Kitamura et al. 2007). Another very recent postmortem human brain report by Alvaro-Bartolome et al. (2011) on cocaine abusers ($N = 10$) demonstrated also no activation of extrinsic and intrinsic apoptotic pathways in the prefrontal cortex and lack of activation of caspase 3 while the Fas-associated death domain (FADD) receptor complex and cytochrome c were downregulated. These authors showed also enhanced degradation of nuclear PARP-1, a hallmark of apoptosis, which was postulated to result from the formation of reactive oxygen species (ROS) by cocaine metabolites due to the induction of contraregulatory adaptations or non-apoptotic (neuroplastic) actions by the psychostimulant.

Manifestations of Genomic and Epigenetic Mechanisms

An early report on brains from humans with a history of cocaine abuse showed marked reductions in the levels of enkephalin mRNA and mu opiate receptor binding within the caudate and putamen, while levels of dynorphin mRNA and kappa opiate receptor binding in the putamen and caudate were elevated (Hurd and Herkenham 1993). As suggested by the latter authors, an imbalance between euphoric (enkephalin and mu opiate receptors) and dysphoric (dynorphin and kappa opiate receptors) markers may be linked with craving phenomenon detected in the human cocaine addict brain.

More recent microarray analysis showed alterations in brain gene expression in the postmortem anterior prefrontal cortex from human cocaine abusers

(Lehrmann et al. 2006). Thus, the drug-specific changes included reduction in calmodulin-related transcripts (CALM1, CALM2, CAMK2B), while transcripts related to cholesterol biosynthesis and trafficking (FDFT1, apolipoprotein L = APOL2, SCARB1) and Golgi/endoplasmic reticulum functions (semaphorin 3B = SEMA3B, GCC1) were increased. As these changes represent common molecular features of drug abuse, they may underlie changes in synaptic function and plasticity that could have important ramifications for decision-making capabilities in drug abusers (cf. Lehrmann et al. 2006). Another gene array analysis in brains from cocaine abusers detected altered expression of some myelin-related genes in the nucleus accumbens, while in situ hybridization to quantify transcript expression of these genes showed only decreased expression of the transcript encoding PLP1. PLP1 is expressed at very high levels in oligodendrocytes and is essential in maintaining stability of myelin sheets, thus implicating adaptive changes following chronic cocaine abuse. Of note, postmortem expression of claudin-11 and transferrin was decreased in the caudate and internal capsule, respectively, in cocaine addicts (Kristiansen et al. 2009).

There are some early evidences about the epigenetic mechanisms due to psychostimulant intake. Chronic cocaine abuse induces alterations in gene expression in the nucleus accumbens of humans (Albertson et al. 2004). Microarray analysis revealed further that expression of cocaine- and amphetamine-regulated transcript (CART) was increased, but some myelin-related genes were decreased. Another study on human postmortem brain revealed transcription and epigenetic changes in the hippocampus (Zhou et al. 2011) with suppression of more than one third of genes responsible for ATP synthesis and increases of the transcript level of DNA methyltransferase (DNMT)3a, cAMP response element binding (CREB)1, HDAC2, and HDAC4. In addition, changes in H3K4me3 distribution were noted near promoters of active genes and away from promoters of protein-coding genes.

4.1.2 Opiates

Changes in Neurotransmission

Chronic opiate exposure impairs brain monoaminergic neurotransmission in human addicts. As evidenced in postmortem nucleus accumbens of 9 chronic heroin users, levels of TH protein and dopamine were reduced. On the other hand, striatal levels of the VMAT and 5-HT were normal (Kish et al. 2001).

Changes in Protein Levels

A recent postmortem report indicated that in the hippocampi of 29 lethally intoxicated heroin addicts, there was a significant increase in polysialic acid neural cell adhesion molecule, being one of the key players involved in plasticity and regeneration, of neural-tissue-positive neurons and glial cells, possibly reflective of an attempt to repair cell damage due to long-term heroin neurotoxicity and impaired plasticity (Weber et al. 2006). This may account for cognitive deficits seen in human addicts (see above).

In the brain of young opiate abusers ($N = 34$, all <40 years age), an excess of AT 8-positive neurofibrillary tangles was found in the subiculum, temporal neocortex,

nucleus basalis of Meynert, and the locus coeruleus, while beta-amyloid precursor protein is increased in the brain stem and temporal lobe (Ramage et al. 2005). The above changes may be risk factors for poorer performance in cognition and memory in opiate addicts; however, the direct proof is still lacking.

Another study performed on brains of chronic opiate addicts ($N = 17$) who had died of a heroin or methadone overdose showed a marked (47 %) reduction in levels of immunoreactive NF-L proteins in the frontal cortex (García-Sevilla et al. 1997); this change was considered as a specific damage of axonal transport resulting from long-term opiate-evoked neuronal damage.

Manifestation of Oxidative Stress

A study performed on samples of 17 human brains obtained 2–4 days after heroin users' death indicates high protein oxidation levels in all lobes of the cortex, brain stem, hippocampus, and white matter and lower peroxyl radical-trapping capacity – a marker reflective of the relationship between ROS amount and antioxidant efficiency (Gutowicz et al. 2006). The latter authors found an elevation of MDA levels, a lipid peroxidation product, in all parts of the brain except the hippocampal formation. Despite the above evidence for neurotoxicity, there was no reduction in the activity of antioxidant enzymes such as SOD or catalase (CAT) (Gutowicz et al. 2006).

Manifestations of Epigenetic Mechanisms

A significant change in the expression of numerous genes encoding proteins involved in presynaptic release of neurotransmitter was reported by Albertson et al. (2006) in heroin abusers. Thus, the transcript encoding the endogenous opioid peptide prodynorphin (*PDYN*) was decreased, while the neurotrophin receptor *NTRK2* (also known as *TrkB*) was significantly upregulated in heroin abusers. In addition, alterations in many genes involved in processes related to neurotransmitter release and recycling, including SNARE proteins, vesicular proteins, and recycling proteins, imply synaptic dysfunction in the brains of human heroin abusers. A very recent report by Michelhaugh et al. (2011) identified alterations of long noncoding RNA (lncRNA)s (such as MIAT, MEG3, NEAT1, and NEAT2) in heroin abusers compared to control subjects.

4.2 Animal Studies

4.2.1 Psychostimulants

Changes in Neurotransmission

Amphetamines produce long-term decreases in markers of biogenic amine neurotransmission (mainly dopamine and 5-HT), and these neurotoxic effects include the decrease in monoamine levels and their major metabolites, reduced level of monoamine transporter binding sites, diminished expression and/or activity of enzymes involved in the metabolism, and synthesis of brain monoamine neurotransmitters – evident in rodents and nonhuman primates

(Yamamoto et al. 2010; Carvalho et al. 2012). Thus, dopamine brain system impairments relate to decreased level of the neurotransmitter (Wagner et al. 1980; Quinton and Yamamoto 2006), the dopamine metabolite, homovanillic acid HVA (Preston et al. 1985), reduced TH activity (Ellison et al. 1978; Hotchkiss and Gibb 1980), and a lower density of the dopamine transporter (Wagner et al. 1980; Krasnova et al. 2001) following high (usually 5–15 mg/kg in rats) doses and/or longitude methamphetamine/amphetamine administration in rodents (Morgan and Gibb 1980; Ricaurte et al. 1982; Gouzoulis-Mayfrank and Daumann 2009) or nonhuman primates (Preston et al. 1985; Woolverton et al. 1989). In a model of a prevalent human methamphetamine exposure, produced by daily multiple doses in monkeys (escalation dosing of methamphetamine over 33 weeks, with final dosages resulting in estimated peak plasma methamphetamine concentrations of 1–3 μM), postmortem analysis of brains showed persistent reductions in striatal dopamine levels and dopamine transporter binding associated with neurotoxicity in presynaptic dopamine system integrity (Melega et al. 2008). Similar long-term reductions in brain dopamine axonal markers were observed in baboons treated with methamphetamine (0.5–2 mg/kg, *i.m.*, 4 times at 2 h intervals) (Villemagne et al. 1998).

On the other hand, the long-term (1.5 years) exposure to self-administered cocaine in monkeys evokes significant increases in dopamine transporter binding sites at the caudal level of the subcortical areas (the ventral caudate, ventral putamen, and nucleus accumbens) (Letchworth et al. 2001). The latter finding – showing similarity to studies in human cocaine addicts – suggests importance of this biomarker to the neurobiological effects of cocaine. Other studies on monkey i.v. self-administered cocaine for 18–22 months showed lowered dopamine D_2 binding site density in the striatum (Moore et al. 1998); these data parallel studies on rodents and humans (see above).

The neurotoxic effects of amphetamines also apply to 5-HT-ergic nerves in the brain. Methamphetamine, although more toxic to DA neurons, leads also to 5-HT system impairments including (1) depletion of 5-HT and its metabolite 5-HIAA levels, (2) diminished tryptophan hydroxylase activity in the striatum and hippocampus, and (3) lower 5-HT transporter densities in rat frontal cortex, amygdala, and striatum (Hotchkiss and Gibb 1980; Ricaurte et al. 1980; Gouzoulis-Mayfrank and Daumann 2009). Another amphetamine derivate, MDMA, causes mainly 5-HT system impairments in rats or nonhuman primates (Quinton and Yamamoto 2006; Gouzoulis-Mayfrank and Daumann 2009; Carvalho et al. 2012) with reduction of 5-HT and 5-HIAA levels, decreased activity of tryptophan hydroxylase, and a diminished density of 5-HT transporter in rat cortical and subcortical brain areas (Battaglia et al. 1987; Itzhak and Achat-Mendes 2004; Cadet et al. 2007; Carvalho et al. 2012). Nevertheless, in mice, MDMA causes primarily dopaminergic neurotoxicity (Green et al. 2003), which demonstrates that not every species has the same sensitivity to amphetamines' actions. According to many studies, nonhuman primates seem to be more sensitive to the neurotoxic effects of MDMA than rodents, resulting in higher rates of 5-HT depletion with smaller doses of MDMA, and persisting hypoinnervation patterns in most neocortical

regions and the hippocampus examined even 7 years posttreatment (Ricaurte et al. 1992; Gouzoulis-Mayfrank and Daumann 2009). In fact, 18 months of MDMA self-administration in primates produced 40–50 % depletion in 5-HT levels in the frontal, parietal, and temporal cortex, while lesser evoked downward trends in 5-HT depletions (approximately 25 %) were apparent in the occipital cortex, thalamus, and hypothalamus (Fantegrossi et al. 2004).

Methamphetamine was found to increase extracellular glutamate level followed by *N*-methyl-D-aspartic acid (NMDA) receptor activation and subsequent increase in intracellular Ca^{2+} concentration (Cunha-Oliveira et al. 2008). As described earlier, a rise in the Ca^{2+} concentration leads to the massive production of nitric oxide that in reaction with O_2 forms $ONOO^-$ (Capela et al. 2009); both NO and $ONOO^-$ lead to nitration of proteins including biosynthetic enzymes of dopamine and 5-HT (Kuhn and Arthur 1997; Kuhn et al. 1999; Carvalho et al. 2012). As hypothesized by Yamamoto et al. (2010), the oxidation and nitration of proteins associated with dopamine and 5-HT terminals appear to be the main mechanisms by which the amphetamine-evoked neurotoxicity arises.

Several studies have addressed the influence of hyperthermia following administration of amphetamines on neurotoxicity in animals. As found, methamphetamine-induced hyperthermia may be a factor increasing the drug-induced neurotoxicity, but it is not a necessary and sufficient condition in neurotoxicity induced by methamphetamine (Kita et al. 2003). Furthermore, hyperthermia might interact with other known mediators of methamphetamine neurotoxicity, such as increased glutamate neurotransmission, to enhance toxicity (see above).

Metabolic Changes

Some changes have been found in brain energy metabolism in rodents after amphetamine treatment. For example, subchronic amphetamine treatment (12–15 mg/kg/day for 7 days followed by 7-daily injections with 6–7 mg/kg/day) in rats caused enhanced local cerebral glucose utilization in the nucleus accumbens (Orzi et al. 1983). Increases in glucose metabolism were reported also after acute amphetamine administration (1–5 mg/kg) in extrapyramidal motor systems in rats (Wechsler et al. 1979; Porrino et al. 1984). Conversely, methamphetamine administration (4 × 12.5 mg/kg, every 2 h) decreased local cerebral glucose utilization in the extrapyramidal system, also in the hippocampus and dorsal raphe nucleus in rats (Huang et al. 1999). These findings clearly demonstrate that amphetamines lead to regional, drug-dependent, and regimen-dependent alterations in brain metabolism.

Morphological Changes

There are several proofs that chronic treatment with psychostimulant drugs of abuse results in neuronal degeneration. Thus, in rats treated continuously with amphetamine (e.g., pellets implanted *s.c.* releasing amphetamine for 10 days) or methamphetamine (e.g., 10 mg/kg × 4 times with 2 h intervals or 15 mg/kg *s.c.* every 6 h for 24 h), anatomical alterations in the form of swollen, dystrophic, or fragmented dopamine axons in the striatum have been found (Ellison et al. 1978; Hanspeter 1981; Ricaurte et al. 1982; Broening et al. 1997). Degenerations of presynaptic

5-HT axon terminals induced by high and/or repeated doses of methamphetamine (Axt and Molliver 1991; Cadet et al. 2003; McCann and Ricaurte 2004) were found throughout the forebrain of rats (O'Hearn et al. 1988), while in squirrel monkeys a reduction in 5-HT innervation was seen 7 years after exposure to the drug (Hatzidimitriou et al. 1999).

Glia and Microglia Activation

As a consequence of amphetamine intake, damage of striatal axonal terminals was accompanied by reactive astrocytosis (Bowyer et al. 1994) and microglial activation (Pubill et al. 2002) via an increase in the expression of proinflammatory molecules (e.g., proinflammatory cytokines, interleukins). Interestingly, even an acute exposure to methamphetamine increased mRNA levels of IL-6, IL-1α, and tumor necrosis factor (TNF)-α in mouse striatum (Sriram et al. 2006; Gonçalves et al. 2008). Neurotoxic methamphetamine exposure (4 times × 10 mg/kg, every 2 h, or 24 times × 1.67 mg/kg once every 15 min) resulted in an increase in glial fibrillary acidic protein level 3 days posttreatment (Herring et al. 2008). Methamphetamine alone or its metabolites might activate glial cells by virtue of elevated cellular dopamine auto-oxidation, resulting in profound formation of dopamine o-quinones, strong microglial activators (Kuhn et al. 2006; Yamamoto et al. 2010; Carvalho et al. 2012).

The ability of MDMA to induce microglial activation is more ambiguous. Thus, microgliosis induced by MDMA administration was found in the male (but not female) mouse striatum and was absent in the rat cortex or striatum. MDMA injections increased also production of the proinflammatory cytokine IL-1β in rat frontal cortex (Orio et al. 2004), while the *i.c.v.* administration of IL-1β intensified MDMA-induced 5-HT toxicity in the cortex. On the other hand, MDMA has been shown to have an immunosuppressive power that involves an increase in IL-10 production, an anti-inflammatory cytokine (Yamamoto et al. 2010).

Manifestations of Oxidative Stress

There is reliable evidence of oxidative damage in animal brain after repeated exposure to amphetamines (see Table 1). Thus, amphetamine, methamphetamine, or MDMA do increase lipid peroxidation (Jayanthi et al. 1998; Gluck et al. 2001; Alves et al. 2007; Miranda et al. 2007) and induce various changes in the levels of GSH (Harold et al. 2000; Carvalho et al. 2001; Frenzilli et al. 2007; Miranda et al. 2007) or activity of several antioxidant enzymes (Jayanthi et al. 1998; Frey et al. 2006; Frenzilli et al. 2007; Miranda et al. 2007), providing evidences for the importance of RS in amphetamine-induced toxicity.

Interestingly, the bidirectional changes in brain SOD activity were demonstrated after amphetamine or its derivatives. For example, subchronic amphetamine administration caused a rise in the SOD activity in rat hippocampus (Frey et al. 2006), while MDMA led to the drop in SOD activity in mouse hippocampus (Frenzilli et al. 2007). The stronger oxidative potential following MDMA derives from its highly reactive quinone intermediate metabolites (cf. Capela et al. 2007). Production of RS appears to be linked in large part with amphetamine-induced thermal instability

Table 1 Preclinical evidence of oxidative stress after amphetamines exposure

Model treatment	Animal	Dose and treatment regimen	Biomarker	Change (brain structure)	Reference
D-Amph, chronic passive administration	Rat	1 mg/kg, 2 mg/kg or 4 mg/kg (*i.p.*) for 7 days	SOD	↑(HIP), ↓(STR) for 1 mg/kg	Frey et al. (2006)
				↑(HIP), ↓(STR) for 4 mg/kg	
			CAT	↑(PFCx), ↑(HIP) for 4 mg/kg	
				↓(HIP), ↓(STR) for 2 mg/kg	
D-Amph, chronic passive administration	Rat	20 mg/kg (*s.c.*) for 14 days	GST	↑(HL)	Carvalho et al. (2001)
			GPx	↑(STR, NAc, mPFCx)	
			GR	↑(HYP, PFCx)	
			CAT	↑ (PFCx)	
METH, acute passive administration	Mouse	1 mg/kg (*i.p.*), 4 × every 2 h	SOD	↓(FCx)	Jayanthi et al. (1998)
			CAT	↓(STR)	
			GPx	↓(FCx, STR)	
			MDA	↑(FCx, STR)	
METH, binge passive administration	Rat	10 mg/kg (*i.p.*), 4 × every 2 h	MDA	↑(STR)	Yamamoto and Zhu (1998)
METH, binge passive administration	Rat	20 mg/kg (*i.p.*), 4 × every 2 h	GSH	↓(STR)	Moszczynska et al. (1998)
			GPx	Ø (STR)	
			GR	Ø (STR)	
			GGTP	Ø (STR)	
			G6PDH	Ø (STR)	
METH, binge passive administration	Rat	10 mg/kg (*i.p.*), 4 × every 2 h	GSH	↑(STR)	Harold et al. (2000)
			GSSG	↑(STR)	
METH, binge passive administration	Mouse	10 mg/kg (*i.p.*), 4 × every 2 h	3-NT	↑(STR)	Imam et al. (2000)
METH, binge passive administration	Mouse	10 mg/kg (*i.p.*), 4 × every 2 h	PC	↑(STR, HIP)	Gluck et al. (2001)

(*continued*)

Table 1 (continued)

Model treatment	Animal	Dose and treatment regimen	Biomarker	Change (brain structure)	Reference
MDMA, binge passive administration	Adolescent rat	10 mg/kg (*i.p.*), 4 × every 2 h	MDA	↑ (Mitochondrial fraction of whole brain)	Alves et al. (2007)
			PC	↑	
MDMA, subchronic passive administration	Mouse	2.5 mg/kg (*i.p.*), for 5 days	GSH	↓(HIP)	Frenzilli et al. (2007)
			SOD	↓(HIP)	
MDMA, subchronic passive administration	Rat	5 mg/kg (*i.p.*), for 7 days	GPx	↓(HIP)	Miranda et al. (2007)
			GSH	Ø (HIP)	
			MDA	↑ (HIP)	

Abbreviations: ↑ increase, ↓ decrease, Ø no effect, D-Amph D-amphetamine, *CAT* catalase, *FCx* rontal cortex, *G6PDH* glucose-6-phosphate dehydrogenase, *GGTP* γ-glutamyltranspeptidase, *GSH* glutathione, *GSSH* reduced glutathione, *GST* glutathione-*S*-transferase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *HIP* hippocampus, *HYP* hypothalamus, *LPO* lipoperoxides, *MDA* malondialdehyde, *MDMA* 3,4-methylenedioxy-*N*-methylamphetamine, *METH* methamphetamine, *NAc* nucleus accumbens, *3-NT* 3-nitrotyrosine, *PFCx* medial prefrontal cortex, *PC* protein carbonyls, *SOD* superoxide dismutase, *STR* striatum

Table 2 Preclinical evidence of oxidative stress after cocaine exposure

Model treatment	Animal	Dose and treatment regimen	Biomarker	Change (brain structure)	Reference
Chronic passive administration	Rat	20 mg/kg *(i.p.)* for 10 days	GPx	↑(FCx, STR), Ø(CER)	Dietrich et al. (2005)
			SOD	↑(FCx), Ø(STR)	
			LP	↑(FCx, STR), Ø(CER)	
Chronic passive administration	Rat	15 mg/kg *(i.p.)* for 20 days	GSH	↓(HIP)	Muriach et al. (2010)
			GPx	↓(HIP)	
Chronic passive administration	Rat	15 mg/kg *(i.p.)*, *2 x in* 1 and 7 days; 30 mg/kg *(i.p.)* for 2–6 days	GSH	↓(NAc)	Uys et al. (2011)
Subchronic passive administration	Prenatal rat	20 mg/kg *(i.p.)* for 4 days	MDA	↑(FCx, HIP)	Bashkatova et al. (2006)
Chronic self-administration and extinction model	Rat	15–18 mg/kg/day *(i.v.)* +10-day extinction	SOD	↑(STR, FCx, HIP)	Pomierny-Chamiolo et al. (2012)
			MDA	↓(STR, FCx, HIP)	
			SOD	↑(STR, FCx, HIP)	
			MDA	↑(STR, FCx, HIP)	
Acute administration	Rat	30 mg/kg *(i.p.)*	MDA	↑(PFCx, NAc)	Numa et al. (2008)
Acute administration	Mouse	10 mg/kg *(i.p.)* 30 mg/kg *(i.p.)* 90 mg/kg *(i.p.)*	CAT	↓(STR), Ø(PFCx)	Macedo et al. (2005)
				↓(STR), Ø(PFCx)	
				↓(STR), ↓(PFCx)	
Acute administration	Rat	90 mg/kg *(i.p.)*	GSH	↑(PFCx, STR)	Macedo et al. (2010)
			MDA	↑(PFCx, STR)	

Abbreviations: ↑ increase, ↓ decrease, Ø no effect, *CER* cerebellum, *FCx* frontal cortex, *GPx* glutathione peroxidase, *GSH* glutathione, *HIP* hippocampus, *LP* lipid peroxidation, *MDA* malondialdehyde, *NAc* nucleus accumbens, *PFCx* prefrontal cortex, *SOD* superoxide dismutase, *STR* striatum

(Krasnova et al. 2001) in animals and in humans, as hyperthermia – particularly if ambient temperature is high – promotes greater RS formation and associated neurotoxicity (Green et al. 2003; Quinton and Yamamoto 2006; Parrott 2012).

Cocaine likewise was found to promote oxidative stress in the brain of experimental animals (see Table 2), and it was recorded as a significant elevation in SOD, glutathione peroxidase (GPx), and CAT activity in the rat cortex and striatum (Dietrich et al. 2005; Macedo et al. 2005, 2010); however, changes in brain CAT were not validated in other studies (Macedo et al. 2005; Muriach et al. 2010). In the hippocampus GSH concentration and GPx activity were reduced in repeatedly cocaine-treated rats; such changes were not evident in the frontal cortex (Muriach et al. 2010). Cocaine intoxication led also to an increase in lipid peroxidation in several brain structures linked to dopamine synthesis and release

(Dietrich et al. 2005; Bashkatova et al. 2006). A very recent report indicates that voluntary cocaine intake (by *i.v.* drug self-administration) evokes a significant reduction in MDA level together with increases in SOD activity in the hippocampus and frontal cortex, while an enhancement in the SOD activity was not seen in yoked cocaine animals (Pomierny-Chamiolo et al. 2012). As suggested, enhanced RS production and oxidative cell status may occur via cocaine or its oxidized metabolites (e.g., norcocaine derivatives; Kovacic 2005).

As stated above, psychostimulants prompt an increase in RS level via enhanced synaptic dopamine concentration or by its oxidation by nonenzymatic or enzymatic pathways (see above). In the mechanisms of drug dependence and neurotoxicity, glutamatergic neurotransmission is strongly involved. In cocaine addiction an increased level of extracellular glutamate concentration in dopamine-innervated areas (Cunha-Oliveira et al. 2008) could be associated with excitotoxic processes through increased activation of NMDA receptors and subsequent increase in intracellular Ca^{2+} level (Rego and Oliveira 2003), leading to the formation of mitochondrial RS and lipid peroxidation (Maciel et al. 2001).

Antioxidant status after chronic exposure may be different than effects of acute exposure due to maladaptations seen in neurotransmitter levels and in antioxidant defense mechanisms that maintain cellular homeostasis (see Table 1 for specific changes in a brain region, drug type, and dosage regimen).

Manifestation of Apoptosis

Several preclinical studies have reported apoptotic features after chronic or even acute exposure to psychostimulants, especially amphetamine derivatives (Cunha-Oliveira et al. 2008). For example, repeated amphetamine administration in mice (4 × 10 mg/kg, every 2 h) caused an increase in p53 protein levels in medium spiny striatal projection neurons with a secondary increase in Bax (proapoptotic gen) and decrease in Bcl-2 (anti-death gene) levels in mouse striatum (Krasnova et al. 2005). Neurotoxicity due to activation of apoptotic pathways has been shown with acute drug treatments. Thus, acute methamphetamine (40 mg/kg, *i.p.*) resulted in an activation proapoptotic Bcl-2 family genes (Bax, Bad and Bid) in mice that was accompanied by a decrease in anti-death genes Bcl-2 and Bcl-X in the frontal cortex (Jayanthi et al. 2001). The same methamphetamine treatment resulted in the release of AIF and smac/DIABLO from the mitochondria in mouse striatum (Jayanthi et al. 2004), followed by activation of the caspase cascade (caspase 9 and 3) (Deng et al. 2002; Jayanthi et al. 2004). Similarly to methamphetamine, acute MDMA exposure caused upregulation of Bax and downregulation of Bcl-2 in the rat hippocampus and cerebellum and respective changes in genes relating to those proteins (Soleimani Asl et al. 2011, 2012). The latter findings suggest that MDMA neurotoxicity can be linked to stimulation of the intrinsic apoptosis pathway. Of note, apoptotic biomarkers were evident also in rat cortical neurons following MDMA (Capela et al. 2006).

In addition to the above mitochondrial apoptosis pathway, acute high-dose (40 mg/kg, *i.p.*) methamphetamine was found to activate cell death via

dysregulation of calcium homeostasis in mice (Jayanthi et al. 2004), initiating calcium-dependent apoptosis via the permeabilization of the outer mitochondrial membrane (Kroemer et al. 2007). Furthermore, acute methamphetamine injection with 20–40 mg/kg to rodents caused activation of calpain – a calcium responsive cytosolic cysteine protease (Jayanthi et al. 2004; Samantaray et al. 2006). In line with the latter observations, proteolysis of the cytoskeletal scaffolding protein spectrin, consequent to calpain activation, has been shown in many studies in rat brain limbic and subcortical structures following methamphetamine exposure (Warren et al. 2005; Staszewski and Yamamoto 2006). Finally, toxic doses of acute methamphetamine (40 mg/kg, *i.p.*) have been shown, in rat striatum, to induce the transcription factors AP-1, Egr, and Nur77 (Jayanthi et al. 2005), which regulate Fas ligand (FasL) expression (Droin et al. 2003; Tóth et al. 2001). Altogether, the literature findings indicate several factors in the amphetamine-related cell death in rodent brain, including activation of either the mitochondria-dependent apoptotic pathway or activation of calpain, or Fas death pathway, or p53 death pathway.

On the other hand, morphological changes of apoptosis induced by cocaine have not been evident (Cunha-Oliveira et al. 2008). As recently reported, chronic cocaine, including its 3-day withdrawal, altered neither Fas–FADD receptor complex, cytochrome c, caspase 3, AIF, and PARP-1 cleavage nor associated signaling in the cerebral cortex. However, some discrete changes (a 28 % decrease in the content of antiapoptotic truncated isoform of dopamine- and cAMP-regulated phosphoprotein of 32 kDa) in rat brain cortex appeared (Alvaro-Bartolomé et al. 2011).

Manifestations of Genomic and Epigenomic Actions

The regulation of changes in gene expression evoked by psychostimulant drugs of abuse is an area of intensive research (Cadet and Krasnova 2009; Piechota et al. 2012). Altered gene expression [e.g., brain-derived neurotrophic factor (BDNF), G-protein signaling 3] in drug addiction is reported in the prefrontal cortex, ventral tegmental area, and striatum (including the nucleus accumbens) (Wong et al. 2011). Another long-term molecular effect relates to upregulation of the cAMP pathway in the nucleus accumbens and dorsal striatum – partially mediated by CREB. Dopamine D_1 receptor stimulation during chronic cocaine administration induces activation of transcription factor CREB, which has many gene targets including BDNF, c-Fos, adenylyl cyclase, corticotropin-releasing factor, dynorphin, and enkephalins (Cunha-Oliveira et al. 2008). A recent study in mice, using whole-genome microarray profiling to evaluate the detailed time course of transcriptional alterations, identified 27 genes (i.e., circadian genes Per1, Per2, and Nr1d1; the glucocorticoid-dependent genes Fkbp5, Sult1a1, and Plin4; the drug-regulated group of transcripts enriched in the nucleus accumbens Pdyn, Cartpt, and Rgs2; as well as new genes Fam40b and Inmt) under regulation by chronic methamphetamine administration (Piechota et al. 2012). As suggested, the above transient gene expression alterations during drug treatment – seen also in the early period of withdrawal – may be contributors to persistent neuroplastic alterations responsible for the development of drug addiction.

A number of findings suggest that epigenetic changes may mediate neuroplasticity in psychostimulant addiction (Kumar et al. 2005; Levine et al. 2005; Romieu et al. 2008; Im et al. 2010; Maze et al. 2010). A recent study has demonstrated that amphetamine induced phosphorylation of methyl CpG binging protein (MeCP2) at Ser421 in mouse accumbal areas, leading to increases in protein activity and enhanced repressive function (Deng et al. 2010). Findings by Renthal and Nestler 2008 show that acute exposure to amphetamine results in ΔFosB binding to the *c-fos* promoter and in repression of its expression through recruitment of HDAC1 and histone methyltransferase (HMT) around the promoter site, leading to downregulation of the gene expression. The same authors showed that chronic amphetamine abuse increases histone H3K9me2 on the *c-fos* promoter, further substantiating its transcriptional silencing (Renthal and Nestler 2008). As suggested, interaction between ΔFosB and *c-fos* may serve as a homeostatic mechanism for the regulation of sensitivity to repeated drug treatment. Other studies indicated global increase in histone H4 acetylation and specific *fosB* promoter-associated H4 hyperacetylation in mouse striatum after repeated amphetamine treatment (Shen et al. 2008). Moreover, co-treatment of amphetamine and HDAC inhibitors (butyric acid and valproic acid) had additive effects on the increase in histone H4 acetylation (Shen et al. 2008; Adachi and Monteggia 2009). The latter study seems to support the notion that amphetamine functions as an HDAC inhibitor, suggesting that HDACs could be used therapeutically for the modulation of psychostimulant action. Acute amphetamine administration provokes an increase in the number of neurons positive for phosphorylation of histone H3 in serine 10 (pH3S10) in a few telencephalic and diencephalic rat brain regions (Rottlant and Armario 2012).

Acute methamphetamine exposure decreased DNMT1 and DNMT2 in the rat hippocampus (Numachi et al. 2004), while bidirectional changes for DNMT1 were observed in the nucleus accumbens in two rat strains (Numachi et al. 2007) following subchronic methamphetamine exposure. The fact that Lewis rats are more susceptible than Fisher rats to methamphetamine sensitization may be reflective of different DNA methylation patterns (Numachi et al. 2007; Kovatsi et al. 2011). Interestingly, a single methamphetamine injection (20 mg/kg) induced time-dependent modifications in the acetylation of histone H3 and H4 as well as increases in AFT2 (enzymes that have HAT activity for histone H4) and HDAC2 expression in rat nucleus accumbens (Martin et al. 2012). Martin and coworkers demonstrated methamphetamine-induced increases of histone H4K5 and H4K8 acetylation as well as decreases in the acetylation of histone H3K9, H3K16, and H3K18. These results suggest that the psychostimulant effect mediated by increased AFT2 and HDAC2 expression may relate to effects on histone acetylation and hypoacetylation. The most recent study has indicated that repeated methamphetamine injections significantly decreased acetylation of histone H4 at lysine 5 (H4K5), lysine 12 (H4K12), and lysine 16 (H4K16) in rat dorsal striatum, at *GluA1* and *GluA2* DNA sequences encoded α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits (Cadet and Jayanthi 2013). Because these changes were seen with the enrichment of methyl CpG binding

protein 2 (MeCP2) at the promoters of both *GluA1* and *GluA2*, the results indicate that methamphetamine induced downregulation of *GluA1* and *GluA2* mRNA levels and did affect glutamatergic function.

Some limited data indicate amphetamines affect expression patterns of microRNAs (miRNA)s. Thus, Lippi and colleagues (2011) have shown that chronic treatment with amphetamine increases the level of *miR-29a/b* in mouse hippocampus. The *miR-29a/b* family directly targets the transcript of *Arpc3*, a subunit of the ARP2/3 actin nucleation complex involved in dendritic spine morphogenesis (Lippi et al. 2011). Current bioinformatic analysis has revealed that amphetamine increases levels of *miR-181a* in a mouse model of chronic drug treatment (Saba et al. 2012), linking epigenetic modification with synaptic plasticity. In fact, *miR-181a* is a negative posttranscriptional regulator of GluA2 expression, and thus it would be an essential regulator of mammalian AMPA-type glutamate receptors, a key determinant of synaptic plasticity.

There are some links between DNA methylation and cocaine addiction. First, cocaine self-administration increased MeCP2 expression in rat ventral and dorsal striatum (Cassel et al. 2006), while lentiviral knockdown of MeCP2 in dorsal striatum decreased drug intake under extended drug conditions (Im et al. 2010). Repeated cocaine administration altered DNMT3a, an enzyme linked to CpG methylation, expression in the mouse nucleus accumbens, with decreases seen during very early phases of withdrawal and sustained increases seen at later time points (LaPlant et al. 2010). Of note, prenatal cocaine exposure alters DNA methylation and gene expression in hippocampal neurons of neonatal and adolescent mice (Novikova et al. 2008).

A few reports point also to interaction between histone acetylation and cocaine. Thus, acute or chronic exposure to this psychostimulant increased cellular levels of H3 and H4 acetylation in the nucleus accumbens (Kumar et al. 2005; McQuown and Wood 2010), while repeated passive cocaine treatment downregulated dimethylation of histone 3 lysine 9 (H3K9me2), a marker of heterochromatin in the same brain area (Maze et al. 2010); this later effect paralleled a decrease in the total amount of heterochromatin and an increase in the volume of these nuclei. Increased H3 acetylation was also observed during cocaine self-administration and conditional place preference, while pharmacological inhibition of sirtuins (their induction is associated with H3 acetylation) reduced cocaine rewarding effects seen in conditioned place preference and self-administration (Renthal et al. 2009).

Findings from the past 3 years indicate that chronic cocaine regulates numerous miRNAs in mouse striatum (Schaefer et al. 2010; Eipper-Mains et al. 2011) as well miRNA-linked proteins that are strongly linked to drug addiction (Li and van der Vaart 2011). For example, cocaine self-administration in rats increased *miR-212* expression in the striatum that was suggested to be – at least partially – responsible for cocaine overconsumption through CREB, MeCP2, and BDNF signaling (Im et al. 2010). Another report identified induction of *miR-212* and *miR-132* in the dorsal striatum after 7 days of cocaine self-administration in rats (Hollander et al. 2010). On the other hand, chronic passive cocaine administration decreased *miR-124*

and increased *miR-181a* in rat brain (Chandrasekar and Dreyer 2009). *miR-124* overexpression in the nucleus accumbens reduced cocaine place conditioning, whereas overexpression of *miR-181a* had the opposite effect (Chandrasekar and Dreyer 2011). Recently, it was found that cocaine mediates the changes of miR-8 family expression in brain and basing on the biostatistical analyses, a suggestion of novel mechanisms for drug-induced alterations in the neuronal cytoskeletal and synaptic structure has been raised (Eipper-Mains et al. 2011). Interestingly, Schaefer et al. (2010) identified an overlapping subset of cocaine-induced miRNAs (*miR-324* and *miR-369*) in dopamine D_2 receptor-expressing neurons of the nucleus accumbens together with reduction in cocaine self-administration.

4.2.2 Opiates

Metabolic Changes

An early report on a single dose of morphine (20 mg/kg) demonstrated increases in glucose concentration (42 %) in rat brain (Miller et al. 1972). The same enhancement in local cerebral metabolic rate for glucose was shown for morphine repeated treatment in 95 brain regions of rats; the most significant changes were seen in the nucleus accumbens and prefrontal cortex (Kraus et al. 1997).

Morphological Changes

Chronic treatment of morphine (from 20 mg/kg to 140 mg/kg twice a day over 14 days at escalating doses) in rats, followed by drug withdrawal, resulted in a dramatic reduction of spine density in second-order dendritic trunks in the accumbens shell (Spiga et al. 2005). Chu et al. (2008) using the same treatment protocol with morphine showed that rough endoplasmic reticulum swelled, membrane configuration of the nucleus and mitochondria were blurred, and structure of myelin sheath changed in the rat ventral tegmental area. These findings support the linkage between opioid-induced abuse and structural functional plasticity of dendritic spines in the brain.

Glia and Microglia Activation

Opioid-induced proinflammatory glial activation (increases in microglial and astrocytic activation markers and release of proinflammatory cytokinesis) was suggested to be involved in the development of opioid dependence and reward (Watkins et al. 2005; Hutchinson et al. 2007). Recently, the glial markers have been implicated also in drug-induced craving. Thus, Lin et al. (2011) showed that repeated exposures to morphine decreased glial fibrillary acidic protein in rat amygdala tissue in the stage of extinction. Another report indicates that heroin self-administration and withdrawal regulate in a time-dependent manner tegmental and accumbal glial cell line-derived neurotrophic factor mRNA expression (Airavaara et al. 2011).

Manifestation of Oxidative Stress

There is evidence that repeated opioid treatments induce oxidative stress in the brain (see Table 3). Heroin injected continuously in increasing dosage

Table 3 Preclinical evidence of oxidative stress after opioid exposure

Model treatment	Animal	Dose and treatment regimen	Biomarker	Change (brain structure)	Reference
Morphine, chronic passive administration	Mouse	5 mg/kg (*s.c.*) for 7 days	GSH	↓Brain homogenate	Abdel-Zaher et al. (2010)
			GPx	↓Brain homogenate	
			MDA	↑Brain homogenate	
Tramadol, chronic passive administration	Mouse	50 mg/kg (*s.c.*) for 7 and 15 days	GSH	↓Brain homogenate	Abdel-Zaher et al. (2011)
			GSH-Px	↓Brain homogenate	
			MDA	↑Brain homogenate	

Abbreviations: ↑ increase, ↓ decrease, *GPx* glutathione peroxidase, *GSH* gluthatione, *MDA* malondialdehyde

(1–35 mg/kg) to mice caused increasingly greater numbers of DNA-damaged cells, protein carbonyl groups, and MDA levels (Qiusheng et al. 2005). Prooxidant status was also marked by decreased activity of antioxidant enzymes (SOD, CAT, GSH) (Qiusheng et al. 2005). It should be stressed that even a single morphine dose can produce oxidative damage in the brain (e.g., decreased GSH levels); however, its manifestation depends on the dose of morphine and age of the animal. Another opioid, tramadol at a 50 mg/kg (*i.p.*) dose, decreased nonenzymatic antioxidants such as intracellular GSH and enzymatic antioxidants such as GPx in mouse brain; repeated tramadol treatments resulted in an increase in the MDA level in the brain (Abdel-Zaher et al. 2011).

Manifestation of Apoptosis

Several studies performed on rodents have shown that sustained exposure to opioids produced cell death in many areas of the brain (Mao et al. 2002; Emeterio et al. 2006), together with increases in the brain expression of proapoptotic factors belonging to both the mitochondrial and the Fas-receptor apoptotic pathways (Boronat et al. 2001; Mao et al. 2002; Emeterio et al. 2006; Tramullas et al. 2007). For example, prolonged (34 days) heroin (5 mg/kg, *s.c.*) administration to mice evoked upregulation of proapoptotic proteins such as Fas, FasL, and Bad, in the cortex and hippocampus, indicating the activation of both the death receptor and the mitochondrial apoptotic pathways. In parallel, the presence of TUNEL (terminal deoxyribonucleotidyl transferase-mediated dUTP nick and labeling method)-positive cells scattered throughout the brain evidenced another indicator of apoptosis linked to DNA fragmentation (Tramullas et al. 2008). The signs of apoptosis were reported even after a single injection of heroin (5 mg/kg), which increased the expression level of Bad in the cortex and FasL in the hippocampus.

These biochemical indices in heroin-treated mice were accompanied by impaired cognitive ability in the Morris water maze and cognitive flexibility-related performance, raising the possibility that chronic exposure to opioid drugs in the brain would interfere with learning and memory through a neurotoxic effect related to activation of apoptotic pathways. Interestingly, heroin-induced apoptosis (caspase 2 and 9 activation) in neuronal-like PC12 cells was associated by increased mitochondrial membrane permeability and decreased mitochondrial potential (Oliveira et al. 2002, 2003).

Similarly to heroin, other opioid drugs can lead to apoptosis in neuronal cells. Thus, tramadol and morphine given to rats in progressive doses of (20, 40, and 80 mg/kg/day of tramadol; 4, 8, and 12 mg/kg/day of morphine) significantly enhanced the presence of red neurons (a histologic marker of apoptosis) as compared with control group (Atici et al. 2004). Chronic morphine or tramadol use in increasing doses caused red neuron degeneration in the rat brain, which probably contributes to cerebral dysfunction. Interestingly, the numbers of red neurons were significantly higher in the temporal and occipital regions in the tramadol group as compared with the morphine group, and these findings are of importance when prolonged opioid treatment is necessary (Atici et al. 2004).

Manifestation of Genetic and Epigenetic Actions

Genetic and epigenetic changes are another area of molecular interactions that could explain the mechanism of opioid neurotoxicity, addiction, and abstinence syndrome. In numerous studies altered adenyl cyclase activity and enhanced intracellular levels of cAMP have been observed during manifestations of opioid withdrawal syndromes (Sharma et al. 1977; Law et al. 1982; Koob and Bloom 1988; Piñeyro and Archer-Lahlou 2007). Recently reported by Piechota et al. (2012), chronic treatment with heroin regulates several genes in mouse striatum, with the most substantial impact for circadian genes (Per1, Per2, and Nr1d1) and glucocorticoid-dependent genes (Fkbp5, Sult1a1, and Plin4), a drug-regulated group of transcripts (Pdyn, Cartpt, and Rgs2, as well as Fam40b and Inmt). The appearance of opioid receptor downregulation and their desensitization were suggested to be the result of histone-deacetylase-mediated epigenetic control of genes (Hu et al. 2001; Hwang et al. 2007). Another paper by Zhou et al. (2006) revealed that morphine-withdrawal-related stress condition increased *oprm1* expression in the rat hypothalamus and striatum as well as lateral hypothalamic orexin mRNA level; this finding supports the hypothesis that orexin activity plays a role in morphine-withdrawal-related behaviors. There is also a finding showing that morphine decreased acetylation of histones H3 and H4, trimethylation of lysine 4 of H3 (H3K4me3) at the promoters of *KCNAB2* and *KCNF1* genes (both encoded subunits of potassium channel), which in turn decreased their transcription in rat ventral tegmental area (Mazei-Robison et al. 2011). Parallel reduction of trimethylation of H4K4me3 at the promoter of *GIRK3* gene (encoded potassium subunit channel) may suggest that morphine downregulates the K^+ channel via epigenetic changes and enhanced excitability of dopamine cell bodies within the mesencephalon.

There are implications for the significance of noncoding RNA, primarily miRNAs, in opioid-based addictive behaviors. Morphine has been implicated in the regulation of *miR-23b* and *let-7* expression that regulates posttranscriptional levels of mu opioid receptor (Wu et al. 2009; He et al. 2010). In the rat hippocampus, morphine activates ERK1/2 and decreases the expression level of *miR-190* (Zheng et al. 2010), which regulates the expression of neurogenic differentiation 1, thereby affecting the morphology of dendrites and adult neurogenesis. In addition, *miR-190* interacts with *pax6* gene-encoded transcription factor regulating neurogenesis (Zheng et al. 2012).

5 Conclusion

Psychostimulants and opioids evoke toxicity within the CNS, which in human research is detected after a particular cumulative drug exposure, while in preclinical studies neurotoxicities are recorded even after acute drug administration. Mechanisms leading to neurotoxicity linked to psychostimulants and opioids are due primarily to the action of the substance (e.g., enhancement of synaptic concentrations of dopamine) or by indirect effects (increases in extrasynaptic glutamate levels, toxic metabolites). Associated with the development of drug addiction, these mechanisms lead to the several pathological processes within the complex network of brain cell-to-cell interactions, such as alteration in neurotransmitter systems, dysregulation of energy metabolism, changes in structure of cellular membrane, apoptosis, generation of RS, glial/microglial cell overactivation, excitotoxicity and prominent deficits in brain morphology (neuronal loss, axonal damage), alterations of transcription factors, immediate early genes and epigenetic mechanisms, as well as neurocognitive and neuropsychological impairments (e.g., risk of Parkinson's disease). The extent of neurotoxic variations depends on drug type, drug dosage (chronic vs acute treatment), species (human vs nonhuman primates or rodents), external factors (environmental temperature), and brain region.

References

Abdel-Zaher, A. O., Abdel-Rahman, M. S., & Elwasei, F. M. (2010). Blockade of nitric oxide overproduction and oxidative stress by Nigella sativa oil attenuates morphine-induced tolerance and dependence in mice. *Neurochemical Research, 35*, 1557–1565.

Abdel-Zaher, A. O., Abdel-Rahman, M. S., & Elwasei, F. M. (2011). Protective effect of Nigella sativa oil against tramadol – induced tolerance and dependence in mice: Role of nitric oxide and oxidative stress. *NeuroToxicology, 32*, 725–733.

Abekawa, T., Ohmori, T., & Koyama, T. (1994). Effects of repeated administration of a high dose of methamphetamine on dopamine and glutamate release in rat striatum and nucleus accumbens. *Brain Research, 643*, 276–281.

Adachi, M., & Monteggia, L. M. (2009). Synergistic interactions between histone deacetylase inhibitors and drugs of abuse. *Neuropsychopharmacology, 34*, 2619–2620.

Ago, M., Ago, K., Hara, K., Kashimura, S., & Ogata, M. (2006). Toxicological and histopathological analysis of a patient who died nine days after a single intravenous dose of methamphetamine: A case report. *Legal Medicine (Tokyo, Japan), 8*, 235–239.

Airavaara, M., Pickens, C. L., Stern, A. L., Wihbey, K. A., Harvey, B. K., Bossert, J. M., Liu, Q. R., Hoffer, B. J., & Shaham, Y. (2011). Endogenous GDNF in ventral tegmental area and nucleus accumbens does not play a role in the incubation of heroin craving. *Addiction Biology, 16*, 261–272.

Albertson, D. N., Pruetz, B., Schmidt, C. J., Kuhn, D. M., Kapatos, G., & Bannon, M. J. (2004). Gene expression profile of the nucleus accumbens of human cocaine abusers: Evidence for dysregulation of myelin. *Journal of Neurochemistry, 88*, 1211–1219.

Albertson, D. N., Schmidt, C. J., Kapatos, G., & Bannon, M. J. (2006). Distinctive profiles of gene expression in the human nucleus accumbens associated with cocaine and heroin abuse. *Neuropsychopharmacology, 31*, 2304–2312.

Alvaro-Bartolomé, M., La Harpe, R., Callado, L. F., Meana, J. J., & García-Sevilla, J. A. (2011). Molecular adaptations of apoptotic pathways and signaling partners in the cerebral cortex of human cocaine addicts and cocaine-treated rats. *Neuroscience, 196*, 1–15.

Alves, E., Summavielle, T., Alves, C. J., Gomes-da-Silva, J., Barata, J. C., Fernandes, E., Bastos Mde, L., Tavares, M. A., & Carvalho, F. (2007). Monoamine oxidase-B mediates ecstasy-induced neurotoxic effects to adolescent rat brain mitochondria. *Journal of Neuroscience, 27*, 10203–10210.

Arendt, R., Angelopoulos, J., Salvator, A., & Singer, L. (1999). Motor development of cocaine-exposed children at age two years. *Pediatrics, 103*, 86–92.

Armenian, P., Mamantov, T. M., Tsutaoka, B. T., Gerona, R. R., Silman, E. F., Wu, A. H., Olson, K. R. (2012). Multiple MDMA (Ecstasy) overdoses at a rave event: A case series. *Journal of Intensive Care Medicine, 28*(4), 252–258. (Epub 2012 May 28).

Atici, S., Cinel, L., Cinel, I., Doruk, N., Aktekin, M., Akca, A., Camdeviren, H., & Oral, U. (2004). Opioid neurotoxicity: Comparison of morphine and tramadol in an experimental rat model. *International Journal of Neuroscience, 114*, 1001–1011.

Axt, K. J., & Molliver, M. E. (1991). Immunocytochemical evidence for methamphetamine-induced serotonergic axon loss in the rat brain. *Synapse, 9*, 302–313.

Banks, M. L., Czoty, P. W., Gage, H. D., Bounds, M. C., Garg, P. K., Garg, S., & Nader, M. A. (2008). Effects of cocaine and MDMA self-administration on serotonin transporter availability in monkeys. *Neuropsychopharmacology, 33*, 219–225.

Barbosa, D. J., Capela, J. P., Oliveira, J. M., Silva, R., Ferreira, L. M., Siopa, F., Branco, P. S., Fernandes, E., Duarte, J. A., de Lourdes Bastos, M., & Carvalho, F. (2012). Pro-oxidant effects of ecstasy and its metabolites in mouse brain synaptosomes. *Bangladesh Journal of Pharmacology, 165*, 1017–1033.

Bart, G., Kreek, M. J., Ott, J., LaForge, K. S., Proudnikov, D., Pollak, L., & Heilig, M. (2005). Increased attributable risk related to a functional mu-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. *Neuropsychopharmacology, 30*, 417–422.

Bashkatova, V., Meunier, J., Vanin, A., & Maurice, T. (2006). Nitric oxide and oxidative stress in the brain of rats exposed in utero to cocaine. *Annals New York Academy of Science, 1074*, 632–642.

Battaglia, G., Yeh, S. Y., O'Hearn, E., Molliver, M. E., Kuhar, M. J., & De Souza, E. B. (1987). 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: Quantification of neurodegeneration by measurement of [^{3}H] paroxetine-labeled serotonin uptake sites. *Journal of Pharmacology and Experimental Therapeutics, 242*, 911–916.

Bechara, A., & Martin, E. M. (2004). Impaired decision making related to working memory deficits in individuals with substance addictions. *Neuropsychology, 18*, 152–162.

Bolla, K. I., McCann, U. D., & Ricaurte, G. A. (1998). Memory impairment in abstinent MDMA ("Ecstasy") users. *Neurology, 51*, 1532–1537.

Boronat, M. A., García-Fuster, M. J., & García-Sevilla, J. A. (2001). Chronic morphine induces up-regulation of the pro-apoptotic Fas receptor and down-regulation of the anti-apoptotic Bcl-2 oncoprotein in rat brain. *British Journal of Pharmacology, 134*, 1263–1270.

Botelho, M. F., Relvas, J. S., Abrantes, M., Cunha, M. J., Marques, T. R., Rovira, E., Fontes Ribeiro, C. A., & Macedo, T. (2006). Brain blood flow SPET imaging in heroin abusers. *Annals of the New York Academy of Sciences, 1074*, 466–477.

Bowyer, J. F., Davies, D. L., Schmued, L., Broening, H. W., Newport, G. D., Slikker, W., Jr., & Holson, R. R. (1994). Further studies of the role of hyperthermia in methamphetamine neurotoxicity. *Journal of Pharmacology and Experimental Therapeutics, 268*, 1571–1580.

Bowyer, J. F., Frame, L. T., Clausing, P., Nagamoto-Combs, K., Osterhout, C. A., Sterling, C. R., & Tank, A. W. (1998). Long-term effects of amphetamine neurotoxicity on tyrosine hydroxylase mRNA and protein in aged rats. *Journal of Pharmacology and Experimental Therapeutics, 286*, 1074–1085.

Broening, H. W., Pu, C., & Vorhees, C. V. (1997). Methamphetamine selectively damages dopaminergic innervation to the nucleus accumbens core while sparing the shell. *Synapse, 27*, 153–160.

Brown, J. M., Hanson, G. R., & Fleckenstein, A. E. (2001). Regulation of the vesicular monoamine transporter-2: A novel mechanism for cocaine and other psychostimulants. *Journal of Pharmacology and Experimental Therapeutics, 296*, 762–767.

Buchert, R., Thomasius, R., Petersen, K., Wilke, F., Obrocki, J., Nebeling, B., Wartberg, L., Zapletalova, P., & Clausen, M. (2006). Reversibility of ecstasy-induced reduction in serotonin transporter availability in polydrug ecstasy users. *European Journal of Nuclear Medicine and Molecular Imaging, 33*, 188–199.

Bunney, E. B., Appel, S. B., & Brodie, M. S. (2001). Electrophysiological effects of cocaethylene, cocaine, and ethanol on dopaminergic neurons of the ventral tegmental area. *Journal of Pharmacology and Experimental Therapeutics, 297*, 696–703.

Büttner, A. (2011). The neuropathology of drug abuse. *Neuropathology and Applied Neurobiology, 37*, 118–134.

Cadet, J. L., & Jayanthi, S. (2013). Epigenetics of methamphetamine induced changes in glutamate function. *Neuropsychopharmacology Reviews, 38*, 248–249.

Cadet, J. L., & Krasnova, I. N. (2009). Molecular bases of methamphetamine induced neurodegeneration. *International Review of Neurobiology, 88*, 101–119.

Cadet, J. L., Jayanthi, S., & Deng, X. (2003). Speed kills: Cellular and molecular bases of methamphetamine-induced nerve terminal degeneration and neuronal apoptosis. *The FASEB Journal, 17*, 1775–1788.

Cadet, J. L., Krasnova, I. N., Jayanthi, S., & Lyles, J. (2007). Neurotoxicity of substituted amphetamines: Molecular and cellular mechanisms. *Neurotoxicity Research, 11*, 183–202.

Caligiuri, M. P., & Buitenhuys, C. (2005). Do preclinical findings of methamphetamine-induced motor abnormalities translate to an observable clinical phenotype? *Neuropsychopharmacology, 30*, 2125–2134.

Capela, J. P., Ruscher, K., Lautenschlager, M., Freyer, D., Dirnagl, U., Gaio, A. R., Bastos, M. L., Meisel, A., & Carvalho, F. (2006). Ecstasy-induced cell death in cortical neuronal cultures is serotonin 2A-receptor-dependent and potentiated under hyperthermia. *Neuroscience, 139*, 1069–1081.

Capela, J. P., Macedo, C., Branco, P. S., Ferreira, L. M., Lobo, A. M., Fernandes, E., Remião, F., Bastos, M. L., Dirnagl, U., Meisel, A., & Carvalho, F. (2007). Neurotoxicity mechanisms of thioether ecstasy metabolites. *Neuroscience, 146*, 1743–1757.

Capela, J. P., Carmo, H., Remião, F., Bastos, M. L., Meisel, A., & Carvalho, F. (2009). Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: An overview. *Molecular Neurobiology, 39*, 210–271.

Carrion, J., Liebling, C. N. B., Reiszel, C., Dalal, R., Dewey, S. L., & Schiffer, W. K. (2009). Imaging the conditioned behavioral effects of methamphetamine in rodents. *Brain Imaging and Behavior, 3*, 176–190.

Carvalho, F., Fernandes, E., Remião, F., Gomes-Da-Silva, J., Tavares, M. A., & Bastos, M. D. (2001). Adaptive response of antioxidant enzymes in different areas of rat brain after repeated d-amphetamine administration. *Addiction Biology, 6*, 213–221.

Carvalho, M., Carmo, H., Costa, V. M., Capela, J. P., Pontes, H., Remião, F., Carvalho, F., & Bastos, M. D. (2012). Toxicity of amphetamines: An update. *Archives of Toxicology, 86*, 1167–1231.

Cassel, S., Carouge, D., Gensburger, C., Anglard, P., Burgun, C., Dietrich, J. B., Aunis, D., & Zwiller, J. (2006). Fluoxetine and cocaine induce the epigenetic factors MeCP2 and MBD1 in adult rat brain. *Molecular Pharmacology, 70*, 487–492.

Castellano, C. (1975). Effects of morphine and heroin on discrimination learning and consolidation in mice. *Psychopharmacologia, 42*, 235–242.

Castellano, C. (1980). Dose-dependent effects of heroin on memory in two inbred strains of mice. *Psychopharmacology, 67*, 235–239.

Chandrasekar, V., & Dreyer, J. L. (2009). microRNAs miR-124, let-7d and miR-181a regulate cocaine-induced plasticity. *Molecular and Cellular Biology, 42*, 350–362.

Chandrasekar, V., & Dreyer, J. L. (2011). Regulation of miR-124, let-7d, and miR-181a in the accumbens affects the expression, extinction, and reinstatement of cocaine-induced conditioned place preference. *Neuropsychopharmacology, 36*, 1149–1164.

Chang, L., Ernst, T., Speck, O., & Grob, C. S. (2005). Additive effects of HIV and chronic methamphetamine use on brain metabolite abnormalities. *The American Journal of Psychiatry, 162*, 361–369.

Chang, L., Alicata, D., Ernst, T., & Volkow, N. (2007). Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. *Addiction, 102*, 16–32.

Chat-Mendes, C., Anderson, K. L., & Itzhak, Y. (2007). Impairment in consolidation of learned place preference following dopaminergic neurotoxicity in mice is ameliorated by N-acetylcysteine but not D_1 and D_2 dopamine receptor agonists. *Neuropsychopharmacology, 32*, 531–541.

Chen, Y. I., Famous, K., Xu, H., Choi, J. K., Mandeville, J. B., Schmidt, H. D., Pierce, R. C., & Jenkins, B. G. (2011). Cocaine self-administration leads to alterations in temporal responses to cocaine challenge in limbic and motor circuitry. *European Journal of Neuroscience, 34*, 800–815.

Chorbov, V. M., Todorov, A. A., Lynskey, M. T., & Cicero, T. J. (2011). Elevated levels of DNA methylation at the OPRM1 promoter in blood and sperm from male opioid addicts. *Journal of Opioid Management, 7*, 258–264.

Chu, N. N., Xia, W., Yu, P., Hu, L., Zhang, R., & Cui, C. L. (2008). Chronic morphine-induced neuronal morphological changes in the ventral tegmental area in rats are reversed by electroacupuncture treatment. *Addiction Biology, 13*, 47–51.

Cipolli, C., & Galliani, I. (1987). Addiction time and intellectual impairment in heroin users. *Psychological Reports, 60*, 1099–1105.

Colado, M. I., O'Shea, E., Granados, R., Murray, T. K., & Green, A. R. (1997). In vivo evidence for free radical involvement in the degeneration of rat brain 5-HT following administration of MDMA ('ecstasy') and p-chloroamphetamine but not the degeneration following fenfluramine. *British Journal of Pharmacology, 121*, 889–900.

Coller, J. K., Christrup, L. L., & Somogyi, A. A. (2009). Role of active metabolites in the use of opioids. *European Journal of Clinical Pharmacology, 65*, 121–139.

Cowan, R. L. (2007). Neuroimaging research in human MDMA users: A review. *Psychopharmacology, 189*, 539–556.

Cowan, R. L., Lyoo, I. K., Sung, S. M., Ahn, K. H., Kim, M. J., Hwang, J., Haga, E., Vimal, R. L. P., Lukas, S. E., & Renshaw, P. F. (2003). Reduced cortical gray matter density in human MDMA (ecstasy) users: A voxel-based morphometry study. *Drug and Alcohol Dependence, 72*, 225–235.

Crowley, J. J., Oslin, D. W., Patkar, A. A., Gottheil, E., DeMaria, P. A., Jr., O'Brien, C. P., Berrettini, W. H., & Grice, D. E. (2003). A genetic association study of the mu opioid receptor and severe opioid dependence. *Psychiatric Genetics, 13*, 169–173.

Crystal, H. A., Hamon, S., Randesi, M., Cook, J., Anastos, K., Lazar, J., Liu, C., Pearce, L., Golub, E., Valcour, V., Weber, K. M., Holman, S., Ho, A., & Kreek, M. J. (2012). A C17T polymorphism in the mu opiate receptor is associated with quantitative measures of drug use in African American women. *Addiction Biology, 17*, 181–191.

Cunha-Oliveira, T., Rego, A. C., & Oliveira, C. R. (2008). Cellular and molecular mechanisms involved in the neurotoxicity of opioid and psychostimulant drugs. *Brain Research Reviews, 58*, 192–208.

Curran, H. V., Kleckham, J., Bearn, J., Strang, J., & Wanigaratne, S. (2001). Effects of methadone on cognition, mood and craving in detoxifying opiate addicts: A dose–response study. *Psychopharmacology, 154*, 153–160.

Czoty, P. W., Morgan, D., Shannon, E. E., Gage, H. D., & Nader, M. A. (2004). Characterization of dopamine D_1 and D_2 receptor function in socially housed cynomolgus monkeys self-administering cocaine. *Psychopharmacology, 174*, 381–388.

Dacher, M., & Nugent, F. S. (2011). Opiates and plasticity. *Neuropharmacology, 61*, 1088–1096.

Daglish, M. R., & Nutt, D. J. (2003). Brain imaging studies in human addicts. *European Neuropsychopharmacology, 13*, 453–458.

Danos, P., Kasper, S., Grünwald, F., Klemm, E., Krappel, C., Broich, K., Höflich, G., Overbeck, B., Biersack, H. J., & Möller, H. J. (1998). Pathological regional cerebral blood flow in opiate-dependent patients during withdrawal: A HMPAO-SPECT study. *Neuropsychobiology, 37*(4), 194–199.

Daumann, J., Fischermann, T., Pilatus, U., Thron, A., Moeller-Hartmann, W., & Gouzoulis-Mayfrank, E. (2004). Proton magnetic resonance spectroscopy in ecstasy (MDMA) users. *Neuroscience Letters, 362*, 113–116.

Daza-Losada, M., Rodríguez-Arias, M., Maldonado, C., Aguilar, M. A., Guerri, C., & Miñarro, J. (2009). Acute behavioural and neurotoxic effects of MDMA plus cocaine in adolescent mice. *Neurotoxicology and Teratology, 31*, 49–59.

de Win, M. M. L., Schilt, T., Reneman, L., Vervaeke, H., Jager, G., Dijkink, S., Booij, J., & van den Brink, W. (2006). Ecstasy use and self-reported depression, impulsivity, and sensation seeking: A prospective cohort study. *Journal of Psychopharmacology, 20*, 226–235.

Deb, I., Chakraborty, J., Gangopadhyay, P. K., Choudhury, S. R., & Das, S. (2010). Single-nucleotide polymorphism (A118G) in exon 1 of OPRM1 gene causes alteration in downstream signaling by mu-opioid receptor and may contribute to the genetic risk for addiction. *Journal of Neurochemistry, 112*, 486–496.

Deng, X., Cai, N. S., McCoy, M. T., Chen, W., Trush, M. A., & Cadet, J. L. (2002). Methamphetamine induces apoptosis in an immortalized rat striatal cell line by activating the mitochondrial cell death pathway. *Neuropharmacology, 42*, 837–845.

Deng, J. V., Rodriguiz, R. M., Hutchinson, A. N., Kim, I. H., Wetsel, W. C., & West, A. E. (2010). MeCP2 in the nucleus accumbens contributes to neural and behavioral responses to psychostimulants. *Nature Neuroscience, 13*, 1128–1136.

Derauf, C., Kekatpure, M., Neyzi, N., Lester, B., & Kosofsky, B. (2009). Neuroimaging of children following prenatal drug exposure. *Seminars in Cell & Developmental Biology, 20*, 441–454.

Di Chiara, G., Tanda, G., Bassareo, V., Pontieri, F., Acquas, E., Fenu, S., Cadoni, C., & Carboni, E. (1999). Drug addiction as a disorder of associative learning. Role of nucleus accumbens shell/extended amygdala dopamine. *Annals of the New York Academy of Sciences, 877*, 461–485.

Dick, D. M., & Foroud, T. (2003). Candidate genes for alcohol dependence: A review of genetic evidence from human studies. *Alcoholism, Clinical and Experimental Research, 27*, 868–879.

Dietrich, J. B., Mangeol, A., Revel, M. O., Burgun, C., Aunis, D., & Zwiller, J. (2005). Acute or repeated cocaine administration generates reactive oxygen species and induces antioxidant enzyme activity in dopaminergic rat brain structures. *Neuropharmacology, 48*, 965–974.

Drakenberg, K., Nikoshkov, A., Horváth, M. C., Fagergren, P., Gharibyan, A., Saarelainen, K., Rahman, S., Nylander, I., Bakalkin, G., Rajs, J., Keller, E., & Hurd, Y. L. (2006). Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 7883–7888.

Droin, N. M., Pinkoski, M. J., Dejardin, E., & Green, D. R. (2003). Egr family members regulate nonlymphoid expression of Fas ligand, TRAIL, and tumor necrosis factor during immune responses. *Molecular and Cellular Biology, 23*, 7638–7647.

Ehlers, C. L., Gizer, I. R., Gilder, D. A., & Wilhelmsen, K. C. (2011). Linkage analyses of stimulant dependence, craving, and heavy use in American Indians. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 156B*, 772–780.

Eipper-Mains, J. E., Kiraly, D. D., Palakodeti, D., Mains, R. E., Eipper, B. A., & Graveley, B. R. (2011). microRNA-Seq reveals cocaine-regulated expression of striatal microRNAs. *RNA, 17*, 1529–1543.

Eisch, A. J., Barrot, M., Schad, C. A., Self, D. W., & Nestler, E. J. (2000). Opiates inhibit neurogenesis in the adult rat hippocampus. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 7579–7584.

Ellison, G., Eison, M., Huberman, H., & Daniel, F. (1978). Long-term changes in dopaminergic innervation of caudate nucleus after continuous amphetamine administration. *Science, 201*, 276–278.

Emeterio, E. P., Tramullas, M., & Hurlé, M. A. (2006). Modulation of apoptosis in the mouse brain after morphine treatments and morphine withdrawal. *Journal of Neuroscience Research, 83*, 1352–1361.

Ernst, T., & Chang, L. (2008). Adaptation of brain glutamate plus glutamine during abstinence from chronic methamphetamine use. *Journal of Neuroimmune Pharmacology, 3*, 165–172.

Ernst, T., Chang, L., Leonido-Yee, M., & Speck, O. (2000). Evidence for long-term neurotoxicity associated with methamphetamine abuse: A ^{1}H MRS study. *Neurology, 54*, 1344–1349.

Eyler, F. D., Behnke, M., Garvan, C. W., Woods, N. S., Wobie, K., & Conlon, M. (2001). Newborn evaluations of toxicity and withdrawal related to prenatal cocaine exposure. *Neurotoxicology and Teratology, 23*, 399–411.

Fantegrossi, W. E., Woolverton, W. L., Kilbourn, M., Sherman, P., Yuan, J., Hatzidimitriou, G., Ricaurte, G. A., Woods, J. H., & Winger, G. (2004). Behavioral and neurochemical consequences of long-term intravenous self-administration of MDMA and its enantiomers by rhesus monkeys. *Neuropsychopharmacology, 29*, 1270–1281.

Ferraro, L., Beggiato, S., Marcellino, D., Frankowska, M., Filip, M., Agnati, L. F., Antonelli, T., Tomasini, M. C., Tanganelli, S., & Fuxe, K. (2010). Nanomolar concentrations of cocaine enhance D_2-like agonist-induced inhibition of the K^+−evoked [^{3}H]-dopamine efflux from rat striatal synaptosomes: A novel action of cocaine. *Journal of Neural Transmission, 117*, 593–597.

Ferris, M. J., Mateo, Y., Roberts, D. C., & Jones, S. R. (2011). Cocaine-insensitive dopamine transporters with intact substrate transport produced by self-administration. *Biological Psychiatry, 69*, 201–207.

Filip, M., Alenina, N., Bader, M., & Przegaliński, E. (2010). Behavioral evidence for the significance of serotoninergic (5-HT) receptors in cocaine addiction. *Addiction Biology, 15*, 227–249.

Fitzmaurice, P. S., Tong, J., Yazdanpanah, M., Liu, P. P., Kalasinsky, K. S., & Kish, S. J. (2006). Levels of 4-hydroxynonenal and malondialdehyde are increased in brain of human chronic users of methamphetamine. *Journal of Pharmacology and Experimental Therapeutics, 319*, 703–709.

Fleckenstein, A. E., Volz, T. J., Riddle, E. L., Gibb, J. W., & Hanson, G. R. (2007). New insights into the mechanism of action of amphetamines. *Annual Review of Pharmacology and Toxicology, 47*, 681–698.

Florian, C., & Roullet, P. (2004). Hippocampal CA3-region is crucial for acquisition and memory consolidation in Morris water maze task in mice. *Behavioural Brain Research, 154*, 365–374.

Fowler, J. S., Volkow, N. D., Kassed, C. A., & Chang, L. (2007). Imaging the addicted human brain. *Science & Practice Perspectives, 3*, 4–16.

Franke, P., Wang, T., Nöthen, M. M., Knapp, M., Neidt, H., Albrecht, S., Jahnes, E., Propping, P., & Maier, W. (2001). Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *American Journal of Medical Genetics, 105*, 114–119.

Franklin, T. R., Acton, P. D., Maldjian, J. A., Gray, J. D., Croft, J. R., Dackis, C. A., O'Brien, C. P., & Childress, A. R. (2002). Decreased gray matter concentration in the insular, orbitofrontal, cingulate, and temporal cortices of cocaine patients. *Biological Psychiatry, 51*, 134–142.

Frenzilli, G., Ferrucci, M., Giorgi, F. S., Blandini, F., Nigro, M., Ruggieri, S., Murri, L., Paparelli, A., & Fornai, F. (2007). DNA fragmentation and oxidative stress in the hippocampal formation: A bridge between 3,4-methylenedioxymethamphetamine (ecstasy) intake and long-lasting behavioral alterations. *Behavioural Pharmacology, 18*, 471–481.

Frey, B. N., Valvassori, S. S., Réus, G. Z., Martins, M. R., Petronilho, F. C., Bardini, K., Dal-Pizzol, F., Kapczinski, F., & Quevedo, J. (2006). Changes in antioxidant defense enzymes after d-amphetamine exposure: Implications as an animal model of mania. *Neurochemical Research, 31*, 699–703.

Galynker, I. I., Watras-Ganz, S., Miner, C., Rosenthal, R. N., Des Jarlais, D. C., Richman, B. L., & London, E. (2000). Cerebral metabolism in opiate-dependent subjects: Effects of methadone maintenance. *The Mount Sinai Journal of Medicine, 67*, 381–387.

Gao, H., Xiang, Y., Sun, N., Zhu, H., Wang, Y., Liu, M., Ma, Y., & Lei, H. (2007). Metabolic changes in rat prefrontal cortex and hippocampus induced by chronic morphine treatment studied ex vivo by high resolution 1H NMR spectroscopy. *Neurochemistry International, 50*, 386–394.

García-Sevilla, J. A., Ventayol, P., Busquets, X., La Harpe, R., Walzer, C., & Guimón, J. (1997). Marked decrease of immunolabelled 68 kDa neurofilament (NF-L) proteins in brains of opiate addicts. *NeuroReport, 8*, 1561–1565.

Gatley, S. J., Volkow, N. D., Gifford, A. N., & Fowler, J. S. (1998). Cocaine: Pharmacology. In R. E. Tarter, R. T. Ammennan, & P. J. Ott (Eds.), *Handbook of substance abuse: Neurobehavioral pharmacology*. New York: Plenum.

Gerra, G., Leonardi, C., Cortese, E., D'Amore, A., Lucchini, A., Strepparola, G., Serio, G., Farina, G., Magnelli, F., Zaimovic, A., Mancini, A., Turci, M., Manfredini, M., & Donnini, C. (2007). Human kappa opioid receptor gene (OPRK1) polymorphism is associated with opiate addiction. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 144B*, 771–775.

Glatt, S. J., Bousman, C., Wang, R. S., Murthy, K. K., Rana, B. K., Lasky-Su, J. A., Zhu, S. C., Zhang, R., Li, J., Zhang, B., Lyon, S. M. J., Faraone, S. V., & Tsuang, M. T. (2007). Evaluation of OPRM1 variants in heroin dependence by family-based association testing and meta-analysis. *Drug and Alcohol Dependence, 90*, 159–165.

Gluck, M. R., Moy, L. Y., Jayatilleke, E., Hogan, K. A., Manzino, L., & Sonsalla, P. K. (2001). Parallel increases in lipid and protein oxidative markers in several mouse brain regions after methamphetamine treatment. *Journal of Neurochemistry, 79*, 152–160.

Goldstein, R. Z., Alia-Klein, N., Tomasi, D., Zhang, L., Cottone, L. A., Maloney, T., Telang, F., Caparelli, E. C., Chang, L., Ernst, T., Samaras, D., Squires, N. K., & Volkow, N. D. (2007a). Is decreased prefrontal cortical sensitivity to monetary reward associated with impaired motivation and self-control in cocaine addiction? *The American Journal of Psychiatry, 164*, 43–51.

Goldstein, R. Z., Tomasi, D., Rajaram, S., Cottone, L. A., Zhang, L., Maloney, T., Telang, F., Alia-Klein, N., & Volkow, N. D. (2007b). Role of the anterior cingulate and medial orbitofrontal cortex in processing drug cues in cocaine addiction. *Neuroscience, 144*, 1153–1159.

Gonçalves, J., Martins, T., Ferreira, R., Milhazes, N., Borges, F., Ribeiro, C. F., Malva, J. O., Macedo, T. R., & Silva, A. P. (2008). Methamphetamine-induced early increase of IL-6 and TNF-alpha mRNA expression in the mouse brain. *Annals of the New York Academy of Sciences, 1139*, 103–111.

Gouzoulis-Mayfrank, E., & Daumann, J. (2006). Neurotoxicity of methylenedioxyamphetamines (MDMA; ecstasy) in humans: How strong is the evidence for persistent brain damage? *Addiction, 101*, 348–361.

Gouzoulis-Mayfrank, E., & Daumann, J. (2009). Neurotoxicity of drugs of abuse–the case of methylenedioxyamphetamines (MDMA, ecstasy), and amphetamines. *Dialogues in Clinical Neuroscience, 11*, 305–317.

Green, A. R., Mechan, A. O., Elliott, J. M., O'Shea, E., & Colado, M. I. (2003). The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacological Reviews, 55*, 463–508.

Gudelsky, G. A., & Yamamoto, B. K. (2008). Actions of 3,4-methylenedioxymethamphetamine (MDMA) on cerebral dopaminergic, serotonergic and cholinergic neurons. *Pharmacology Biochemistry and Behavior, 90*, 198–207.

Guerra, D., Solé, A., Camí, J., & Tobeña, A. (1987). Neuropsychological performance in opiate addicts after rapid detoxification. *Drug and Alcohol Dependence, 20*, 261–270.

Guo, M., Xu, N. J., Li, Y. T., Yang, J. Y., Wu, C. F., & Pei, G. (2005). Morphine modulates glutamate release in the hippocampal CA1 area in mice. *Neuroscience Letters, 381*, 12–15.

Gutowicz, M., Sadurska, B., Chołojczyk, M., Pokorska-Lis, M., Siwińska-Ziółkowska, A., & Barańczyk-Kuźma, A. (2006). Antioxidant status in different regions of heroin addicts' brain. *Environmental Toxicology and Pharmacology, 21*, 80–85.

Hanspeter, L. (1981). Fluorescence histochemistry indicates damage of striatal dopamine nerve terminals in rats after multiple doses of methamphetamine. *Life Sciences, 28*, 911–916.

Harold, C., Wallace, T., Friedman, R., Gudelsky, G., & Yamamoto, B. (2000). Methamphetamine selectively alters brain glutathione. *European Journal of Pharmacology, 400*, 99–102.

Harvey, J. A. (2004). Cocaine effects on the developing brain: Current status. *Neuroscience and Biobehavioral Reviews, 27*, 751–764.

Haselhorst, R., Dürsteler-MacFarland, K. M., Scheffler, K., Ladewig, D., Müller-Spahn, F., Stohler, R., Seelig, J., & Seifritz, E. (2002). Frontocortical N-acetylaspartate reduction associated with long-term i.v. heroin use. *Neurology, 58*, 305–307.

Hashimoto, K., Tsukada, H., Nishiyama, S., Fukumoto, D., Kakiuchi, T., & Iyo, M. (2007). Protective effects of minocycline on the reduction of dopamine transporters in the striatum after administration of methamphetamine: A positron emission tomography study in conscious monkeys. *Biological Psychiatry, 61*, 577–581.

Hatzidimitriou, G., McCann, U. D., & Ricaurte, G. A. (1999). Altered serotonin innervation patterns in the forebrain of monkeys treated with (+/−)3,4-methylenedioxymethamphetamine seven years previously: Factors influencing abnormal recovery. *Journal of Neuroscience, 19*, 5096–5107.

He, Y., Yang, C., Kirkmire, C. M., & Wang, Z. J. (2010). Regulation of opioid tolerance by let-7 family microRNA targeting the mu opioid receptor. *Journal of Neuroscience, 30*, 10251–10258.

Henry, P. K., Murnane, K. S., Votaw, J. R., & Howell, L. L. (2010). Acute brain metabolic effects of cocaine in rhesus monkeys with a history of cocaine use. *Brain Imaging and Behavior, 4*, 212–219.

Hepner, I. J., Homewood, J., & Taylor, A. J. (2002). Methadone disrupts performance on the working memory version of the Morris water task. *Physiology & Behavior, 76*, 41–49.

Herring, N. R., Schaefer, T. L., Gudelsky, G. A., Vorhees, C. V., & Williams, M. T. (2008). Effect of +−methamphetamine on path integration learning, novel object recognition, and neurotoxicity in rats. *Psychopharmacology, 199*, 637–650.

Hoehe, M. R., Köpke, K., Wendel, B., Rohde, K., Flachmeier, C., Kidd, K. K., Berrettini, W. H., & Church, G. M. (2000). Sequence variability and candidate gene analysis in complex disease: Association of mu opioid receptor gene variation with substance dependence. *Human Molecular Genetics, 9*, 2895–2908.

Hollander, J. A., Im, H. I., Amelio, A. L., Kocerha, J., Bali, P., Lu, Q., Willoughby, D., Wahlestedt, C., Conkright, M. D., & Kenny, P. J. (2010). Striatal microRNA controls cocaine intake through CREB signalling. *Nature, 466*, 197–202.

Holman, B. L., Carvalho, P. A., Mendelson, J., Teoh, S. K., Nardin, R., Hallgring, E., Hebben, N., & Johnson, K. A. (1991). Brain perfusion is abnormal in cocaine-dependent polydrug users: A study using technetium-99m-HMPAO and ASPECT. *Journal of Nuclear Medicine, 32*, 1206–1210.

Holman, B. L., Mendelson, J., Garada, B., Teoh, S. K., Hallgring, E., Johnson, K. A., & Mello, N. K. (1993). Regional cerebral blood flow improves with treatment in chronic cocaine polydrug users. *Journal of Nuclear Medicine, 34*, 723–727.

Hotchkiss, A. J., & Gibb, J. W. (1980). Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. *Journal of Pharmacology and Experimental Therapeutics, 214*, 257–262.

Howell, L. L., & Murnane, K. S. (2011). Nonhuman primate positron emission tomography neuroimaging in drug abuse research. *Journal of Pharmacology and Experimental Therapeutics, 337*, 324–334.

Hu, X., Bi, J., Loh, H. H., & Wie, L. N. (2001). An intronic Ikaros-binding element mediates retinoic acid suppression of the kappa opioid receptor gene, accompanied by histone deacetylation on the promoters. *Journal of Biological Chemistry, 276*, 4597–4603.

Huang, Y. H., Tsai, S. J., Su, T. W., & Sim, C. B. (1999). Effects of repeated high-dose methamphetamine on local cerebral glucose utilization in rats. *Neuropsychopharmacology, 21*, 427–434.

Hurd, Y. L., & Herkenham, M. (1993). Molecular alterations in the neostriatum of human cocaine addicts. *Synapse, 13*, 357–69.

Hutchinson, M. R., Bland, S. T., Johnson, K. W., Rice, K. C., Maier, S. F., & Watkins, L. R. (2007). Opioid-induced glial activation: Mechanisms of activation and implications for opioid analgesia, dependence, and reward. *The Scientific World Journal, 7*, 98–111.

Hwang, C. K., Song, K. Y., Kim, C. S., Choi, H. S., Guo, X. H., Law, P. Y., Wei, L. N., & Loh, H. H. (2007). Evidence of endogenous mu opioid receptor regulation by epigenetic control of the promoters. *Molecular and Cellular Biology, 27*, 4720–4736.

Ide, S., Kobayashi, H., Tanaka, K., Ujike, H., Sekine, Y., Ozaki, N., Inada, T., Harano, M., Komiyama, T., Yamada, M., Iyo, M., Ikeda, K., & Sora, I. (2004). Gene polymorphisms of the mu opioid receptor in methamphetamine abusers. *Annals of the New York Academy of Sciences, 1025*, 316–324.

Ide, S., Kobayashi, H., Ujike, H., Ozaki, N., Sekine, Y., Inada, T., Harano, M., Komiyama, T., Yamada, M., Iyo, M., Iwata, N., Tanaka, K., Shen, H., Iwahashi, K., Itokawa, M., Minami, M., Satoh, M., Ikeda, K., & Sora, I. (2006). Linkage disequilibrium and association with methamphetamine dependence/psychosis of mu-opioid receptor gene polymorphisms. *The Pharmacogenomics Journal, 6*, 179–188.

Im, H. I., Hollander, J. A., Bali, P., & Kenny, P. J. (2010). MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nature Neuroscience, 13*, 1120–1127.

Imam, S. Z., Islam, F., Itzhak, Y., Slikker, W., Jr., & Ali, S. F. (2000). Prevention of dopaminergic neurotoxicity by targeting nitric oxide and peroxynitrite: Implications for the prevention of methamphetamine-induced neurotoxic damage. *Annals of the New York Academy of Sciences, 914*, 157–171.

Imam, S. Z., el-Yazal, J., Newport, G. D., Itzhak, Y., Cadet, J. L., Slikker, W., Jr., & Ali, S. F. (2001). Methamphetamine-induced dopaminergic neurotoxicity: Role of peroxynitrite and neuroprotective role of antioxidants and peroxynitrite decomposition catalysts. *Annals of the New York Academy of Sciences, 939*, 366–380.

Inoue, H., Ikeda, N., Kudo, K., Ishida, T., Terada, M., & Matoba, R. (2006). Methamphetamine-related sudden death with a concentration which was of a 'toxic level'. *Legal Medicine (Tokyo), 8*, 150–155.

Ishigami, A., Kubo, S., Gotohda, T., & Tokunaga, I. (2003). The application of immunohistochemical findings in the diagnosis in methamphetamine-related death-two forensic autopsy cases. *The Journal of Medical Investigation, 50*, 112–116.

Itzhak, Y., & Achat-Mendes, C. (2004). Methamphetamine and MDMA (ecstasy) neurotoxicity: 'of mice and men'. *IUBMB Life, 56*, 249–255.

Jager, G., de Win, M., Vervaeke, H., Schilt, T., Kahn, R., van den Brink, W., van Ree, J., & Ramsey, N. (2007). Incidental use of ecstasy: No evidence for harmful effects on cognitive brain function in a prospective fMRI study. *Psychopharmacology, 193*, 403–414.

Jayanthi, S., Ladenheim, B., & Cadet, J. L. (1998). Methamphetamine-induced changes in antioxidant enzymes and lipid peroxidation in copper/zinc-superoxide dismutase transgenic mice. *Annals of the New York Academy of Sciences, 844*, 92–102.

Jayanthi, S., Deng, X., Bordelon, M., McCoy, M. T., & Cadet, J. L. (2001). Methamphetamine causes differential regulation of pro-death and anti-death Bcl-2 genes in the mouse neocortex. *The FASEB Journal, 15*, 1745–1752.

Jayanthi, S., Deng, X., Noailles, P. A., Ladenheim, B., & Cadet, J. L. (2004). Methamphetamine induces neuronal apoptosis via cross-talks between endoplasmic reticulum and mitochondria-dependent death cascades. *The FASEB Journal, 18*, 238–251.

Jayanthi, S., Deng, X., Ladenheim, B., McCoy, M. T., Cluster, A., Cai, N. S., & Cadet, J. L. (2005). Calcineurin/NFAT-induced up-regulation of the Fas ligand/Fas death pathway is involved in methamphetamine-induced neuronal apoptosis. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 868–873.

Jenkins, A. J., & Cone, E. J. (1998). Pharmacokinetics: Drug absorption, distribution and elimination. In S. B. Karch (Ed.), *Drug abuse handbook* (pp. 151–201). Boca Raton, FL: CRC Press.

Jones, N. A., Field, T., Davalos, M., & Hart, S. (2004). Greater right frontal EEG asymmetry and nonemphatic behavior are observed in children prenatally exposed to cocaine. *International Journal of Neuroscience, 114*, 459–480.

Kahlig, K. M., Binda, F., Khoshbouei, H., Blakely, R. D., McMahon, D. G., Javitch, J. A., & Galli, A. (2005). Amphetamine induces dopamine efflux through a dopamine transporter channel. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 3495–3500.

Kalivas, P. W. (2004). Recent understanding in the mechanisms of addiction. *Current Psychiatry Reports, 6*, 347–351.

Kapur, S., Sharad, S., Singh, R. A., & Gupta, A. K. (2007). A118g polymorphism in mu opioid receptor gene (oprm1): Association with opiate addiction in subjects of Indian origin. *Journal of Integrative Neuroscience, 6*, 511–522.

Kaur, G., & Kaur, G. (2001). Role of cholinergic and GABAergic neurotransmission in the opioids-mediated GnRH release mechanism of EBP-primed OVX rats. *Molecular and Cellular Biochemistry, 219*, 13–19.

Kimmel, H. L., Nye, J. A., Voll, R., Mun, J., Stehouwer, J., Goodman, M. M., Votaw, J. R., Carroll, F. I., & Howell, L. L. (2012). Simultaneous measurement of extracellular dopamine and dopamine transporter occupancy by cocaine analogs in squirrel monkeys. *Synapse, 66*, 501–508.

Kish, S. J., Furukawa, Y., Ang, L., Vorce, S. P., & Kalasinsky, K. S. (2000). Striatal serotonin is depleted in brain of a human MDMA (ecstasy) user. *Neurology, 55*, 294–296.

Kish, S. J., Kalasinsky, K. S., Derkach, P., Schmunk, G. A., Guttman, M., Ang, L., Adams, V., Furukawa, Y., & Haycock, J. W. (2001). Striatal dopaminergic and serotonergic markers in human heroin users. *Neuropsychopharmacology, 24*, 561–567.

Kish, S. J., Fitzmaurice, P. S., Boileau, I., Schmunk, G. A., Ang, L. C., Furukawa, Y., Chang, L. J., Wickham, D. J., Sherwin, A., & Tong, J. (2009). Brain serotonin transporter in human methamphetamine users. *Psychopharmacology, 202*, 649–661.

Kish, S. J., Fitzmaurice, P. S., Chang, L. J., Furukawa, Y., & Tong, J. (2010). Low striatal serotonin transporter protein in a human polydrug MDMA (ecstasy) user: A case study. *Journal of Psychopharmacology, 24*, 281–284.

Kita, T., Wagner, G. C., & Nakashima, T. (2003). Current research on methamphetamine-induced neurotoxicity: Animal models of monoamine disruption. *Journal of Pharmacological Sciences, 92*, 178–195.

Kitamura, O. (2009). Detection of methamphetamine neurotoxicity in forensic autopsy cases. *Legal Medicine (Tokyo), 11*, S63–S65.

Kitamura, O., Tokunaga, I., Gotohda, T., & Kubo, S. (2007). Immunohistochemical investigation of dopaminergic terminal markers and caspase-3 activation in the striatum of human methamphetamine users. *International Journal of Legal Medicine, 121*, 163–168.

Kitamura, O., Takeichi, T., Wang, E. L., Tokunaga, I., Ishigami, A., & Kubo, S. (2010). Microglial and astrocytic changes in the striatum of methamphetamine abusers. *Legal Medicine (Tokyo), 12*, 57–62.

Kobayashi, H., Hata, H., Ujike, H., Harano, M., Inada, T., Komiyama, T., Yamada, M., Sekine, Y., Iwata, N., Iyo, M., Ozaki, N., Itokawa, M., Naka, M., Ide, S., Ikeda, K., Numachi, Y., & Sora, I. (2006). Association analysis of delta-opioid receptor gene polymorphisms in methamphetamine dependence/psychosis. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 141B*, 482–446.

Koob, G. F., & Bloom, F. E. (1988). Cellular and molecular mechanisms of drug dependence. *Science, 242*, 715–723.

Koob, G. F., & Swerdlow, N. R. (1988). The functional output of the mesolimbic dopamine system. *Annals of the New York Academy of Sciences, 537*, 216–227.

Kovacic, P. (2005). Role of oxidative metabolites of cocaine in toxicity and addiction: Oxidative stress and electron transfer. *Medical Hypotheses, 64*, 350–356.

Kovatsi, L., Njau, S., Nikolaou, K., Topouridou, K., Papamitsou, T., & Koliakos, G. (2010). Evaluation of prooxidant-antioxidant balance in chronic heroin users in a single assay: An identification criterion for antioxidant supplementation. *The American Journal of Drug and Alcohol Abuse, 36*, 228–232.

Kovatsi, L., Fragou, D., Samanidou, V., Njau, S., & Kouidou, S. (2011). Drugs of abuse: Epigenetic mechanisms in toxicity and addiction. *Current Medicinal Chemistry, 18*, 1765–1774.

Krasnova, I. N., & Cadet, J. L. (2009). Methamphetamine toxicity and messengers of death. *Brain Research Review, 60*, 379–407.

Krasnova, I. N., Ladenheim, B., Jayanthi, S., Oyler, J., Moran, T. H., Huestis, M. A., & Cadet, J. L. (2001). Amphetamine-induced toxicity in dopamine terminals in CD-1 and C57BL/6J mice: Complex roles for oxygen-based species and temperature regulation. *Neuroscience, 107*, 265–274.

Krasnova, I. N., Ladenheim, B., & Cadet, J. L. (2005). Amphetamine induces apoptosis of medium spiny striatal projection neurons via the mitochondria-dependent pathway. *The FASEB Journal, 19*, 851–853.

Kraus, M. A., Piper, J. M., & Kornetsky, C. (1997). Persistent increases in basal cerebral metabolic activity induced by morphine sensitization. *Pharmacology, Biochemistry, and Behavior, 57*, 89–100.

Kristiansen, L. V., Bannon, M. J., & Meador-Woodruff, J. H. (2009). Expression of transcripts for myelin related genes in postmortem brain from cocaine abusers. *Neurochemical Research, 34*, 46–54.

Kroemer, G., Galluzzi, L., & Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. *Physiological Reviews, 87*, 99–163.

Kuczenski, R. (1975). Effects of catecholamine releasing agents on synaptosomal dopamine biosynthesis: Multiple pools of dopamine or multiple forms of tyrosine hydroxylase. *Neuropharmacology, 14*, 1–10.

Kuhn, D. M., & Arthur, R., Jr. (1997). Molecular mechanism of the inactivation of tryptophan hydroxylase by nitric oxide: Attack on critical sulfhydryls that spare the enzyme iron center. *Journal of Neuroscience, 17*, 7245–7251.

Kuhn, D. M., Aretha, C. W., & Geddes, T. J. (1999). Peroxynitrite inactivation of tyrosine hydroxylase: Mediation by sulfhydryl oxidation, not tyrosine nitration. *Journal of Neuroscience, 19*, 10289–10294.

Kuhn, D. M., Francescutti-Verbeem, D. M., & Thomas, D. M. (2006). Dopamine quinones activate microglia and induce a neurotoxic gene expression profile: Relationship to methamphetamine-induced nerve ending damage. *Annals of the New York Academy of Sciences, 1074*, 31–41.

Kumar, A., Choi, K., Renthal, W., Tsankova, N. M., Theobald, D. E. H., Truong, H. T., Russo, S. J., LaPlant, Q., Sasaki, T. S., Whistler, K. N., Neve, R. L., Self, D. W., & Nestler, E. J. (2005). Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron, 48*, 303–314.

Lane, S. D., Steinberg, J. L., Ma, L., Hasan, K. M., Kramer, L. A., Zuniga, E. A., Narayana, P. A., & Moeller, F. G. (2010). Diffusion tensor imaging and decision making in cocaine dependence. *PLoS One, 5*, 11591.

LaPlant, Q., Vialou, V., Covington, H. E., 3rd, Dumitriu, D., Feng, J., Warren, B. L., Maze, I., Dietz, D. M., Watts, E. L., Iñiguez, S. D., Koo, J. W., Mouzon, E., Renthal, W., Hollis, F., Wang, H., Noonan, M. A., Ren, Y., Eisch, A. J., Bolaños, C. A., Kabbaj, M., Xiao, G., Neve, R. L., Hurd, Y. L., Oosting, R. S., Fan, G., Morrison, J. H., & Nestler, E. J. (2010). Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nature Neuroscience, 13*, 1137–1143.

Law, P. Y., Koehler, J. E., & Loh, H. H. (1982). Comparison of opiate inhibition of adenylate cyclase activity in neuroblastoma N18tG2 and neuroblastoma x glioma NG108-15 hybrid cell lines. *Molecular Pharmacology, 21*, 483–491.

Lecca, D., Cacciapaglia, F., Valentini, V., Acquas, E., & Di Chiara, G. (2007). Differential neurochemical and behavioral adaptation to cocaine after response contingent and noncontingent exposure in the rat. *Psychopharmacology, 191*, 653–667.

Lehrmann, E., Colantuoni, C., Deep-Soboslay, A., Becker, K. G., Lowe, R., Huestis, M. A., Hyde, T. M., Kleinman, J. E., & Freed, W. J. (2006). Transcriptional changes common to human cocaine, cannabis and phencyclidine abuse. *PLoS One, 1*, 114.

Letchworth, S. R., Nader, M. A., Smith, H. R., Friedman, D. P., & Porrino, L. J. (2001). Progression of changes in dopamine transporter binding site density as a result of cocaine self-administration in rhesus monkeys. *Journal of Neuroscience, 21*, 2799–2807.

Levin, J. M., & Kaufman, M. J. (1995). Functional studies in substance abuse: Imaging and beyond. *Journal of Nuclear Medicine, 36*, 1298–1300.

Levin, J. M., Mendelson, J. H., Holman, B. L., Teoh, S. K., Garada, B., Schwartz, R. B., & Mello, N. K. (1995). Improved regional cerebral blood flow in chronic cocaine polydrug users treated with buprenorphine. *Journal of Nuclear Medicine, 36*, 1211–1215.

Levine, A. A., Guan, Z., Barco, A., Xu, S., Kandel, E. R., & Schwartz, J. H. (2005). CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 19186–19891.

Levran, O., O'Hara, K., Peles, E., Li, D., Barral, S., Ray, B., Borg, L., Ott, J., Adelson, M., & Kreek, M. J. (2008). ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. *Human Molecular Genetics, 17*, 2219–2227.

Levran, O., Londono, D., O'Hara, K., Randesi, M., Rotrosen, J., Casadonte, P., Linzy, S., Ott, J., Adelson, M., & Kreek, M. J. (2009). Heroin addiction in African Americans: A hypothesis-driven association study. *Genes, Brain, and Behavior, 8*, 531–540.

Levran, O., Awolesi, O., Shen, P. H., Adelson, M., Kreek, M. J. (2012). Estimating ancestral proportions in a multi-ethnic US sample: Implications for studies of admixed populations. *Human Genomics*, 6, 2 (Epub ahead of print).

Li, M. D., & van der Vaart, A. D. (2011). MicroRNAs in addiction: Adaptation's Middlemen? *Molecular Psychiatry, 16*, 1159–1168.

Licata, S. C., & Renshaw, P. F. (2010). Neurochemistry of drug action Insights from proton magnetic resonance spectroscopic imaging and their relevance to addiction. *Annals of the New York Academy of Sciences, 1187*, 148–171.

Lin, X., Wang, Q., Cheng, Y., Ji, J., & Yu, L. C. (2011). Changes of protein expression profiles in the amygdala during the process of morphine-induced conditioned place preference in rats. *Behavioural Brain Research, 221*, 197–206.

Lippi, G., Steinert, J. R., Marczylo, E. L., D'Oro, S., Fiore, R., Forsythe, I. D., Schratt, G., Zoli, M., Nicotera, P., & Young, K. W. (2011). Targeting of the Arpc3 actin nucleation factor by miR-29a/b regulates dendritic spine morphology. *The Journal of Cell Biology, 194*, 889–904.

Little, K. Y., Krolewski, D. M., Zhang, L., & Cassin, B. J. (2003). Loss of striatal vesicular monoamine transporter protein (VMAT2) in human cocaine users. *The American Journal of Psychiatry, 160*, 47–55.

Little, K. Y., Ramssen, E., Welchko, R., Volberg, V., Roland, C. J., & Cassin, B. (2009). Decreased brain dopamine cell numbers in human cocaine users. *Psychiatry Research, 168*, 173–180.

London, E. D., Simon, S. L., Berman, S. M., Mandelkern, M. A., Lichtman, A. M., Bramen, J., Shinn, A. K., Miotto, K., Learn, J., Dong, Y., Matochik, J. A., Kurian, V., Newton, T., Woods, R., Rawson, R., & Ling, W. (2004). Mood disturbances and regional cerebral metabolic abnormalities in recently abstinent methamphetamine abusers. *Archives of General Psychiatry, 61*, 73–84.

Lyons, D., Friedman, D. P., Nader, M. A., & Porrino, L. J. (1996). Cocaine alters cerebral metabolism within the ventral striatum and limbic cortex of monkeys. *Journal of Neuroscience, 16*, 1230–1238.

Lyoo, I. K., Pollackm, M. H., Silveri, M. M., Ahn, K. H., Diaz, C. I., Hwang, J., Kim, S. J., Yurgelun-Todd, D. A., Kaufman, M. J., & Renshaw, P. F. (2006). Prefrontal and temporal gray matter density decreases in opiate dependence. *Psychopharmacology, 184*, 139–144.

Macedo, D. S., deVasconcelos, S. M., dosSantos, R. S., Aguiar, L. M., Lima, V. T., Viana, G. S., & deSousa, F. C. (2005). Cocaine alters catalase activity in prefrontal cortex and striatum of mice. *Neuroscience Letters, 387*, 53–56.

Macedo, D. S., deVasconcelos, S. M., Andrate-Neto, M., Belchior, L. D., Honorino, J. E. R., Goncalves, D. O., Fonteles, M. M. F., Silva, M. I. G., Aguiar, L. M. V., Viana, G. S. B., & Florenco de Sousa, F. C. (2010). Cocaine-induced status epilepticus and death generate oxidative stress in prefrontal cortex and striatum of mice. *Neurochemistry International, 56*, 183–187.

Maciel, E. N., Vercesi, A. E., & Castilho, R. F. (2001). Oxidative stress in Ca(2+)-induced membrane permeability transition in brain mitochondria. *Journal of Neurochemistry, 79*, 1237–1245.

Madayag, A., Lobner, D., Kau, K. S., Mantsch, J. R., Abdulhameed, O., Hearing, M., Grier, M. D., & Baker, D. A. (2007). Repeated N-acetylcysteine administration alters plasticity-dependent effects of cocaine. *Journal of Neuroscience, 27*, 13968–13976.

Malison, R. T., Best, S. E., van Dyck, C. H., McCance, E. F., Wallace, E. A., Laruelle, M., Baldwin, R. M., Seibyl, J. P., Price, L. H., Kosten, T. R., & Innis, R. B. (1998). Elevated striatal dopamine transporters during acute cocaine abstinence as measured by [^{123}I] beta-CIT SPECT. *The American Journal of Psychiatry, 155*, 832–834.

Mao, J., Sung, B., Ji, R. R., & Lim, G. (2002). Neuronal apoptosis associated with morphine tolerance: Evidence for an opioid-induced neurotoxic mechanism. *Journal of Neuroscience, 22*, 7650–7661.

Marcellino, D., Navarro, G., Sahlholm, K., Nilsson, J., Agnati, L. F., Canela, E. I., Lluís, C., Århem, P., Franco, R., & Fuxe, K. (2010). Cocaine produces D_2R-mediated conformational changes in the adenosine A(2A)R-dopamine D_2R heteromer. *Biochemical and Biophysical Research Communications, 394*, 988–992.

Martin, M., Hurley, R. A., & Taber, K. H. (2007). Is opiate addiction associated with longstanding neurobiological changes? *Journal of Neuropsychiatry and Clinical Neurosciences, 19*, 242–248.

Martin, T. A., Jayanthi, S., McCoy, M. T., Brannock, C., Ladenheim, B., Garrett, T., Lehrmann, E., Becker, K. G., & Cadet, J. L. (2012). Methamphetamine causes differential alterations in gene expression and patterns of histone acetylation/hypoacetylation in the rat nucleus accumbens. *PLoS One, 7*, e34236.

Martinez, D., Greene, K., Broft, A., Kumar, D., Liu, F., Narendran, R., Slifstein, M., Van Heertum, R., & Kleber, H. D. (2009). Lower level of endogenous dopamine in patients with cocaine dependence: Findings from PET imaging of D(2)/D(3) receptors following acute dopamine depletion. *The American Journal of Psychiatry, 166*, 1170–1177.

Martinez, C. M., Neudörffer, A., & Largeron, M. (2012). A convenient biomimetic synthesis of optically active putative neurotoxic metabolites of MDMA ("ecstasy") from R-(−)- and S-(+)-N-methyl-α-methyldopamine precursors. *Organic & Biomolecular Chemistry, 10*, 3739–3748.

Martin-Soelch, C., Leenders, K. L., Chevalley, A. F., Missimer, J., Künig, G., Magyar, S., Mino, A., & Schultz, W. (2001). Reward mechanisms in the brain and their role in dependence: Evidence from neurophysiological and neuroimaging studies. *Brain Research Reviews, 36*, 139–149.

Maze, I., Covington, H. E., 3rd, Dietz, D. M., LaPlant, Q., Renthal, W., Russo, S. J., Mechanic, M., Mouzon, E., Neve, R. L., Haggarty, S. J., Ren, Y., Sampath, S. C., Hurd, Y. L., Greengard, P., Tarakhovsky, A., Schaefer, A., & Nestler, E. J. (2010). Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science, 327*, 213–216.

Mazei-Robison, M. S., Koo, J. W., Friedman, A. K., Lansink, C. S., Robison, A. J., Vinish, M., Krishnan, V., Kim, S., Siuta, M. A., Galli, A., Niswender, K. D., Appasani, R., Horvath, M. C., Neve, R. L., Worley, P. F., Snyder, S. H., Hurd, Y. L., Cheer, J. F., Han, M. H., Russo, S. J., & Nestler, E. J. (2011). Role for mTOR signaling and neuronal activity in morphine-induced adaptations in ventral tegmental area dopamine neurons. *Neuron, 72*, 977–990.

McCann, U. D., & Ricaurte, G. A. (2004). Amphetamine neurotoxicity: Accomplishments and remaining challenges. *Neuroscience and Biobehavioral Reviews, 27*, 821–826.

McCann, U. D., Ridenour, A., Shaham, Y., & Ricaurte, G. A. (1994). Serotonin neurotoxicity after (+/−)3,4-methylenedioxymethamphetamine (MDMA; "ecstasy"): A controlled study in humans. *Neuropsychopharmacology, 10*, 129–138.

McCann, U. D., Szabo, Z., Scheffel, U., Dannals, R. F., & Ricaurte, G. A. (1998a). Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet, 352*, 1433–1437.

McCann, U. D., Wong, D. F., Yokoi, F., Villemagne, V., Dannals, R. F., & Ricaurte, G. A. (1998b). Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: Evidence from positron emission tomography studies with [^{11}C]WIN-35,428. *Journal of Neuroscience, 18*, 8417–8422.

McCann, U. D., Mertl, M., Eligulashvili, V., & Ricaurte, G. A. (1999). Cognitive performance in (+/−) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") users: A controlled study. *Psychopharmacology, 143*, 417–425.

McCann, U. D., Kuwabara, H., Kumar, A., Palermo, M., Abbey, R., Brasic, J., Ye, W., Alexander, M., Dannals, R. F., Wong, D. F., & Ricaurte, G. A. (2008). Persistent cognitive and dopamine transporter deficits in abstinent methamphetamine users. *Synapse, 62*, 91–100.

McFarland, K., Lapish, C. C., & Kalivas, P. W. (2003). Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *Journal of Neuroscience, 23*, 3531–3537.

McKetin, R., McLaren, J., Riddell, S., & Robins, L. (2006). The prevalence of psychotic symptoms among methamphetamine users. *Addiction, 101*, 1473–1478.

McQuown, S. C., & Wood, M. A. (2010). Epigenetic regulation in substance use disorders. *Current Psychiatry Reports, 12*, 145–153.

Melega, W. P., Raleigh, M. J., Stout, D. B., Lacan, G., Huang, S. C., & Phelps, M. E. (1997). Recovery of striatal dopamine function after acute amphetamine- and methamphetamine-induced neurotoxicity in the vervet monkey. *Brain Research, 766*, 113–120.

Melega, W. P., Lacan, G., Desalles, A. A., & Phelps, M. E. (2000). Long-term methamphetamine-induced decreases of [(11)C] WIN 35,428 binding in striatum are reduced by GDNF: PET studies in the vervet monkey. *Synapse, 35*, 243–249.

Melega, W. P., Jorgensen, M. J., Lacan, G., Way, B. M., Pham, J., Morton, G., Cho, A. K., & Fairbanks, L. A. (2008). Long-term methamphetamine administration in the vervet monkey models aspects of a human exposure: Brain neurotoxicity and behavioral profiles. *Neuropsychopharmacology, 33*, 1441–1452.

Michelhaugh, S. K., Lipovich, L., Blythe, J., Jia, H., Kapatos, G., & Bannon, M. J. (2011). Mining affymetrix microarray data for long noncoding RNAs: Altered expression in the nucleus accumbens of heroin abusers. *Journal of Neurochemistry, 116*, 459–466.

Miguéns, M., Del Olmo, N., Higuera-Matas, A., Torres, I., García-Lecumberri, C., & Ambrosio, E. (2008). Glutamate and aspartate levels in the nucleus accumbens during cocaine self-administration and extinction: A time course microdialysis study. *Psychopharmacology, 196*, 303–313.

Milhazes, N., Cunha-Oliveira, T., Martins, P., Garrido, J., Oliveira, C., Rego, A. C., & Borges, F. (2006). Synthesis and cytotoxic profile of 3,4-methylenedioxymethamphetamine ("ecstasy") and its metabolites on undifferentiated PC12 cells: A putative structure-toxicity relationship. *Chemical Research in Toxicology, 19*, 1294–1304.

Miller, A. L., Hawkins, R. A., Harris, R. L., & Veech, R. L. (1972). The effects of acute and chronic morphine treatment and of morphine withdrawal on rat brain in vivo. *Biochemical Journal, 129*, 463–469.

Milroy, C. M., Clark, J. C., & Forrest, A. R. W. (1996). Pathology of deaths associated with "ecstasy" and "eve" misuse. *Journal of Clinical Pathology, 49*, 149–153.

Miranda, M., Bosch-Morell, F., Johnsen-Soriano, S., Barcia, J., Almansa, I., Asensio, S., Araiz, J., Messeguer, A., & Romero, F. J. (2007). Oxidative stress in rat retina and hippocampus after chronic MDMA ('ecstasy') administration. *Neurochemical Research, 32*, 1156–1162.

Mirecki, A., Fitzmaurice, P., Ang, L., Kalasinsky, K. S., Peretti, F. J., Aiken, S. S., Wickham, D. J., Sherwin, A., Nobrega, J. N., Forman, H. J., & Kish, S. J. (2004). Brain antioxidant systems in human methamphetamine users. *Journal of Neurochemistry, 89*, 1396–1408.

Moeller, F. G., Steinberg, J. L., Schmitz, J. M., Ma, L., Liu, S., Kjome, K. L., Rathnayaka, N., Kramer, L. A., & Narayana, P. A. (2010). Working memory fMRI activation in cocaine-dependent subjects: Association with treatment response. *Psychiatry Research, 181*, 174–182.

Moore, R. J., Vinsant, S. L., Nader, M. A., Porrino, L. J., & Friedman, D. P. (1998). Effect of cocaine self-administration on striatal dopamine D_1 receptors in rhesus monkeys. *Synapse, 28*, 1–9.

Morgan, M. E., & Gibb, J. W. (1980). Short-term and long-term effects of methamphetamine on biogenic amine metabolism in extra-striatal dopaminergic nuclei. *Neuropharmacology, 19*, 989–995.

Moszczynska, A., Turenne, S., & Kish, S. J. (1998). Rat striatal levels of the antioxidant glutathione are decreased following binge administration of methamphetamine. *Neuroscience Letters, 255*, 49–52.

Moszczynska, A., Fitzmaurice, P., Ang, L., Kalasinsky, K. S., Schmunk, G. A., Peretti, F. J., Aiken, S. S., Wickham, D. J., & Kish, S. J. (2004). Why is parkinsonism not a feature of human methamphetamine users? *Brain, 127*, 363–370.

Muriach, M., Lopez-Pedrajas, R., Barcia, J. M., Sanchez-Villarejo, M. V., Almansa, I., & Romero, F. J. (2010). Cocaine causes memory and learning impairments in rats: Involvement of nuclear factor kappa B and oxidative stress, and prevention by topiramate. *Journal of Neurochemistry, 114*, 675–684.

Murnane, K. S., & Howell, L. L. (2011). Neuroimaging and drug taking in primates. *Psychopharmacology, 216*, 153–171.

Nader, M. A., Daunais, J. B., Moore, T., Nader, S. H., Moore, R. J., Smith, H. R., Friedman, D. P., & Porrino, L. J. (2002). Effects of cocaine self-administration on striatal dopamine systems in rhesus monkeys: Initial and chronic exposure. *Neuropsychopharmacology, 27*, 35–46.

Nader, M. A., Morgan, D., Gage, H. D., Nader, S. H., Calhoun, T. L., Buchheimer, N., Ehrenkaufer, R., & Mach, R. H. (2006). PET imaging of dopamine D_2 receptors during chronic cocaine self-administration in monkeys. *Nature Neuroscience, 9*, 1050–1056.

Narendran, R., Lopresti, B. J., Martinez, D., Mason, N. S., Himes, M., May, M. A., Daley, D. C., Price, J. C., Mathis, C. A., & Frankle, W. G. (2012). In vivo evidence for low striatal vesicular monoamine transporter 2 (VMAT2) availability in cocaine abusers. *The American Journal of Psychiatry, 169*, 55–63.

Nassogne, M. C., Evrard, P., & Courtoy, P. J. (1998). Selective direct toxicity of cocaine on fetal mouse neurons. Teratogenic implications of neurite and apoptotic neuronal loss. *Annals of the New York Academy of Sciences, 846*, 51–68.

Nicol, B., Rowbotham, D. J., & Lambert, D. G. (1996). mu- and kappa-opioids inhibit K^+ evoked glutamate release from rat cerebrocortical slices. *Neuroscience Letters, 218*, 79–82.

Nielsen, D. A., Yuferov, V., Hamon, S., Jackson, C., Ho, A., Ott, J., & Kreek, M. J. (2009). Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. *Neuropsychopharmacology, 34*, 867–873.

Novikova, S. I., He, F., Bai, J., Cutrufello, N. J., Lidow, M. S., & Undieh, A. S. (2008). Maternal cocaine administration in mice alters DNA methylation and gene expression in hippocampal neurons of neonatal and prepubertal offspring. *PLoS One, 3*, e1919.

Numa, R., Kohen, R., Poltyrev, T., & Yaka, R. (2008). Tempol diminishes cocaine-induced oxidative damage and attenuates the development and expression of behavioral sensitization. *Neuroscience, 155*, 649–658.

Numachi, Y., Yoshida, S., Yamashita, M., Fujiyama, K., Naka, M., Matsuoka, H., Sato, M., & Sora, I. (2004). Psychostimulant alters expression of DNA methyltransferase mRNA in the rat brain. *Annals of the New York Academy of Sciences, 1025*, 102–109.

Numachi, Y., Shen, H., Yoshida, S., Fujiyama, K., Toda, S., Matsuoka, H., Sora, I., & Sato, M. (2007). Methamphetamine alters expression of DNA methyltransferase 1 mRNA in rat brain. *Neuroscience Letters, 414*, 213–217.

O'Hearn, E., Battaglia, G., De Souza, E. B., Kuhar, M. J., & Molliver, M. E. (1988). Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: Immunocytochemical evidence for neurotoxicity. *Journal of Neuroscience, 8*, 2788–2803.

O'Leary, G., Nargiso, J., & Weiss, R. D. (2001). 3,4-Methylenedioxymethamphetamine (MDMA): A review. *Current Psychiatry Report, 3*, 477–483.

Oliveira, M. T., Rego, A. C., Morgadinho, M. T., Macedo, T. R., & Oliveira, C. R. (2002). Toxic effects of opioid and stimulant drugs on undifferentiated PC12 cells. *Annals New York Academy of Science, 965*, 487–496.

Oliveira, M. T., Rego, A. C., Macedo, T. R., & Oliveira, C. R. (2003). Drugs of abuse induce apoptotic features in PC12 cells. *Annals New York Academy of Science, 1010*, 667–670.

Orio, L., O'Shea, E., Sanchez, V., Pradillo, J. M., Escobedo, I., Camarero, J., Moro, M. A., Green, A. R., & Colado, M. I. (2004). 3,4-Methylenedioxymethamphetamine increases interleukin-1beta levels and activates microglia in rat brain: Studies on the relationship with acute hyperthermia and 5-HT depletion. *Journal of Neurochemistry, 89*, 1445–1453.

Orzi, F., Dow-Edwards, D., Jehle, J., Kennedy, C., & Sokoloff, L. (1983). Comparative effects of acute and chronic administration of amphetamine on local cerebral glucose utilization in the conscious rat. *Journal of Cerebral Blood Flow and Metabolism, 3*, 154–160.

Parrott, A. C. (2012). MDMA and temperature: A review of the thermal effects of 'Ecstasy' in humans. *Drug and Alcohol Dependence, 121*, 1–9.

Pettit, H. O., & Justice, J. B., Jr. (1989). Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis. *Pharmacology Biochemistry and Behavior, 34*, 899–904.

Piechota, M., Korostynski, M., Sikora, M., Golda, S., Dzbek, J., & Przewlocki, R. (2012). Common transcriptional effects in the mouse striatum following chronic treatment with heroin and methamphetamine. *Genes, Brain, and Behavior, 11*, 404–414.

Piñeyro, G., & Archer-Lahlou, E. (2007). Ligand-specific receptor states: Implications for opiate receptor signalling and regulation. *Cellular Signalling, 19*, 8–19.

Pomierny-Chamiolo, L., Moniczewski, A., Wydra, K., Suder, A., & Filip, M. (2012). Oxidative stress biomarkers in some rat brain structures and peripheral organs underwent cocaine. *Neurotoxicity Research, 23*, 92–102.

Pontieri, F. E., Tanda, G., Orzi, F., & Di Chiara, G. (1996). Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. *Nature, 382*, 255–257.

Porrino, L. J., Lucignani, G., Dow-Edwards, D., & Sokoloff, L. (1984). Correlation of dose-dependent effects of acute amphetamine administration on behavior and local cerebral metabolism in rats. *Brain Research, 307*, 311–320.

Porrino, L. J., Daunais, J. B., Smith, H. R., & Nader, M. A. (2004). The expanding effects of cocaine: Studies in a nonhuman primate model of cocaine self-administration. *Neuroscience and Biobehavioral Reviews, 27*, 813–820.

Porter, J. N., Olsen, A. S., Gurnsey, K., Dugan, B. P., Jedema, H. P., & Bradberry, C. W. (2011). Chronic cocaine self-administration in rhesus monkeys: Impact on associative learning, cognitive control, and working memory. *Journal of Neuroscience, 31*, 4926–4934.

Preston, K. L., Wagner, G. C., Schuster, C. R., & Seiden, L. S. (1985). Long-term effects of repeated methylamphetamine administration on monoamine neurons in the rhesus monkey brain. *Brain Research, 338*, 243–248.

Pu, L., Bao, G. B., Xu, N. J., Ma, L., & Pei, G. (2002). Hippocampal long-term potentiation is reduced by chronic opiate treatment and can be restored by re-exposure to opiates. *Journal of Neuroscience, 22*, 1914–1921.

Pubill, D., Verdaguer, E., Sureda, F. X., Camins, A., Pallàs, M., Camarasa, J., & Escubedo, E. (2002). Carnosine prevents methamphetamine-induced gliosis but not dopamine terminal loss in rats. *European Journal of Pharmacology, 448*, 165–168.

Puckett, R. E., & Lubin, F. D. (2011). Epigenetic mechanisms in experience-driven memory formation and behavior. *Epigenomics, 3*, 649–664.

Qiusheng, Z., Yuntao, Z., Rongliang, Z., Dean, G., & Changling, L. (2005). Effects of verbascoside and luteolin on oxidative damage in brain of heroin treated mice. *Pharmazie, 60*, 539–543.

Quinton, M. S., & Yamamoto, B. K. (2006). Causes and consequences of methamphetamine and MDMA toxicity. *The AAPS Journal, 8*, 337–347.

Ramage, S. N., Anthony, I. C., Carnie, F. W., Busuttil, A., Robertson, R., & Bell, J. E. (2005). Hyperphosphorylated tau and amyloid precursor protein deposition is increased in the brains of young drug abusers. *Neuropathology and Applied Neurobiology, 31*, 439–448.

Ramonet, D., Rodríguez, M. J., Fredriksson, K., Bernal, F., & Mahy, N. (2004). In vivo neuroprotective adaptation of the glutamate/glutamine cycle to neuronal death. *Hippocampus, 14*, 586–594.

Ramsay, R. R., & Hunter, D. J. (2002). Inhibitors alter the spectrum and redox properties of monoamine oxidase A. *Biochimica et Biophysica Acta, 1601*, 178–184.

Rego, A. C., & Oliveira, C. R. (2003). Mitochondrial dysfunction and reactive oxygen species in excitotoxicity and apoptosis: Implications for the pathogenesis of neurodegenerative diseases. *Neurochemical Research, 28*, 1563–1574.

Reneman, L., Majoie, C. B., Schmand, B., van den Brink, W., & den Heeten, G. J. (2001). Prefrontal N-acetylaspartate is strongly associated with memory performance in (abstinent) ecstasy users: Preliminary report. *Biological Psychiatry, 50*, 550–554.

Reneman, L., Booij, J., Lavalaye, J., de Bruin, K., Reitsma, J. B., Gunning, B., den Heeten, G. J., & van Den Brink, W. (2002). Use of amphetamine by recreational users of ecstasy (MDMA) is associated with reduced striatal dopamine transporter densities: A [^{123}I]beta-CIT SPECT study—preliminary report. *Psychopharmacology, 159*, 335–340.

Renthal, W., & Nestler, E. J. (2008). Epigenetic mechanisms in drug addiction. *Trends in Molecular Medicine, 14*, 341–350.

Renthal, W., Maze, I., Krishnan, V., Covington, H. E., 3rd, Xiao, G., Kumar, A., Russo, S. J., Graham, A., Tsankova, N., Kippin, T. E., Kerstetter, K. A., Neve, R. L., Haggarty, S. J., McKinsey, T. A., Bassel-Duby, R., Olson, E. N., & Nestler, E. J. (2007). Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron, 56*, 517–529.

Renthal, W., Kumar, A., Xiao, G., Wilkinson, M., Covington, H. E. I. I. I., Maze, I., Sikder, D., Robison, A. J., LaPlant, Q., Dietz, D. M., Russo, S. J., Vialou, V., Chakravarty, S., Kodadek, T. J., Stack, A., Kabbaj, M., & Nestler, E. J. (2009). Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. *Neuron, 62*, 335–348.

Ricaurte, G. A., Schuster, C. R., & Seiden, L. S. (1980). Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: A regional study. *Brain Research, 193*, 153–163.

Ricaurte, G. A., Guillery, R. W., Seiden, L. S., Schuster, C. R., & Moore, R. Y. (1982). Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Research, 235*, 93–103.

Ricaurte, G. A., Finnegan, K. T., Irwin, I., & Langston, J. W. (1990). Aminergic metabolites in cerebrospinal fluid of humans previously exposed MDMA: Preliminary observations. *Annals in New York Academy of Science, 600*, 699–710.

Ricaurte, G. A., Martello, A. L., Katz, J. L., & Martello, M. B. (1992). Lasting effects of (+−)-3,4 methylenedioxymeth¬amphetamine (MDMA) on central serotonergic neurons in nonhuman primates: Neurochemical observations. *Journal of Pharmacology and Experimental Therapeutics, 261*, 616–622.

Ricaurte, G. A., McCann, U. D., Szabo, Z., & Scheffel, U. (2000). Toxicodynamics and long-term toxicity of the recreational drug, 3, 4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'). *Toxicology Letters, 112–113*, 143–146.

Riddle, E. L., Topham, M. K., Haycock, J. W., Hanson, G. R., & Fleckenstein, A. E. (2002). Differential trafficking of the vesicular monoamine transporter-2 by methamphetamine and cocaine. *European Journal of Pharmacology, 449*, 71–74.

Robison, A. J., & Nestler, E. J. (2011). Transcriptional and epigenetic mechanisms of addiction. *Nature Reviews Neuroscience, 12*, 623–637.

Rocher, C., & Gardier, A. M. (2001). Effects of repeated systemic administration of d-fenfluramine on serotonin and glutamate release in rat ventral hippocampus: Comparison with methamphetamine using in vivo microdialysis. *Naunyn-Schmiedeberg's Archives of Pharmacology, 363*, 422–428.

Romero, M. J., Asensio, S., Palau, C., Sanchez, A., & Romero, F. J. (2010). Cocaine addiction: Diffusion tensor imaging study of the inferior frontal and anterior cingulate white matter. *Psychiatry Research, 181*, 57–63.

Romieu, P., Host, L., Gobaille, S., Sandner, G., Aunis, D., & Zwiller, J. (2008). Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. *Journal of Neuroscience, 28*, 9342–9348.

Rose, J. S., Branchey, M., Buydens-Branchey, L., Stapleton, J. M., Chasten, K., Werrell, A., & Maayan, M. L. (1996). Cerebral perfusion in early and late opiate withdrawal: A technetium-99m-HMPAO SPECT study. *Psychiatry Research, 67*, 39–47.

Rose-Jacobs, R., Cabral, H., Posner, M. A., Epstein, J., & Frank, D. A. (2002). Do "we just know"? Masked assessors' ability to accurately identify children with prenatal cocaine exposure. *Journal of Developmental and Behavioral Pediatrics, 23*, 340–346.

Rothman, R. B., & Baumann, M. H. (2003). Monoamine transporters and psychostimulant drugs. *European Journal of Pharmacology, 479*, 23–40.

Rottlant, D., & Armario, A. (2012). Brain pattern of histone H3 phosphorylation after acute amphetamine administration: Its relationship to brain c-fos induction is strongly dependent on the particular brain area. *Neuropharmacology, 62*, 1073–1081.

Ryan, A., Molloy, F. M., Farrell, M. A., & Hutchinson, M. (2005). Fatal toxic leukoencephalopathy: Clinical, radiological, and necropsy findings in two patients. *Journal of Neurology, Neurosurgery, and Psychiatry, 76*, 1014–1016.

Saba, R., Storchel, P. H., Aksoy-Aksel, A., Kepura, F., Lippi, G., Plant, T. D., & Schratt, G. M. (2012). Dopamine-regulated MicroRNA MiR-181a controls GluA2 surface expression in hippocampal neurons. *Molecular and Cellular Biology, 32*, 619–632.

Saddoris, M. P., Stamatakis, A., & Carelli, R. M. (2011). Neural correlates of Pavlovian-to-instrumental transfer in the nucleus accumbens shell are selectively potentiated following cocaine self-administration. *European Journal of Neuroscience, 33*, 2274–2287.

Samantaray, S., Ray, S. K., Ali, S. F., & Banik, N. L. (2006). Calpain activation in apoptosis of motoneurons in cell culture models of experimental parkinsonism. *Annals New York Academy of Science, 1074*, 349–256.

Sawynok, J. (1986). The therapeutic use of heroin: A review of the pharmacological literature. *Canadian Journal of Physiology and Pharmacology, 64*, 1–6.

Schaefer, A., Im, H. I., Venø, M. T., Christie, D., Fowler, C. D., Min, A., Intrator, A., Kjems, J., Kenny, P. J., O'Carroll, D., & Greengard, P. (2010). Argonaute 2 in dopamine 2 receptor–expressing neurons regulates cocaine addiction. *The Journal of Experimental Medicine, 207*, 1843–1851.

Scheffel, U., Szabo, Z., Mathews, W. B., Finley, P. A., Dannals, R. F., Ravert, H. T., Szabo, K., Yuan, J., & Ricaurte, G. A. (1998). *In vivo* detection of short- and long-term MDMA neurotoxicity – a positron emission tomography study in the living baboon brain. *Synapse, 29*, 183–192.

Scott, J. C., Woods, S. P., Matt, G. E., Meyer, R. A., Heaton, R. K., Atkinson, J. H., & Grant, I. (2007). Neurocognitive effects of methamphetamine: A critical review and meta-analysis. *Neuropsychology Review, 17*, 275–297.

Sekine, Y., Minabe, M., Kawai, K., Suzuki, M., Iyo, H., Isoda, H., Sakahara, C. R., Jr, A., Takei, N., & Mori, N. (2002). Metabolite alterations in basal ganglia associated with methamphetamine-related psychiatric symptoms. A proton MRS study. *Neuropsychopharmacology, 27*, 453–461.

Sekine, Y., Minabe, Y., Ouehi, Y., Takei, N., lyo, M., Nakamura, K., Suzuki, K., Tsukada, H., Okada, H., Yoshikawa, E., Futatsubashi, M., & Mori, N. (2003). Association of dopamine transporter loss in the orbitofrontal and dorsolateral prefrontal cortices with methamphetamine-related psychiatric symptoms. *The American Journal of Psychiatry, 160*, 1699–1701.

Sekine, Y., Ouchi, Y., Takei, N., Yoshikawa, E., Nakamura, K., Futatsubashi, M., Okada, H., Minabe, Y., Suzuki, K., Iwata, Y., Tsuchiya, K. J., Tsukada, H., lyo, M., & Mori, N. (2006). Brain serotonin transporter density and aggression in abstinent methamphetamine abusers. *Archives of Generals Psychiatry, 63*, 90–100.

Sekine, Y., Ouchi, Y., Sugihara, G., Takei, N., Yoshikawa, E., Nakamura, K., Iwata, Y., Tsuchiya, K. J., Suda, S., Suzuki, K., Kawai, M., Takebayashi, K., Yamamoto, S., Matsuzaki, H., Ueki, T., Mori, N., Gold, M. S., & Cadet, J. L. (2008). Methamphetamine causes microglial activation in the brains of human abusers. *Journal of Neuroscience, 28*, 5756–5761.

Semple, D. M., Ebmeier, K. P., Glabus, M. F., Ebmeier, K. P., Glabus, M. F., O'Carroll, R. E., Johnstone, E. C., O'Carroll, R. E., & Johnstone, E. C. (1999). Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ('ecstasy') users. *The British Journal of Psychiatry, 175*, 63–69.

Sepúlveda, J., Oliva, P., & Contreras, E. (2004). Neurochemical changes of the extracellular concentrations of glutamate and aspartate in the nucleus accumbens of rats after chronic administration of morphine. *European Journal of Pharmacology, 483*, 249–258.

Sharma, S. K., Klee, W. A., & Nirenberg, M. (1977). Opiate-dependent modulation of adenylate cyclase. *Proceedings of the National Academy of Sciences of the United States of America, 74*, 3365–3369.

Sharma, H. S., Muresanu, D., Sharma, A., & Patnaik, R. (2009). Cocaine-induced breakdown of the blood–brain barrier and neurotoxicity. *International Review of Neurobiology, 88*, 297–334.

Shen, H. Y., Kalda, A., Yu, L., Ferrara, J., Zhu, J., & Chen, J. F. (2008). Additive effects of histone deacetylase inhibitors and amphetamine on histone H4 acetylation, cAMP responsive element binding protein phosphorylation and DeltaFosB expression in the striatum and locomotor sensitization in mice. *Neuroscience, 157*, 644–655.

Sim, M. E., Lyoo, I. K., Streeter, C. C., Covell, J., Sarid-Segal, O., Ciraulo, D. A., Kim, M. J., Kaufman, M. J., Yurgelun-Todd, D. A., & Renshaw, P. F. (2007). Cerebellar gray matter volume correlates with duration of cocaine use in cocaine-dependent subjects. *Neuropsychopharmacology, 32*, 2229–2237.

Sitte, H. H., Huck, S., Reither, H., Boehm, S., Singer, E. A., & Pifl, C. (1998). Carrier-mediated release, transport rates, and charge transfer induced by amphetamine, tyramine, and dopamine in mammalian cells transfected with the human dopamine transporter. *Journal of Neurochemistry, 71*, 1289–1297.

Sizemore, G. M., Co, C., & Smith, J. E. (2000). Ventral pallidal extracellular fluid levels of dopamine, serotonin, gamma amino butyric acid, and glutamate during cocaine self-administration in rats. *Psychopharmacology, 150*, 391–398.

Smith, L. M., Chang, L., Yonekura, M. L., Gilbride, K., Kuo, J., Poland, R. E., Walot, I., & Ernst, T. (2001). Brain proton magnetic resonance spectroscopy and imaging in children exposed to cocaine in utero. *Pediatrics, 107*, 227–231.

Smith, R. J., Doyle, G. A., Han, A. M., Crowley, J. J., Oslin, D. W., Patkar, A. A., Mannelli, P., Demaria, P. A., Jr., O'Brien, C. P., & Berrettini, W. H. (2005). Novel exonic mu-opioid receptor gene (OPRM1) polymorphisms not associated with opioid dependence. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 133B*, 105–109.

Soleimani Asl, S., Falahati, P., Shekar Riz, N., Molavi, N., Esmaeili, F., Azimi, Z., Sajadi, F., Hayat, P., Samzadeh-Kermani, A., & Mehdizadeh, M. (2011). Protective effects of N-acetyl-L-cysteine on 3,4-methylenedioxymethamphetamine-induced neurotoxicity in cerebellum of male rats. *Basic and Clinical Neuroscience, 3*, 44–47.

Soleimani Asl, S., Farhadi, M. H., Moosavizadeh, K., Samadi Kuchak Saraei, A., Soleymani, M., Behnameldin Jamei, S., Joghataei, M. T., Samzadeh-Kermani, A., Hashemi-Nasl, H., & Mehdizadeh, M. (2012). Evaluation of Bcl-2 family gene expression in hippocampus of 3, 4-methylenedioxymethamphetamine treated rats. *Cell Journal, 13*, 275–280.

Soloway, A. H., & Patil, P. N. (2011). Potential role of reactive metabolites of addictive drugs at the receptor: A novel hypothesis. *Medical Hypotheses, 77*, 889–894.

Soto-Montenegro, M. L., Vaquero, J. J., Arango, C., Ricaurte, G., García-Barreno, P., & Desco, M. (2007). Effects of MDMA on blood glucose levels and brain glucose metabolism. *European Journal of Nuclear Medicine and Molecular Imaging, 34*, 916–925.

Spain, J. W., & Newsom, G. C. (1991). Chronic opioids impair acquisition of both radial maze and Y-maze choice escape. *Psychopharmacology, 105*, 101–106.

Spiga, S., Puddu, M. C., Pisano, M., & Diana, M. (2005). Morphine withdrawal-induced morphological changes in the nucleus accumbens. *European Journal of Neuroscience, 22*, 2332–2340.

Sriram, K., Miller, D. B., & O'Callaghan, J. P. (2006). Minocycline attenuates microglial activation but fails to mitigate striatal dopaminergic neurotoxicity: Role of tumor necrosis factor-alpha. *Journal of Neurochemistry, 96*, 706–718.

Staszewski, R. D., & Yamamoto, B. K. (2006). Methamphetamine-induced spectrin proteolysis in the rat striatum. *Journal of Neurochemistry, 96*, 1267–1276.

Stephans, S. E., & Yamamoto, B. K. (1994). Methamphetamine-induced neurotoxicity: Roles for glutamate and dopamine efflux. *Synapse, 17*, 203–209.

Sulzer, D. (2011). How addictive drugs disrupt presynaptic dopamine neurotransmission. *Neuron, 69*, 628–649.

Sulzer, D., Chen, T. K., Lau, Y. Y., Kristensen, H., Rayport, S., & Ewing, A. (1995). Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *Journal of Neuroscience, 15*, 4102–4108.

Sung, Y. H., Cho, S. C., Hwang, J., Kim, S. J., Kim, H., Bae, S., Kim, N., Chang, K. H., Daniels, M., Renshaw, P. F., & Lyoo, I. K. (2007). Relationship between N-acetyl-aspartate in gray and white matter of abstinent methamphetamine abusers and their history of drug abuse: A proton magnetic resonance spectroscopy study. *Drug and Alcohol Dependence, 88*, 28–35.

Szabo, Z., McCann, U. D., Wilson, A. A., Scheffel, U., Owonikoko, T., Mathews, W. B., Ravert, H. T., Hilton, J., Dannals, R. F., & Ricaurte, G. A. (2002). Comparison of (+)-(11)C-McN5652 and (11)C-DASB as serotonin transporter radioligands under various experimental conditions. *Journal of Nuclear Medicine, 43*, 678–692.

Tang, X. C., McFarland, K., Cagle, S., & Kalivas, P. W. (2005). Cocaine-induced reinstatement requires endogenous stimulation of mu-opioid receptors in the ventral pallidum. *Journal of Neuroscience, 25*(18), 4512–4520.

Thomasius, R., Zapletalova, P., Petersen, K., Buchert, R., Andresen, B., Wartberg, L., Nebeling, B., & Schmoldt, A. (2006). Mood, cognition and serotonin transporter availability in current and former ecstasy (MDMA) users: The longitudinal perspective. *Journal of Psychopharmacology, 20*, 211–225.

Thompson, P. M., Hayashi, K. M., Simon, S. L., Geaga, J. A., Hong, M. S., Sui, Y., Lee, J. Y., Toga, A. W., Ling, W., & London, E. D. (2004). Structural abnormalities in the brains of human subjects who use methamphetamine. *Journal of Neuroscience, 24*, 6028–6036.

Timar, J., Gyarmati, S., Szabo, A., & Fürst, S. (2003). Behavioural changes in rats treated with a neurotoxic dose regimen of dextrorotatory amphetamine derivatives. *Behavioural Pharmacology, 14*, 199–206.

Tóth, R., Szegezdi, E., Reichert, U., Bernardon, J. M., Michel, S., Ancian, P., Kis-Tóth, K., Macsári, Z., Fésüs, L., & Szondy, Z. (2001). Activation-induced apoptosis and cell surface expression of Fas (CD95) ligand are reciprocally regulated by retinoic acid receptor alpha and gamma and involve nur77 in T cells. *European Journal of Immunology, 31*, 1382–1391.

Tramullas, M., Martínez-Cué, C., & Hurlé, M. A. (2007). Chronic methadone treatment and repeated withdrawal impair cognition and increase the expression of apoptosis-related proteins in mouse brain. *Psychopharmacology, 193*, 107–120.

Tramullas, M., Martínez-Cué, C., & Hurlé, M. A. (2008). Chronic administration of heroin to mice produces up-regulation of brain apoptosis-related proteins and impairs spatial learning and memory. *Neuropharmacology, 54*, 640–652.

Trexler, A. J., & Rhoades, E. (2012). Function and dysfunction of α-synuclein: Probing conformational changes and aggregation by single molecule fluorescence. *Molecular Neurobiology, 47*(2), 622–31.

Tzschentke, T. M., & Schmidt, W. J. (2003). Glutamatergic mechanisms in addiction. *Molecular Psychiatry, 8*, 373–382.

Uys, J. D., Knackstedt, L., Hurt, P., Tew, K. D., Manevich, Y., Hutchens, S., Townsend, D. M., & Kalivas, P. W. (2011). Cocaine-induced adaptations in cellular redox balance contributes to enduring behavioral plasticity. *Neuropsychopharmacology, 36*, 2551–2560.

Vella, S., Kreis, R., Lovblad, K. O., & Steinlin, M. (2003). Acute leukoencephalopathy after inhalation of a single dose of heroin. *Neuropediatrics, 34*, 100–104.

Villemagne, V., Yuan, J., Wong, D. F., Dannals, R. F., Hatzidimitriou, G., Mathews, W. B., Ravert, H. T., Musachio, J., McCann, U. D., & Ricaurte, G. A. (1998). Brain dopamine neurotoxicity in baboons treated with doses of methamphetamine comparable to those recreationally abused by humans: Evidence from [^{11}C]WIN-35,428 positron emission tomography studies and direct in vitro determinations. *Journal of Neuroscience, 18*, 419–427.

Vindenes, V., Handal, M., Ripel, A., Boix, F., & Mørland, J. (2006). Conditioned place preference induced by morphine and morphine-6-glucuronide in mice. *Pharmacology, Biochemistry, and Behavior, 85*, 292–297.

Volkow, N. D., & Fowler, J. S. (2000). Addiction, a disease of compulsion and drive: Involvement of the orbitofrontal cortex. *Cerebral Cortex, 10*, 318–325.

Volkow, N. D., Mullani, N., Gould, K. L., Adler, S., & Krajewski, K. (1988). Cerebral blood flow in chronic cocaine users: A study with positron emission tomography. *The British Journal of Psychiatry, 152*, 641–648.

Volkow, N. D., Fowler, J. S., Wolf, A. P., Hitzemann, R., Dewey, S., Bendriem, B., Alpert, R., & Hoff, A. (1991). Changes in brain glucose metabolism in cocaine dependence and withdrawal. *The American Journal of Psychiatry, 148*, 621–626.

Volkow, N. D., Wang, G. J., & Fowler, J. S. (1997). Imaging studies of cocaine in the human brain and studies of the cocaine addict. *Annals of New York Academy of Science, 820*, 41–54.

Volkow, N. D., Chang, L., & Wang, G. J. (2001a). Loss of dopamine transportersin methamphetamine abusers recovers with protracted abstinence. *Journal of Neuroscience, 21*, 9414–9418.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Franceschi, D., Sedler, M. J., Gatley, S. J., Hitzemann, R., Ding, Y. S., Wong, C., & Logan, J. (2001b). Higher cortical and lower subcortical metabolism in detoxified methamphetamine abusers. *The American Journal of Psychiatry, 158*, 383–389.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Leonido-Yee, M., Franceschi, D., Sedler, M. J., Gatley, S. J., Hitzemann, R., Ding, Y. S., Logan, J., Wong, C., & Miller, E. N. (2001c). Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *The American Journal of Psychiatry, 158*, 377–382.

Volkow, N. D., Fowler, J. S., & Wang, G.-J. (2003). The addicted human brain: Insights from imaging studies. *Journal of Clinical Investigation, 111*, 1444–1451.

Volkow, N. D., Fowler, J. S., Wang, G. J., & Swanson, J. M. (2004). Dopamine in drug abuse and addiction: Results from imaging studies and treatment implications. *Molecular Psychiatry, 9*, 557–569.

Volkow, N. D., Tomasi, D., Wang, G. J., Fowler, J. S., Telang, F., Goldstein, R. Z., Alia-Klein, N., & Wong, C. (2011). Reduced metabolism in brain "control networks" following cocaine-cues exposure in female cocaine abusers. *PLoS One, 6*, e16573.

Wagner, G. C., Ricaurte, G. A., Johanson, C. E., Schuster, C. R., & Seiden, L. S. (1980). Amphetamine induces depletion of dopamine and loss of dopamine uptake sites in caudate. *Neurology, 30*, 547–550.

Walsh, S. L., & Wagner, G. C. (1992). Motor impairments after methamphetamine-induced neurotoxicity in the rat. *Journal of Pharmacology and Experimental Therapeutics, 263*, 617–626.

Wan, F. J., Tung, C. S., Shiah, I. S., & Lin, H. C. (2006). Effects of alpha-phenyl-N-tert-butyl nitrone and N-acetylcysteine on hydroxyl radical formation and dopamine depletion in the rat striatum produced by d-amphetamine. *European Neuropsychopharmacology, 16*, 147–153.

Wang, G. J., Volkow, N. D., Chang, L., Miller, E., Sedler, M., Hitzemann, R., Zhu, W., Logan, J., Ma, Y., & Fowler, J. S. (2004). Partial recovery of brain metabolism in methamphetamine abusers after protracted abstinence. *The American Journal of Psychiatry, 161*, 242–248.

Warren, M. W., Kobeissy, F. H., Liu, M. C., Hayes, R. L., Gold, M. S., & Wang, K. K. (2005). Concurrent calpain and caspase-3 mediated proteolysis of alpha II-spectrin and tau in rat brain after methamphetamine exposure: A similar profile to traumatic brain injury. *Life Science, 78*, 301–309.

Watkins, L. R., Hutchinson, M. R., Johnston, I. N., & Maier, S. F. (2005). Glia: Novel counter-regulators of opioid analgesia. *Trends in Neurosciences, 28*, 661–669.

Weber, M., Modemann, S., Schipper, P., Trauer, H., Franke, H., Illes, P., Geiger, K. D., Hengstler, J. G., & Kleemann, W. J. (2006). Increased polysialic acid neural cell adhesion molecule expression in human hippocampus of heroin addicts. *Neuroscience, 138*, 1215–1223.

Wechsler, L. R., Savaki, H. E., & Sokoloff, L. (1979). Effects of d- and l-amphetamine on local cerebral glucose utilization in the conscious rat. *Journal of Neurochemistry, 32*, 15–22.

Wilson, J. M., Kalasinsky, K. S., Levey, A. I., Bergeron, C., Reiber, G., Anthony, R. M., Schmunk, G. A., Shannak, K., Haycock, J. W., & Kish, S. J. (1996). Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *Nature Medicine, 2*, 699–703.

Winsauer, P. J., McCann, U. D., Yuan, J., Delatte, M. S., Stevenson, M. W., Ricaurte, G. A., & Moerschbaecher, J. M. (2002). Effects of fenfluramine, m-CPP and triazolam on repeated-acquisition in squirrel monkeys before and after neurotoxic MDMA administration. *Psychopharmacology, 159*, 388–396.

Wise, R. A. (1987). The role of reward pathways in the development of drug dependence. *Pharmacology & Therapeutics, 35*, 227–263.

Wong, C. C., Mill, J., & Fernandes, C. (2011). Drugs and addiction: An introduction to epigenetics. *Addiction, 106*, 480–489.

Woolverton, W. L., Ricaurte, G. A., Forno, L. S., & Seiden, L. S. (1989). Long-term effects of chronic methamphetamine administration in rhesus monkeys. *Brain Research, 486*, 73–78.

Wu, C. W., Ping, Y. H., Yen, J. C., Chang, C. Y., Wang, S. F., Yeh, C. L., Chi, C. W., & Lee, H. C. (2007). Enhanced oxidative stress and aberrant mitochondrial biogenesis in human neuroblastoma SH-SY5Y cells during methamphetamine induced apoptosis. *Toxicology and Applied Pharmacology, 220*, 243–251.

Wu, Q., Zhang, L., Law, P. Y., Wei, L. N., & Loh, H. H. (2009). Long-term morphine treatment decreases the association of mu-opioid receptor (MOR1) mRNA with polysomes through miRNA23b. *Molecular Pharmacology, 75*, 744–750.

Wydra, K., Golembiowska, K., Zaniewska, M., Kamińska, K., Ferraro, L., Fuxe, K., & Filip, M. (2013). Accumbal and pallidal dopamine, glutamate and GABA overflow during cocaine self-administration and its extinction in rats. *Addiction Biology, 18*(2), 307–24.

Xi, Z. X., Ramamoorthy, S., Shen, H., Lake, R., Samuvel, D. J., & Kalivas, P. W. (2003). GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine. *Journal of Neuroscience, 23*, 3498–3505.

Xiao, Z. W., Cao, C. Y., Wang, Z. X., Li, J. X., Liao, H. Y., & Zhang, X. X. (2006). Changes of dopamine transporter function in striatum during acute morphine addiction and its abstinence in rhesus monkey. *Chinese Medical Journal (English Edition), 119*, 1802–1807.

Xu, N. J., Bao, L., Fan, H. P., Bao, G. B., Pu, L., Lu, Y. J., Wu, C. F., Zhang, X., & Pei, G. (2003). Morphine withdrawal increases glutamate uptake and surface expression of glutamate transporter GLT1 at hippocampal synapses. *Journal of Neuroscience, 23*, 4775–4784.

Xu, J., DeVito, E. E., Worhunsky, P. D., Carroll, K. M., Rounsaville, B. J., & Potenza, M. N. (2010). White matter integrity is associated with treatment outcome measures in cocaine dependence. *Neuropsychopharmacology, 35*, 1541–1549.

Yamamoto, B. K., & Zhu, W. (1998). The effects of methamphetamine on the production of free radicals and oxidative stress. *Journal of Pharmacology and Experimental Therapeutics, 287*, 107–14.

Yamamoto, B. K., Moszczynska, A., & Gudelsky, G. A. (2010). Amphetamine toxicities: Classical and emerging mechanisms. *Annals of the New York Academy of Sciences, 1187*, 101–21.

Yücel, M., Lubman, D. I., Harrison, B. J., Fornito, A., Allen, N. B., Wellard, R. M., Roffel, K., Clarke, K., Wood, S. J., Forman, S. D., & Pantelis, C. (2007). A combined spectroscopic and functional MRI investigation of the dorsal anterior cingulate region in opiate addiction. *Molecular Psychiatry, 12*(611), 691–702.

Yuferov, V., Fussell, D., LaForge, K. S., Nielsen, D. A., Gordon, D., Ho, A., Leal, S. M., Ott, J., & Kreek, M. J. (2004). Redefinition of the human kappa opioid receptor gene (OPRK1) structure and association of haplotypes with opiate addiction. *Pharmacogenetics, 14*, 793–804.

Zheng, H., Chu, J., Zeng, Y., Loh, H. H., & Law, P. Y. (2010). Yin Yang 1 phosphorylation contributes to the differential effects of μ-opioid receptor agonists on microRNA-190 expression. *Journal of Biological Chemistry, 285*, 21994–22002.

Zheng, H., Law, P. Y., & Loh, H. H. (2012). Non-coding RNAs regulating morphine function: With emphasis on the in vivo and in vitro functions of miR-190. *Frontiers in Genetics, 3*, 113.

Zhou, Y., Bendor, J., Hofmann, L., Randesi, M., Ho, A., & Kreek, M. J. (2006). Mu opioid receptor and orexin/hypocretin mRNA levels in the lateral hypothalamus and striatum are enhanced by morphine withdrawal. *Journal of Endocrinology, 191*, 137–145.

Zhou, Z., Yuan, Q., Mash, D. C., & Goldman, D. (2011). Substance-specific and shared transcription and epigenetic changes in the human hippocampus chronically exposed to cocaine and alcohol. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 6626–6631.

Zweben, J. E., Cohen, J. B., Christian, D., Galloway, G. P., Salinardi, M., Parent, D., & Iguchi, M. (2004). Methamphetamine treatment project. Psychiatric symptoms in methamphetamine users. *American Journal of Addiction, 13*, 181–190.

Molecular Mechanism and Effects of Clostridial Neurotoxins

Bal Ram Singh, Raj Kumar, and Shuowei Cai

Contents

Abstract

The genus Clostridia, gram-positive anaerobic bacteria, produce two of the deadliest toxins known to mankind: tetanus and botulinum. Toxicity of these molecules comes from their action on very specific substrates, SNARE proteins, in the neuronal cells. They cleave SNAREs, which are the crucial components of neuroexocytosis, causing inhibition of release of neurotransmitters at the synapses. Botulinum neurotoxin molecule has evolved itself to have high stability, specificity, and selectivity. It maneuvers and exploits itself in each step of intoxication and preserves its active structure to achieve an exquisite toxicity. The mechanisms of action and biological functions of clostridial neurotoxins

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still have several unresolved questions. Several unique characteristics of these neurotoxins attract scientists to understand the mysteries of neurotoxin molecules. Despite high toxicity, botulinum neurotoxin has a wide range of medicinal applications. In the last few decades, toxin transformed itself from life-threatening disease to a wonder drug. Therapeutic use of botulinum neurotoxin is based on three unique features: specificity, potency, and duration of action. Active research is underway to transform botulinum neurotoxins into the most versatile and useful drugs for neuromuscular disorders, and as cosmeceuticals. This chapter summarizes current knowledge on its structure and mechanism of intoxication. It also describes basic understanding of the evolution of this molecule to achieve this extremely high biological activity in terms of toxicity or therapeutics. Understanding the true nature and function of its dynamic structure still remains a work in progress.

1 Toxins

In general toxins can be small molecules, peptides, or proteins. These molecules are toxic because they are capable to evade protection mechanisms of the infected host cells and affect important cellular processes such as protein synthesis, inhibition of other cellular processes, and promotion of hemolysis. Toxins can be generally grouped according to their source (e.g., bacterial toxins), their purpose (e.g., phototoxins), and their site of action or affected organs (e.g., hepatotoxins, neurotoxins). Among these the most complex and deadly toxins are the bacterial toxins. Pathogenic bacteria use many ways to interact with target tissues or cells. Toxins produced by bacteria can act locally or at a distant location from the sites of infection, leading to various diseases in human and animals. Bacterial toxins are classified as two types: exotoxins, which are secreted by the bacteria in the environment, and endotoxins, which may be released after the lysis of bacterial cell wall. Clostridial neurotoxins produced by anaerobic *Clostridium botulinum* and *Clostridium tetani* are exotoxins, which can be produced in infected tissues, such as wounds or infant guts. These neurotoxins are the most toxic substances currently known to mankind. There are many unique features associated with these neurotoxins and also with the bacteria which produce them, all of which together play a role in the mechanism of pathogenicity, making this class of pathogens highly unique.

2 Clostridium Family

The genus *Clostridium* represents gram-positive, ubiquitous, anaerobic, and endospore-forming bacteria. Clostridium has about 100 species consisting of ubiquitous bacteria and pathogens. Members of this group of bacteria exhibit large straight or slightly curve-shaped bacterial cells. Members of this genus produce some of the

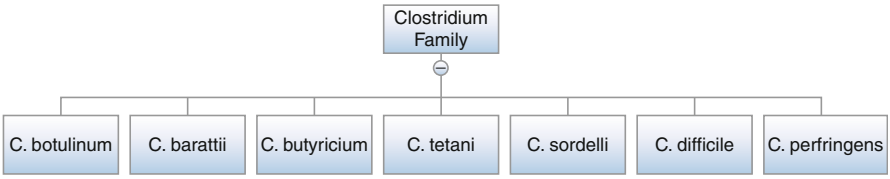

Fig. 1 Members of *Clostridium* family, known for producing toxins

most potent toxins discovered so far. Dormant cells are highly resistant to heat, desiccation, toxic chemicals, and detergents. Spores do not germinate normally unless they have suitable environment to grow. Most of clostridial species are saprophytes, but a few are pathogenic to humans and animals, for example, *C. difficile*, *C. botulinum*, *C. tetani*, and *C. perfringens* (Fig. 1). The pathogens are primarily saprophytes in nature. *C. tetani* and *C. botulinum* produce the most potent biological toxins known to mankind. In their active form they secrete exotoxins which cause various diseases, such as tetanus and botulism.

3 The *Clostridium botulinum*

Clostridium botulinum, which was first isolated from a contaminated ham and from the spleen of a patient by van Ermengem in Belgium in 1895 (Van Ermengem 1979; Foster 1993) grows anaerobically at 37 °C or 30 °C under pH 6.5–7.0 conditions. Most of *C. botulinum* as well as some isolates of *C. butyricum* and *C. baratii* can produce botulinum neurotoxins (BoNTs). The strains of *C. botulinum* are traditionally classified into seven serotypes, A to G, according to the antigenic properties of neurotoxins they produce. Generally, one strain of *C. botulinum* can only produce one type of neurotoxin, but several strains that produce more than one type of neurotoxin have been identified over the years. One type C strain (C6813) was found to produce the mosaic neurotoxin in which one-third of the C-terminal region was similar to that of type D neurotoxin (Moriishi et al. 1996). One type A strain, called type AB now, was shown to produce both type A and type B neurotoxins (Fujinaga et al. 1995). *C. botulinum* type G organism differs phenotypically from other *C. botulinum* types and is now designated as *C. argentinense* (Gimenez and Ciccarelli 1970; Campbell et al. 1993b). *Clostridium butyricum* and *Clostridium baratii* were found producing type E (BoNT/E) and type F (BoNT/F) neurotoxins, respectively (Fuji and Kimura 1993; Thompson et al. 1993).

BoNT is synthesized as a single and relatively inactive polypeptide chain, which undergoes a proteolytic cleavage at its nicking site to form a biologically active dichain structure linked by a disulfide bond. The proteolytic strains of *C. botulinum* produce endogenous proteases and secrete the neurotoxin as an active dichain form by cleaving it with endogenous protease, but non-proteolytic strains of *C. botulinum* require exogenous proteases, such as trypsin in the intestinal tract of hosts, to cleave the progenitor toxin to activate it (Call et al. 1995, Fig. 2).

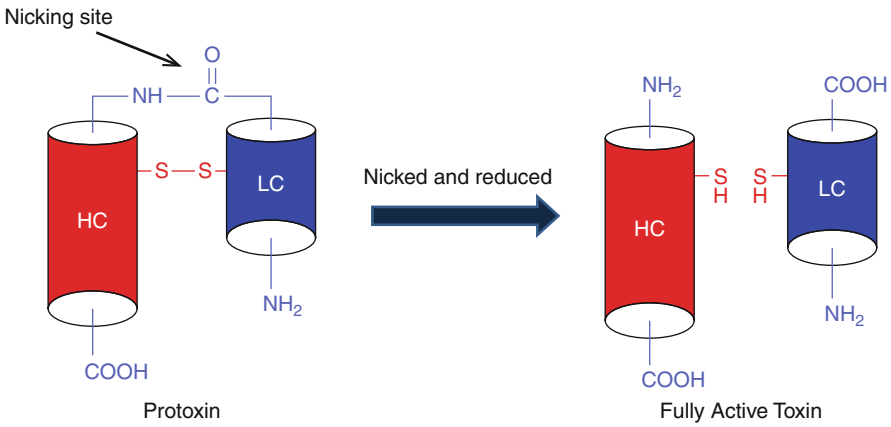

Fig. 2 Botulinum neurotoxin: BoNTs are produced as a single chain (progenitor or protoxin) which is processed exogenously or endogenously as a dichain. Reduction of disulfide bond makes fully active toxin

Strains of *C. botulinum* can also be divided into four phylogenetically and physiologically distinct groups (I to IV) based on biochemical properties and nucleic acid hybridization studies. Group I includes type A, proteolytic strains of types B and F. Group II includes type E, non-proteolytic strains of types B and F. Group III consists of types C and D, and group IV consists of type G (Campbell et al. 1993a). Rainey and Stackebrandt (1993) and Campbell et al. (1993b) used a very high level of 23S rRNA sequence similarity to show that groups I, II, III, and IV *C. botulinum* represent a single genetic unit phylogenetically remote from each other and that the marked genotypic variation within the species does not correlate with the neurotoxins produced. *C. baratii* that produces type F neurotoxin belongs to a new added group, group V, and *Clostridium butyricum* that produces type E neurotoxin belonged to the latest group, group VI (see Table 1; Chang 2011). The same conclusions were also drawn from 16S rRNA sequence similarities (Hutson et al. 1993). However, the genetic relationship inferred from the BoNT sequences differs markedly from above conclusions and it is clearly established now that all three types of BoNT/F (proteolytic, non-proteolytic, and *C. baratii* type F) are genealogically highly related.

The spores of proteolytic *C. botulinum* are heat resistant and do not grow at temperatures below 10 °C, but the spores of non-proteolytic *C. botulinum*, which are less heat resistant, can germinate relatively rapidly at temperatures in the range of 2–10 °C (Lund and Peck 1994). Type A *C. botulinum* has 0.39 h generation time and reaches its exponential growth after 5 h of anaerobic incubation of spores (Call et al. 1995). The bacterial growth curve of type A *C. botulinum* (strain Hall) shows a lag period of 4 h and log phase at 10 h before reaching the stationary phase (Roberts and Hobbs 1968; Billon et al. 1997; Shukla and Singh 1999, 2009; Stringer et al. 2009).

Table 1 Summarized characteristics of six physiologically distinct Clostridia that produce the botulinum neurotoxin (BoNT)[a]

	Group I			Group II			Group III		Group IV	Group V	Group VI
Endogenous Protease Activity	Proteolytic			Non Proteolytic			Non Proteolytic		Proteolytic	Non Proteolytic	Non Proteolytic
Clostridial Neurotoxin Serotype	A	B	F	B	F	E	C	D	G	F	E
Accumulated Subtypes so far	A1-A5	B1-B3 Bivalent B	F1-F5	B4,B5	F6	E1-E3 E6				F7	E4,E5
Belong to Type-I: Ha(+)/Orf-X(–)	A1, A5	B		B			C	D	G		
Belong to Type-II: Ha(+)/Orf-X(–)	A1-A4		F1-F5		F6	E				F7	E
Neuronal Receptors	SV2A, SV2B, SV2C	Syt I and II,	glycosylated SV2A,			SV2A, SV2B	Unknown		Syt I and II,		
	GT1b, GD1a	GT1b, GD1a	GT1b, GD1a			GT1b, GT1a		PE	all ganglioside		
	GD1b	GD1b	GD1b								
Toxins Specific Substrate	SNAP-25	VAMP (also called Synaptobrevin)				SNAP-25	SNAP-25 Syntaxin		VAMP (also called Synaptobrevin)		SNAP-25
Type E and F that come from different groups all belong to neurotoxin gene cluster type II; *cnt* R is absent from all type E and *cnt* C is absent from all type G.)											
Harbored Neurotoxin genes	Single and Bivalent			Single							
Bivalent Gene Types if contained	Ab, Af, Ba, Bf, A(B)			Hasn't Found Yet							
Mean Genome Size	4.02 Mb			3.73 Mb			2.96 Mb				
Mean % G + C of Genome	28.23%			27.45%			29.00%				
Neurotoxin Gene Site	Chromosome or Plasmid			Chromosome or Plasmid			acteriophage		Plasmid	Transposon	Transposon
Non-Neurotoxigenic Equivalent clostridia	C. sporogenes			Hasn't given name yet			C. noyyi C. hemolyticum		C. subterminate C. proteolyticus	Typical C. Baratii	Typical C. butyricum
Growth Temperature Attribution	Mesophilic			Psychrotropic			Mesophilic		Mesophilic	Mesophilic	Mesophilic
Spore Heat Resistance	$D_{121°C}$ = 0.21 min			$D_{82.2°C}$ = 2.4 min			$D_{104°C}$ = 0.91 min		$D_{104°C}$ = 1.1 min		$D_{100°C}$ < 0.1 min
	High Heat Resistance			Moderate Heat Resistance			Moderate		Moderate	Moderate	Moderate
Associated Botulism Types	Foodborne Botulism			Foodborne Botulism			Avian Botulism		Toxin is toxic in vitro	Foodborne Botulism	Infant Botulism
	Wound Botulism						Foxes Botulism		but hasn't found	Infant Botulism	Adult-Intestinal
	Infant/Intestinal Botulism						Minks Botulism		case report yet	Adult-Intestinal	
Optimal Growth °C	37°C			25°C			40°C		37°C	30~45°C	30~37°C
Minimum Growth °C	10~12°C			2.5~3.0°C			15°C			10~15°C	12°C
Minimal pH for Growth	4.6			5			5.1				4.8
[NaCl%] Preventing Growth	10%			5%					6.50%		
Lipase Production	+			+			+		–	–	–
Glucose Fermentation	+			+			+		–	+	+
Fructose Fermentation	+/–			+			+/–		–	+	+
Maltose Fermentation	+/–			+			+/–		–	+	+
Sucrose Fermentation	–			–			–		–	+	+
Remark	Source: Data from Smith & Sugiyama (1988), Lund and Peck (2000), Stringer and Peck (2008), Poulain et al. (2008)										
	means all strians ; +/– means some strains are positive while others are negative; – means all strains negative.										
	Spore Heat Resistance is determined in phosphate buffer (pH 7.0) without lysozyme										
	Group II could be separated into 2 major groups in the future, one containing E the other containing B & F.										
	The genome of a phage contains BoNT/C gene cluster has been sequenced. It is a linear dsDNA of 186 kb with a low %G+C (26.2%)										
	Type G neurotoxin gene cluster is located at a 123 kB Plasmid.										

* Modified table from Chang (2011) PhD thesis dissertation

The studies with a monoclonal antitoxin conjugated with colloidal gold demonstrated that the type A BoNT is synthesized in the cell cytoplasm at late-exponential phase (17 h) and reaches its maximum concentration at stationary phase (24–25 h). The BoNT is translocated across the cytoplasmic membrane and then exported to the extracellular medium via cell wall exfoliation. The greatest proportion of BoNT is located within the cell wall at 45 h (Call et al. 1995).

The spores of *C. botulinum* are widely distributed in soil, mud, and surface waters over much of the globe, but different types of *C. botulinum* prevail in different geographical locations. In the western United States, Brazil, Argentina, and China, type A spores predominate, while proteolytic type B spores tend to predominate in soils of the eastern United States and non-proteolytic type B in Britain and Europe. Type E is often associated with freshwater and marine sediments, and northern regions such as northern Canada and Japan (McClure et al. 1994). Initially, the strains of *C. botulinum* type A were divided into two groups, infant type A and type A, based on the cases of infant botulism in Japan and the classical food-borne botulism (Sakaguchi et al. 1990). But several studies (Collins et al. 1994; Cordoba and Collins 1995; Aranda et al. 1997) have revealed that the strains of *C. botulinum* type A could be divided into two groups (subtypes), A1 and A2, based on different geographical locations isolated and did not correlate with their origins as the cases of both infant botulism and food-borne botulism. Strains isolated from infant botulism in Japan and several strains isolated from classical food-borne botulism in United Kingdom and in Mauritius fell into group A2, while all strains examined from the USA and two strains from UK, from both infant and food-borne botulism fell, into group A1. Group A1 is typical *C. botulinum* type A containing hemagglutinin genes and shows hemagglutinin activity. Several group A1 strains contain an unexpressed type B neurotoxin gene (Ab type). Group A2 lacks the hemagglutinin gene and possesses no hemagglutinin activity. Along with the increasing number of sequenced type A strains, we can now identify more subtypes based on the criteria of sequence variations between 2.6 % and 32 % at the amino acid level, while compared to up to 70 % between different main serotypes of neurotoxin (Marks 2004; Smith et al. 2005; Arndt et al. 2006). Currently there are five subtypes of type A neurotoxin (termed A1, A2, A3, A4, A5), five type B subtypes, six type E subtypes (Arndt et al. 2006; Chen et al. 2007a; Hill et al. 2007; Smith and Hill 2007; Carter et al. 2009), and seven type F subtypes (Raphael et al. 2010) have been classified (see details in Table 1, Chang 2011). Interestingly all five type A subtypes belong to the same group (group I), while the other subtypes belong to more than one groups. Overall, the subtypes A1 $\sim$ A5, B1 $\sim$ B3, and F1 $\sim$ F5 belong to group I. The subtypes B4 $\sim$ B5, F6, E1 $\sim$ E3, and E6 belong to group II. The subtypes E4 $\sim$ E5 belong to group VI, and only subtype F7 belongs to group V. The existence of these subtypes is a manifestation of host adaption and divergent evolution of neurotoxin gene, and it will be important more and more in the development of neurotoxin antiserum and antidotes.

3.1 Botulism

Historically, botulism – derived from botulinus meaning sausage – was discovered long before the name appeared in the literature. Earliest reference to possible botulism-related disease is found in an edict by Emperor Leo VI of Byzantium (886–911) in which manufacturing of blood sausages was forbidden (Erbguth 2004). However, the first systematic study of "sausage poisoning" was carried out in 1815 by a health officer in the town of Herrenberg of Württemberg (Germany) by the name of J. G. Steinbuch and later independently by Justinus Kerner, another medical officer in the town of Welzheim in Württemberg (Erbguth 2004). Kerner's work was published in journals and monographs in early 1800s. In 1822, Kerner described his observations and experiments with sausage toxin, also referred as fat poison, in a monograph. Kerner wrote "The nerve conduction is brought by the toxin into a condition in which its influence on the chemical process of life is interrupted. The capacity of nerve conduction is interrupted by the toxin in the same way as in an electrical conductor by rust." Interestingly, Kerner had also hypothesized on the idea of sausage toxin being used as a therapeutic agent (Erbguth 1996, 1998).

Botulism is a severe disease characterized by flaccid paralysis and caused by botulism neurotoxins, extremely potent food poisons with a mouse lethal dose of 0.1 ng/kg (Montecucco and Schiavo 1993). Three types of botulism have been observed, so far. Classical food-borne botulism is caused by ingestion of food where the bacteria have produced the neurotoxin. Infant botulism result due to the production of neurotoxin in the intestine after germination and outgrowth of ingested spores. Wound botulism maybe due to the outgrowth of bacteria in the wound or due to unknown origin (Cordoba and Collins 1995; Hatheway and Ferreira 1996). Classical food-borne botulism in humans is caused mainly by *C. botulinum* types A, B, E, and rarely by type F neurotoxins. Classical food-borne botulism in animals is always caused by *C. botulinum* types C1 and D neurotoxins (Fach et al. 1993; Cordoba and Collins 1995). In contrast, the causative agents for infant botulism can be *C. botulinum* types A or B neurotoxins or *Clostridium butyricum* type E and *Clostridium baratii* type F neurotoxins. The wound botulism is rare in humans, and the causative agents are either type A or type B from group I (Hatheway 1993), but is increasingly significantly in recent years among drug users (Merrison et al. 2002). Neurotoxins can also be used in the therapy of a variety of human muscle disorders such as strabismus, hemifacial spasm, and blepherospasm (Schantz and Johnson 1992; Eleopra et al. 2004).

Epidemiologically, different serotypes of BoNT are known to selectively cause the disease in different animals. For example, BoNT/C is mostly associated with avian botulism; BoNT/C and BoNT/D with cattle, sheep, and horse; and BoNT/A, BoNT/B, and BoNT/E are associated with human, cattle, and horse botulism, while BoNT/E is particularly associated with fish botulism (Huss and Eskildsen 1974; Burns and Williams 1975; Ala-Huikku et al. 1977; Fernandez et al. 1989; Baldassi et al. 1991; Haagsma 1991; Jeffrey et al. 1994; Ashie et al. 1996;

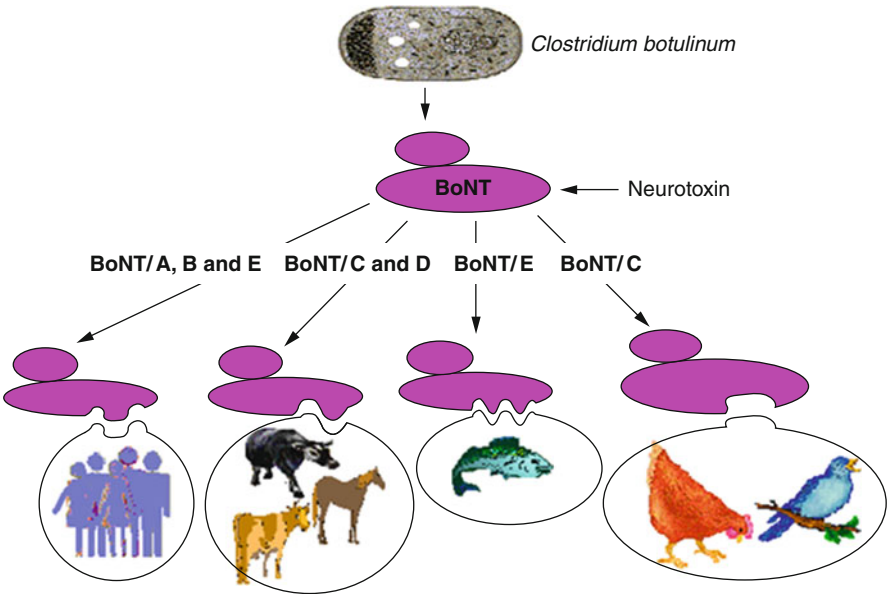

Fig. 3 Schematic representation of different binding topography of various serotypes of BoNT for their interactions with unique protein receptors in different species (Adapted from Singh 1999)

Ortolani et al. 1997). It is believed that botulism by a selective serotype of BoNT in a given animal is in part related to the presence of a high-affinity receptor for that serotype in the host animal system (Fig. 3). The true case number of food-borne botulism is probably underreported in the world. First, botulism is not a reportable disease in all countries (Therre 1999), and secondly the efficiency of investigating potential outbreaks varies from country to country (Peck 2009). It is estimated that the botulism case reporting efficiency in the USA is about 50 % only (Mead et al. 1999).

4 Clostridium Neurotoxins

Clostridial bacteria produce two of the deadliest neurotoxins known: tetanus and botulinum. Tetanus neurotoxin is produced by *C. tetani* and *C. botulinum* produces botulinum toxin. *C. tetani* produces a single toxic species, whereas strains of *Clostridium botulinum* produce at least seven serologically distinct neurotoxins: A–G. BoNTs are food poisons, whereas TeNT is not, and this has been attributed in part to the existence of the complex form of BoNT, consisting of toxin as well as neurotoxin-associated proteins (NAPs). There is no protein associated with tetanus toxin (Singh et al. 1995; Innoue et al. 1996; Minton 1995). NAPs are supposed to protect and enhance the activity of neurotoxin (Sharma and Singh 2004;

Kukreja and Singh 2007). However, Maksymowych et al. (1999) showed that there is no role of NAPs in enhancing the toxin activity. The extreme potency of these toxins lies in their specificity to the neuronal cells.

Clostridial neurotoxins belong to a group of bacterial toxins known as A-B type toxins consisting of at least two separate functional domains: one is responsible for binding and internalization and other is responsible for the intercellular action of the toxins. Both BoNT and TeNT are known to exert neuroparalytic effect in vertebrates (Humeau et al. 2000). Tetanus is structurally similar to BoNT but produces different symptoms upon intoxication. The action site is also different: BoNTs act on peripheral nervous system, while TeNTs act on central nervous system (Caleo and Schiavo 2009). Tetanus toxin induces spastic paralysis, while botulinum toxin induces flaccid paralysis. BoNTs inhibit the release of acetylcholine at the neuromuscular junctions (Simpson 1986, 2004), whereas TeNT inhibits the release of glycine and γ-amino butyric acid in the central nervous system (Schiavo et al. 2000). However, BoNT/A suppresses the release of acetylcholine, glutamate, noradrenaline, glycine, serotonin, GABA, and dopamine (Bozzi et al. 2006; Ashton and Dolly 1988; Verderio et al. 2004, 2007; Sutton et al. 2004; Costantin et al. 2005; Antonucci et al. 2008) in neuronal cells and synaptosomes. The differential site of BoNT and TeNT action may be due to differences in the binding affinity of the BoNT and TeNT for peripheral nerves vs. central nerves and also due to differential retrograde action of the toxins once internalized into nerve cells (Schiavo et al. 1993b, 1994b, 2000; Wang et al. 2012).

Clostridial neurotoxins are Zn-metalloprotease having very high specificity for its substrate: SNARE proteins involved in neurotransmitter release in the neuronal cells. The toxins selectively cleave the SNARE proteins; syntaxin, synaptobrevin (also known as vesicle-associated membrane protein or VAMP) and SNAP-25, but the final results are same – the hydrolysis of the target protein, blockage of neurotransmitter release, and a resultant paralysis. While BoNTs remain in the nerve terminal, the tetanus toxin is transported retrograde axonal transport into the cell body. Tetanus and botulinum toxins affect both somatic and autonomic nervous systems, but autonomic features are more common in tetanus.

All seven serotypes of BoNT are structurally similar. They are synthesized as a single-polypeptide chain of 150 kDa each which is processed by endogenous or exogenous proteases into an activated dichain form (Dekleva and Das Gupta 1989; Krieglstein et al. 1994; Singh and DasGupta 1989, Fig. 2). In each case, the dichain neurotoxin is composed of 100 kDa heavy chain (HC) and a 50 kDa light chain (LC), which is linked through a disulfide bond. Heavy chain consists of two domains of 50 kDa each: H_C and H_N; H_C is binding domain and H_N is the translocation domain. Structurally each toxin molecule has all three domains: binding domain (H_C), translocation domain (H_N), and catalytic domain (LC). Hc comprises two subdomains – a β-sheets domain (H_{CN}) and a β-tree-foil (H_{CC}).

The three domains of BoNTs appear to be naturally designed for toxin delivery system, as binding domain (BD) acts a targeting unit, the translocation domain (TD)

can deliver cargo into a cell, and the catalytic domain acts as the cargo (Foster 2009; Bade et al. 2004; Chaddock and Marks 2006; Chaddock and Acharya 2011). The arrangement and the function of subunit in BoNTs are analogy to those in diphtheria toxin and anthrax toxin complex (Humeau et al. 2000; Monteccuco and Schiavo 1994; Montecucco et al. 1994; Montecucco and Schiavo 1995; Schiavo et al. 1994a, b; Singh 2000). In BoNT structure, in addition to the disulfide link between HC and LC, there is a belt, a loop from the HC that wraps around LC to hold the two chains together (Lacy and Stevens 1999; Chen et al. 2007b). The active site of LC is partially occluded by the belt in unreduced toxin (Simpson et al. 2004; Ahmed and Smith 2000), and the belt is predicted to be intermolecular chaperone for the light chain (Brunger et al. 2007). All the domains work together with high precision and accuracy. That is perhaps part of the reason why BoNTs are highly effective biologics, making them the most toxic substances.

The BD of HC binds to nerve cells, and after internalization of the BoNT through endocytosis, the TD forms a pore in the endosomal membrane, through which the LC domain crosses into the cytosol. This leads to intracellular blocking of neurotransmitters release by proteolysis of one of the three proteins of SNARE complex by Zn-endopeptidase activity of the LC. Isolated LC cleaves SNARE protein but it cannot readily internalize on its own, and therefore remain nontoxic. Also, isolated H_C or H_N cannot cleave SNARE proteins, so remain nontoxic. So only when three domains come together they create most the toxic substance known. It is notable that this unique assembly allows us to explore the possibility of combinatorial profiling of multidomain assemblies, for example, LC-TD for retargeting LC function (Darios et al. 2010).

The crystal structure of BoNT/A (PDB: 3BTA), BoNT/B (PDB: 1EPW), and BoNT/E (PDB: 3FFZ) supports the hypothesis of modular assembly of BoNT (Fig. 4). Crystal structure of TeNT is not available. BoNT/A and BoNT/B show similar structural organization, whereas BoNT/E has some significant differences. Nevertheless, BoNT/E also has all the three domains, and while all other serotypes are made of two chains endogenously, BoNT/E is produced entirely as a single-chain protein. However, BoNT/B and BoNT/F are also produced as a single chain by non-proteolytic strains of the *Clostridium botulinum* (East et al. 1998). All the three domains are working together to achieve intoxication and act as a chaperone for the others (Brunger et al. 2007; Montal 2010). BoNT's functional complexities emerge from its modular design and tight interplay of individual component actions. Above-described design helps BoNT in using a very vital neuronal cell process, endocytosis, to reach to its targets. Exquisite toxicity of BoNTs is achieved by its unique structure which this molecule exploits very well. BoNT is considered an astonishing nanomachine that unites recognition, trafficking, unfolding, translocation, refolding, and catalysis in a single entity.

As stated earlier, TeNT is also synthesized by bacteria as a single-chain polypeptide, and because of the presence of bacterial protease, the toxin is isolated in a nicked form like botulinum toxin. Like A-B toxin, both separated heavy

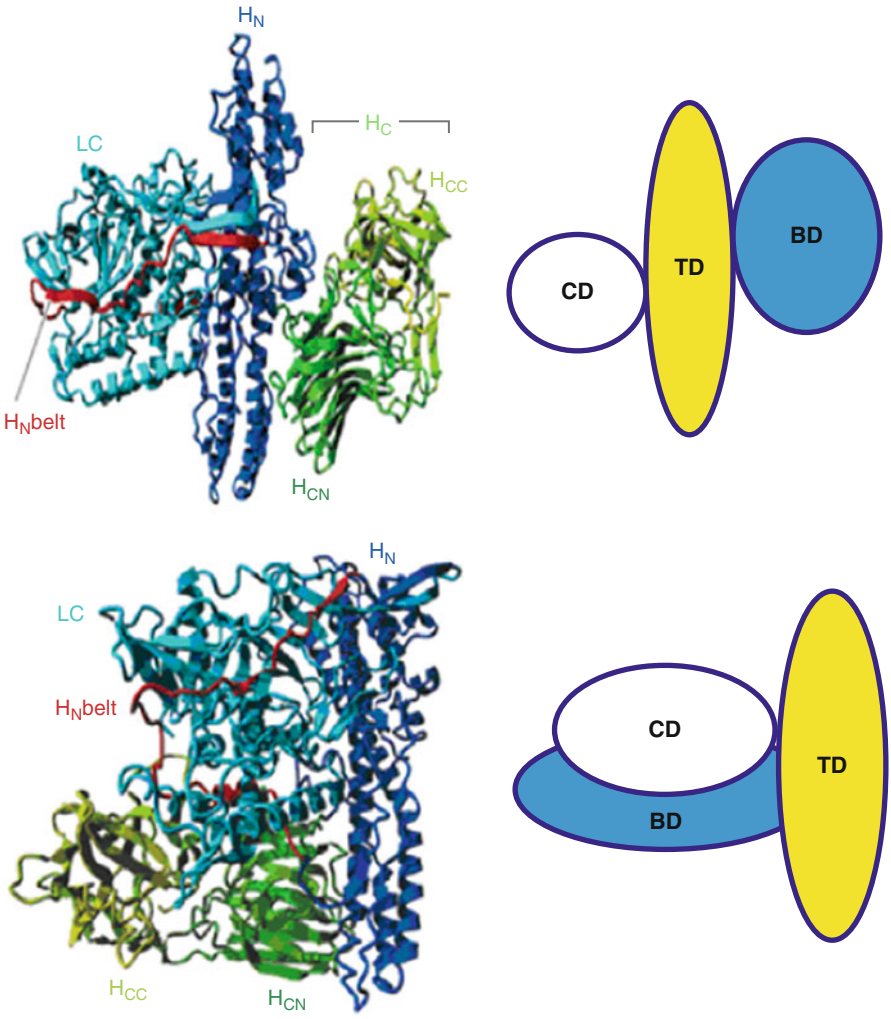

Fig. 4 Crystal structure of BoNT/A (3BTA) and BoNT/E (3FFZ). Hc is depicted in *green*, H_N is in *blue*, and LC is in *gray* color. Hc belt is depicted in *red*. BoNT/B (1EPW) is similar to BoNT/A. In case of BoNT/E LC (CD) and HC (BD) are on the same side of translocation domain (*TD*), whereas in BoNT/A CD and BD are on the opposite side of translocation domain (Kumaran et al. 2009)

chain and light chain of TeNT are nontoxic. But there is a second protease-sensitive site in heavy chain which splits the heavy chain approximately in half. In nonreducing condition, two chains can be isolated: one consists of 50 kDa heavy chain linked to 50 kDa light chain (referred to as fragment B), and other contains rest of heavy chain (50 kDa, fragment C). Fragment B is toxic to animal, and like botulinum toxin, it induces flaccid paralysis (Monteccuco and Schiavo 1994).

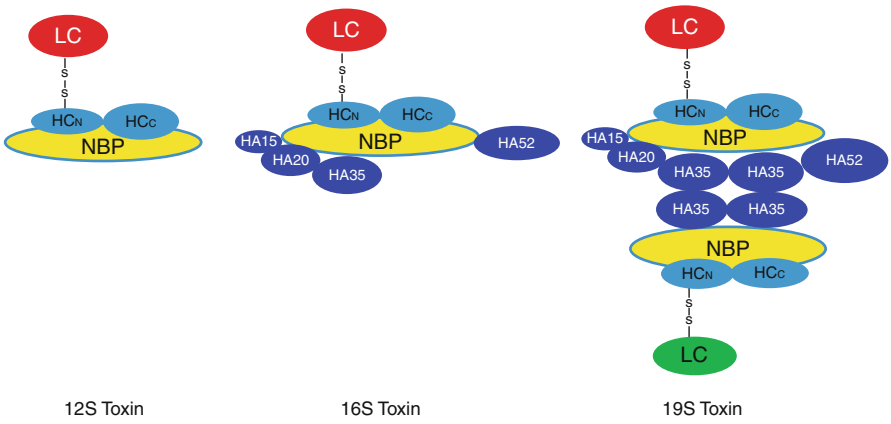

Fig. 5 Structure of botulinum neurotoxin complex. The toxins are produced by bacteria as progenitor toxins, composed of toxin molecule associated with NBP and other NAPs (hemagglutinins or HAs). Based on the organization of these proteins, botulinum toxins are termed as LL (19S), L (16S), and M (12S) toxin. HA35 has the highest molar ratio in complex compared to toxin. According to Innoue et al. (1996), molar ratio of toxin to HA35 is 1.00:3.99 and 1.00:7.76 in 16S and 19S toxin, respectively

4.1 Neurotoxin-Associated Proteins

In bacterial cultures, BoNTs are released in the form of multimeric complexes, with the set of nontoxic neurotoxin-associated proteins (NAPs) coded by genes adjacent to neurotoxin gene. These complexes are termed progenitor toxins (Innoue et al. 1996; Minton 1995). Some BoNT-associated proteins have hemagglutinating activity (Fujita et al. 1995). A large nontoxic non-hemagglutinating protein of 139 kDa coded for the gene by a gene upstream to the BoNT gene is present in all seven serotypes of BoNTs called neurotoxin binding protein (NBP) (Innoue et al. 1996; Minton 1995; Fujita et al. 1995; Singh et al. 1995). One of the interesting facts about the NBP is that it has similar structural domains as BoNT (Gu et al. 2012). Three forms of progenitor toxins have been characterized: extra-large size (LL complex, sediments at 19 S, about 900 kDa), large size (L Complex, sediments at 16 S, about 500 kDa), and medium size (M complex, sediments at 12 S, about 300 kDa). The M complex is BoNT-NBP complex; L Complex of BoNT-NBP, and several components of NAPs with hemagglutinin activity, and the LL complex are believed to be a dimer of L complex associated with each other (Innoue et al. 1996). The four hemagglutinin (HA) proteins found in BoNT/A complex are HA15/17(15 or 17 kDa), HA33/35 (33 or 35 kDa), HA19/20 (19 or 20 kDa), and HA70 (70 kDa). Notably, HA52 and HA15/17 are fractions of HA70 (Fig. 5). Innoue et al. (1996) determined the molar composition of different protein in complex. HA33/35 has been purified from BoNT/A complex (Fu et al. 1998), and also has been expressed as a recombinant protein.

While BoNT/A exists in three complex forms, M, L, or LL, types B, C, and D BoNTs exist in two forms, L and M. Type E BoNT is now known to exist in L and

M forms, although NAPs composition of the L form is different (Singh and Zhang 2004; Kukreja and Singh 2007). Type F BoNTs are reported only in M form, and type G exists only in L form (Fujita et al. 1995, Fig. 5).

Since every BoNT serotype is associated with one or more NAPs, it is also important to examine biological role, if any, of these proteins. BoNTs in complex forms are more stable than in purified form. NAPs protect BoNTs against proteolysis and denaturation (Chen et al. 1998; Sakaguchi 1983; Kukreja and Singh 2007). Survival of the complex in harsh GI-tract conditions is believed to be a major reason for BoNTs to be food poison. Oral toxicity of BoNTs is more in complex form than their purified forms (Sakaguchi 1983). At least one of the NAPs has been shown to exhibit a series of biological functions. It has been shown to possess heat-shock protein characteristics (Shukla and Singh 1999, 2009) and is believed to act as molecular chaperone (Shukla et al. 1997). HA33 is also known to block neuronal apoptosis (Kumar et al. 2012). Furthermore, HA33 is known to assist in the translocation of BoNT across the gut wall after ingestion of the BoNT complex (Matsumura et al. 2008; Fujinaga et al. 2009). These results indicate that associated protein play an important role in the toxico-infection process of botulism.

5 Mechanism of Action of Clostridial Neurotoxin

It has been proposed (Simpson 1986, 2004; Hilger and Bigalke 1995) that the biological action of clostridial neurotoxins involves three steps: (a) binding and internalization of the neurotoxin via the HC through receptor-mediated endocytosis (cellular process by which cell absorbs and engulfing external molecule), (b) membrane translocation of LC into the cytosol purportedly through a pH-induced membrane channel formed by the HC, and (c) intracellular blocking of neurotransmitter release by proteolysis of one of the three SNARE proteins of by Zn-endopeptidase activity of the LC (Fig. 6). Isolated LC cleaves SNARE protein but it cannot readily internalize on its own and therefore remains nontoxic. Also, isolated H_C or H_N cannot cleave SNARE proteins, so remain nontoxic. So only when three domains are together they create the most toxic substance known to humanity. Interestingly, this unique assembly allows us to explore the possibility of combinatorial profiling of multidomain assemblies, for example, LC-TD for targeting LC function (Darios et al. 2010).

5.1 Receptor Binding and Internalization

As described above, BoNTs have trimodular architecture, and this unique architecture has a physiological meaning. This unique structural organization helps it to achieve its goal in harsh cellular environment of host. Its mechanism of action starts with the high-affinity interaction with a surface protein receptor, via ganglioside co-receptor. So far, protein receptors identified are SV2A for BoNT/A (Dong et al. 2006; Mahrhold et al. 2006); glycosylated SV2A and SV2B for BoNT/E

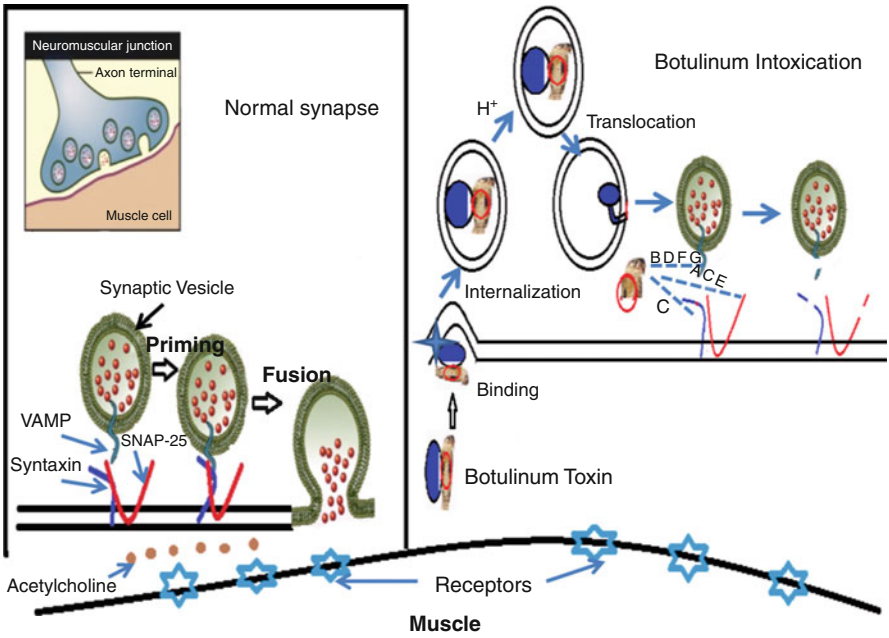

Fig. 6 Mode of action of botulinum neurotoxin. *Left*, pre and postsynaptic events in normal neuronal cells. *Right*, steps involved in botulinum intoxication; binding, internalization, translocation

(Dong et al. 2008); glycosylated SV2A, SV2B, and SV2C for BoNT/F (Fu et al. 2009; Rummel et al. 2009); and synaptotagmin I and II for BoNT/B (Dong et al. 2003; Nishiki et al. 1994, 1996) and BoNT/G (Rummel et al. 2004a). Interestingly, the BoNT serotypes which exhibit highest sequence similarity in binding region share the same protein receptor, i.e., A, E, and F bind to SV2 and B and G bind to SytI and SytII. The protein receptor(s) for BoNT/C and BoNT/D remain unknown. Binding of BoNTs to the peripheral neuromuscular junction is the initial step of intoxication. Apart from these receptors BoNTs have high-affinity (with nM dissociation constants) binding with ganglioside co-receptor (Binz and Rummel 2009). BoNT types A, B, C, and F bind GT1b, GD1a, and GD1b, respectively. BoNT/E binds GT1b and GD1a, whereas BoNT/G recognizes all gangliosides with similar affinity. BoNT/D binds phosphatidylethanolamine (PE). The ganglioside binding pockets lie in the Hcc domain. Crystal structure of Hcc fold is highly conserved for all BoNTs (~60 % homology). GT1b binds on a crevice formed by Trp1266 and Tyr1267 on one face and Glu1203, His1253, and Phe1252 on the other face. Generally, lactose-binding motif has all these key residues (H...SXWY...G) (Rummel et al. 2004b), and this is conserved among all BoNT serotypes (Stenmark et al. 2008). This particular motif provides hydrophobic character to the binding cavity. In contrast, BoNT/D, which interacts with PE, (phosphatidylethanolamine) lacks the key residues and shows a dependency on Lys1117 and Lys1135 for PE

binding. Study of Hcc structure in the presence and absence of the SytII peptide (Jin et al. 2006; Rummel et al. 2004b) is similar. Also in the presence and absence of GT1b, no change in conformation was inferred (Stenmark et al. 2008), which implies not only the Hcc fold is rigid but that the binding sites for GT1b and SytII are nonoverlapping. This still needs to be looked at carefully as there is no structure available for a BoNT/A-SV2 complex or of a BoNT/B-GT1b complex. Nevertheless, we have BoNT/A Hcc-GT1b complex (PDB 2VU9) and BoNT/B Hcc-SytII peptide complex (PDB 2NM1), which allow us to understand the lipid and receptor binding. It is also believed that solvent accessible surface of each BoNT serotype is unique. Each ternary complex (BoNT/A-SV2-GT1b or BoNT/E-glycosylated SV2A-GT1b) may expose distinct charge groups. PIPs (phosphatidylinositol 4,5-bisphsophate) are minor phospholipids in cell membrane and involve in recruitment/regulation of protein in membrane and in signaling. PIP binding site is identified in BoNT/A H_{CN}. Existence of two lipid binding sites, PIP and GT1b, suggests a dual anchorage mechanism for binding and internalization of this toxin (Muraro et al. 2009). Together with phospholipids, toxin acquires a new surface that confers a propensity for novel interaction which assisted in membrane insertion. Evidences suggest that after binding toxin does not enter the cell directly through plasma membrane, but is endocytosed into endosomes (enter into a lumen vesicular structure) in a temperature- and energy-dependent process (Dolly et al. 1984; Critchley et al. 1985; Black and Dolly 1986; Schiavo et al. 2000). The Hc domain of toxin plays an important role in internalization.

5.2 Pore Formation and Translocation

Second step after receptor-mediated endocytosis and internalization is the translocation of LC into the cytosol. LC must cross the hydrophobic barrier to reach cytosol. It is important to note that internalization and translocation both are distinct steps of cell intoxication. Translocation is a crucial step in the neurotoxicity of BoNTs. It is believed that H_N is translocation domain. H_N exhibits high-sequence conservation among all the serotypes with exception of the belt region that diverges significantly (Lacy and Stevens 1999). H_N helps in making pore in the endosomal membrane to facilitate LC translocation. LC translocation by HC stringently depends on the following conditions: (a) a pH gradient, acidic on the inside and neutral in the cytosol; (b) a redox gradient, oxidizing on the inside and reducing on the cytosolic side; and (c) a transmembrane potential (Montal 2010; Koriazova and Montal 2003; Fischer and Montal 2006, 2007).

There is no general consensus on how the translocation of LC occurs. According to one hypothesis, the LC unfolds at low pH and permeates through the transmembrane pore formed by H_N. After exposure to the neutral pH of the cytosol, the LC refolds, and it is released from the vesicle by reduction of the interchain disulfide bond. This is known as "tunnel" model (Boquet et al. 1976; Montecucco and Schiavo 1995), and formation of transmembrane ion-conducting pore is the prerequisite for this model. This model has some problems: (1) the light chains of toxin

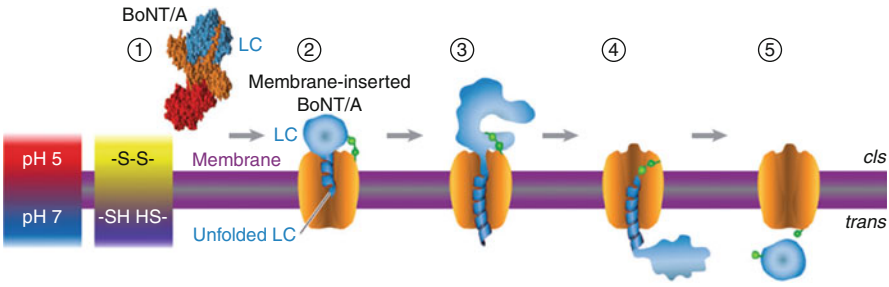

Fig. 7 Schematic representation of BoNT/A LC translocation. BoNT/A prior to insertion (*1*), membrane inserted BoNT/(*2*), partial unfolding of light chain and translocation (*3*) and (*4*), and exit to cytosol (*5*) (Copyright (2007) © National Academy of Science, USA)

may be exposed to the fatty acid chains of lipids, and (2) channel activity values of the order of a few tens of picoseconds do not account for the dimensions expected for a protein channel that has to accommodate a polypeptide chain with lateral groups of different volume, charge, and hydrophilicity.

A second model, proposed by Beise et al. (1994) suggests a decrease in the internal pH of endocytotic vehicle carrying toxin by ATPase proton pump, followed by neurotoxin insertion into the lipid bilayer. Electrochemical gradients and osmotic lysis cause destabilization of the lipid bilayers, breaking the membrane barrier, and the cargo of toxin molecules are released into the cytosol. This model explains the translocation of toxins fairly well, but some experimental findings are inconsistent with it. It is already shown that TeNT does not lyse the plasmalemma of neuronal cell at pH 5.0 and also diphtheria toxin forms channel without lysing the cell (Beaumelle et al. 1992).

Third model is the cleft model (Humeau et al. 2000; Simpson 2004) in which there is conformational change at low pH in both HC and LC in such a way that they expose their hydrophobic surfaces and enter into lipid bilayer by getting in contact with its hydrophobic core. The HC forms a transmembrane hydrophilic cleft that nests the passage of the partially unfolded LC with its hydrophobic segments facing the lipids. This process may be driven by electrochemical gradient, such as pH gradient across endosomal membrane. Neutral pH of cytosol and/or cytosolic chaperones help LC to fold and regain its water-soluble neutral conformation after reduction of the interchain disulfide bond. In the cleft model, the ion channel is a consequence of membrane translocation rather than a prerequisite.

A recent study by Montal (2010) suggests a very interesting mechanism of translocation, suggesting the role of interchain disulfide bond. This explanation suggests occlusion of heavy chain channel by light chain, if disulfide bond is reduced prior to translocation. Also, disulfide bond reduction within the bilayers aborts translocation. Translocation is only complete when disulfide bridge and the C-terminus of the LC enter the cytosol. According to this observation, it may be suggested that channel formation and translocation are two different events and not associated with each other (Montal 2010, Fig. 7).

Examination of BoNT crystal structure also reveals a transmembrane region (548–872) and a loop (561–568; completely disordered) connecting translocation belt (492–545 in BoNT/A and 481–532 in BoNT/B) and HN, which may change its conformation at low pH. The translocation domain appeared to be sensitive to environment variables, as the pH of this region is 4.66 (as compared to 9.1 for the binding domain and 6.0 for the whole toxin). There are several clusters of negative charges as well as proximal flexible structures in HN which can assist the pore formation (Lacy and Stevens 1999).

Evidences show that there are similarities in the action of botulinum and tetanus neurotoxins. TeNT, like BoNT, is a 150 kDa single-polypeptide protein with a heavy chain (100 kDa) and a light chain (50 kDa) region held together by a disulfide bridge and by noncovalent protein-protein interactions; TeNT also binds to peripheral nerve terminals, internalizes through endocytosis, and retrogrades to central nerves, where the light chain translocates into cytosol, leading to biochemical action that blocks neurotransmitter release.

The carboxyl terminal of TeNT heavy chain binds to cholinergic receptors of the presynaptic membranes of the central and peripheral nervous system, and possibly to sympathetic adrenergic fibers. Polysialogangliosides are an example of cholinergic receptors. TeNT binds tightly to such receptors because of the protein's multiple interactions with sugar and protein binding sites. It is retrogradely transported within the axon to the cell body. TeNT is released from the postsynaptic dendrites and taken up by the presynaptic nerve terminals. The entire journey of TeNT from peripheral neurons to its ultimate site of action, inhibitory neurons of central nervous system, involves binding, internalization, and transport within a cell. Transport of the toxin seems to occur within smooth vesicles, cisternae, and tubules. The toxin acts specifically on the interneurons of the spinal cord that inhibit voluntary muscle action. The N-terminus of the heavy chain is the translocation domain because it forms pores or toxin channels in the vesicle lipid bilayers of spinal cord neurons. The light (50 kDa) chain of TeNT is the toxic part of the protein. This portion is a specific protease that cleaves synaptobrevin, a vesicle-associated membrane protein (VAMP), at a single site. VAMPs help mediate membrane fusion, vesicle transport, and neurotransmitter release. Cleavage of synaptobrevin would not only denature the protein, but it would also prevent the exocytosis of the glycine and γ-aminobutyric acid neurotransmitters that control muscle contraction. With such actions inhibited, the nerve cells that control voluntary muscle action fire constantly and lead to the characteristic unopposed muscle spasm leading to spastic paralysis.

5.3 Blockage of Neurotransmitter Release

5.3.1 SNAREs and Neurotransmission

To understand the intracellular mechanism of action of clostridium neurotoxins, we first need to understand its substrate SNARE proteins and its role in neurotransmitter release. SNAREs are set of three proteins: synaptic vesicle protein, VAMP

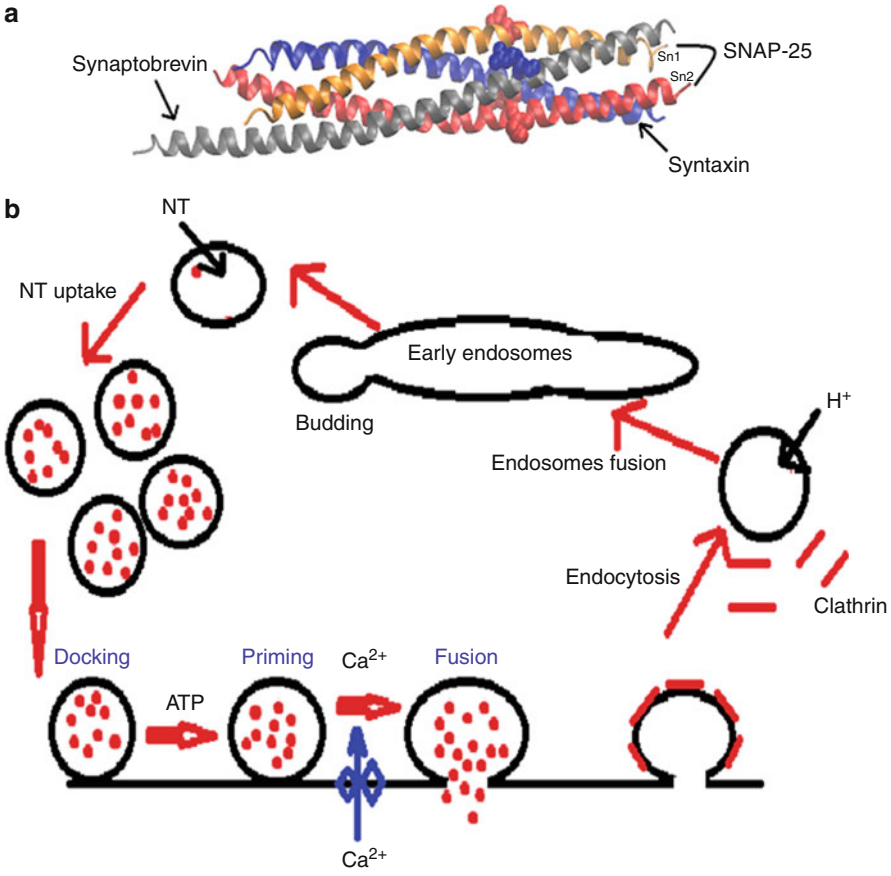

Fig. 8 (**a**) Tetrahelical assembly structure of SNARE complex, (**b**) schematic representation of the processes of endocytosis and exocytosis. Docking, priming, and fusion of exocytosis are represented in this figure. SNARE proteins (syntaxin, SNAP-25, and synaptobrevin) are critically involved in different steps of the exocytosis process. Synaptic vesicles loaded with neurotransmitter dock near the plasma membrane. Partial SNARE complex formation occurs in the priming step. Primed vesicles fuse very rapidly with the plasma membrane after calcium influx upon the arrival of the action potential. Energy for the fusion comes from SNARE assembly

(vesicle-associated membrane protein also called as synaptobrevin), and plasma membrane proteins, syntaxin, and SNAP-25 (synaptosome-associated protein of 25 kDa) (Fig. 8a). These three proteins are membrane receptors for NSF (*N*-ethylamide-sensitive factors) and SNAPs (soluble NSF attachment proteins and are named as SNARE (SNAP receptors) proteins. SNAREs can be divided into two categories based on their localization inside the cells: v-SNAREs, localized in vesicle membrane, and t-SNAREs, localized in target membrane or plasma membrane. They are also sometimes termed as R-SNARE (middle group arginine) and Q-SNARE (middle group glutamines). SNARE proteins are characterized by

a conserved 60–70 amino acid motif. In addition to this motif SNAREs also have sequences that anchor them to the membrane. Syntaxin and synaptobrevin are anchored through their respective transmembrane domains, whereas SNAP-25 associates with the plasma membrane via the palmitoylation of cysteine residues in the linker region. These proteins play a very important role in the membrane fusion and neuronal exocytosis, one of the important physiological processes in neuronal cells. SNAP-25 and syntaxin are highly conserved in their isoforms. SNAP-25 is also needed for axonal growth and regeneration (Osen et al. 1993). One of the unique characteristic features of SNARE proteins is their assembly in 1:1:1 stoichiometry to form a very stable ternary complex, which is resistant to dissociation by sodium dodecyl sulfate (SDS). During the ternary complex formation, carboxy-terminal domain of syntaxin (amino acids 199–243; H-3 helix) binds to SNAP-25. Complex of SNAP-25 and syntaxin binds to the central part of the synaptobrevin (amino acids 27–96). All of SNAP-25 is required for binding with synaptobrevin (VAMP2, 29–60) and amino- and carboxy-terminal domains participate in binding to syntaxin (syntaxin 1, 199–243). Assembly of SNARE complex brings the two opposing membrane bilayers of synaptic vesicle and plasma membrane closure and acts as a bridge. The arrival of action potential triggers inflow of Ca^{2+} ion, allowing the vesicle to dock at the active zone. After docking, second Ca^{2+} influx in the active zone creates a fusion pore involving two other proteins: synaptophysin and physophillin. Other vital components in this process are synaptotagmin and complexin. Complexin interacts with tetrahelical strands (Fig. 8a) of SNARE complex and prevents neurotransmitter release until an action potential arrives at synapse to facilitate quantal release (Kummel et al. 2012). Synaptotagmin binds to Ca^{2+} ion and acts as a sensor for vesicle fusion and quantal neurotransmitter release (Kummel et al. 2012). Formation of initial pore leads to complete fusion of vesicle membrane to plasma membrane, and the content of vesicle (neurotransmitter) is released into the synaptic cleft. The whole process is called exocytosis (Fig. 8a and b).

5.3.2 Motifs and Conformations Involved in Unique Substrate Recognition of BoNT Endopeptidase

All the light chains are Zn-metalloprotease of thermolysin family but are unique among Zn proteases due to their stringent specificity for substrates or at least long peptide segments containing highly selective cleavage sites. BoNT LCs require 16 to >50 residue long substrates whereas other Zn proteases can hydrolyze as short as two residue peptides (Shone et al. 1993; Cornille et al. 1997; Shone and Roberts 1994). The known substrate for BoNTs is SNARE proteins with stringent substrate specificity. TeNT and BoNTs target and peptide bond specificity is displayed in Table 2.

Each of the clostridial toxins cleaves their targets at distinct sites, except BoNT/B and TeNT which cleave the same peptide bond on the same substrate. This exquisite selectivity is believed to be due to the presence of highly conserved motifs in SNARE proteins. These motifs are termed as S1 to S4 in SNAP-25; V1 and V2 in VAMP; and X1 and X2 in syntaxin. Although BoNT/B and TeNT attack the same

Table 2 TeNT and BoNT target and peptide bond specificities. Cleavage site is at P1–P1'

Toxin type	Substrate	P4-P3-P2-P1-P1′-P2′-P3′-P4′
TeNT	VAMP	G- A- S- Q- F- E- T- S
BoNT/A	SNAP-25	E- A- N- Q- R- A- T- K
BoNT/B	VAMP	G- A- S- Q- F- G- T- S
BoNT/C	SNAP-25	A- N- Q-R- A- T- K-M
BoNT/C	Syntaxin	D- T- K- K- A- V- K- F
BoNT/D	VAMP	R- D- Q- K-L- S- E- L
BoNT/E	SNAP-25	Q- I- D- R- I- M- E-K
BoNT/F	VAMP	E- R- D-Q- K-L- S- E
BoNT/G	VAMP	E- T- S- A- A-K- L-K

Gln-Phe peptide bond, these toxins display distinct affinities for V1 and V2. For efficient cleavage, BoNT/A and BoNT/E require C-terminal region of SNAP-25 which has S4 motifs. However, S1 (leu20-Arg30), S2 (leu36-Ile45), and S3 (Met50-Glu59) motives partially substitute for S4 (Glu145-Ser154) motif (Washbourne et al. 1997). SNARE proteins are protected from proteolytic attack if they are bound to ternary complex (Poulain et al. 2008). This is probably due to masking of the cleavage site or recognition motifs.

Several evidences suggest that neurotoxins recognize tertiary structure of their substrates:

(a) Clostridial neurotoxins require long substrates compared to other Zn-metalloprotease.
(b) They cleave their substrates exclusively at single-specific site out of several identical peptide bonds present in their respective target proteins.
(c) Synthetic peptides of same cleavage segments are not hydrolyzed by the neurotoxins; they are shown to be good inhibitors of proteolytic activity of the toxins. This means although they can bind, they are not cleaved by toxins (Schiavo et al. 1992a, b, 1993a, b; Dayanithi et al. 1994).
(d) Light chains of these toxins cleave very specific bonds even if the same bonds are present in other part of the substrate sequence (Gln15-Arg16 of SNAP-25 in case of BoNT/A and Arg59-Ile60 and Arg191-Ile192 in case of BoNT/E).

Apart from SNARE, several homologues of these proteins are known as a substrate for BoNT, which can be cleaved to different degrees. Murine SNAP-23 can be cleaved by BoNT/E and to some extent by BoNT/A, but human SNAP-23 is resistant to both because of mutation Lys185Asp and Pro185Arg (Washbourne et al. 1997; Vaidyanathan et al. 1999; Pickett and Perrow 2011). Mutations in amino acid sequences of the toxin targets make them resistant to proteolysis and can account for almost all cases of species insensitivity to BoNT or TeNT.

LC of BoNT is a compact globular structure composed of a combination of β-sheets and α-helices (PDB ID 1XTG; 1 F82; 1T3A; 2ISE). All the crystal structures reported so far are derived from a truncated version of the LCs.

Fig. 9 Active site motifs of clostridial neurotoxins

BoNT/A	**D**	**P**	**A**	**V**	**T**	**L**	**A**	**H**	**E**	**L**	**I**	**H**	**A**	**G**	**H**	**R**	**L**	**Y**	**G**
BoNT/B	**D**	**P**	**A**	**L**	**I**	**L**	**M**	**H**	**E**	**L**	**I**	**H**	**Y**	**L**	**H**	**G**	**L**	**Y**	**G**
BoNT/C	**D**	**P**	**I**	**L**	**I**	**L**	**M**	**H**	**E**	**L**	**N**	**H**	**A**	**N**	**H**	**N**	**L**	**Y**	**G**
BoNT/D	**D**	**P**	**V**	**I**	**A**	**L**	**M**	**H**	**E**	**L**	**I**	**H**	**S**	**L**	**N**	**Q**	**L**	**Y**	**G**
BoNT/E	**D**	**P**	**A**	**L**	**T**	**L**	**M**	**H**	**E**	**L**	**I**	**H**	**S**	**L**	**H**	**G**	**L**	**Y**	**G**
BoNT/F	**D**	**P**	**A**	**I**	**S**	**L**	**A**	**H**	**E**	**L**	**I**	**H**	**A**	**L**	**N**	**G**	**L**	**Y**	**G**
BoNT/G	**D**	**P**	**A**	**L**	**T**	**L**	**M**	**H**	**E**	**L**	**I**	**H**	**Y**	**L**	**H**	**G**	**L**	**T**	**G**
TeNT	**D**	**P**	**A**	**L**	**I**	**L**	**M**	**H**	**E**	**L**	**I**	**H**	**Y**	**L**	**H**	**G**	**L**	**Y**	**G**
Zn endopeptidase								**H**	**E**	**X**	**X**	**H**							

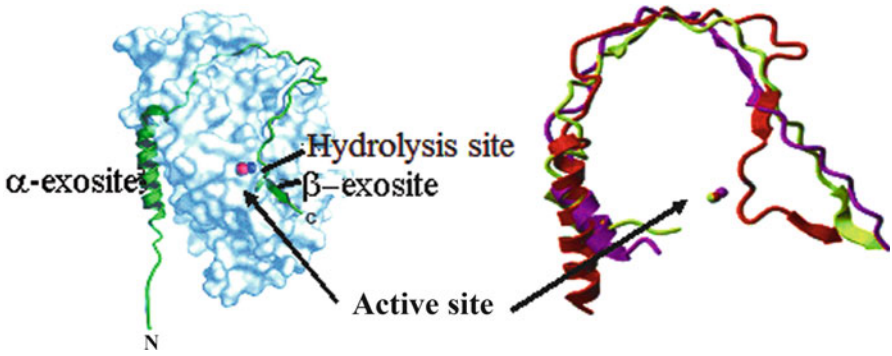

Fig. 10 Structure of BoNT/A LC in complex with truncated SNAP-25 (Sn2, *left*), showing interaction of SNAP-25 (Sn2 segment) with α- and β-exosites and active site. *Right* figure shows overlapped structure of SNAP-25 (Sn2 segment, *red*) and HC belt of BoNT/A (*magenta*) and BoNT B (*lime*) (Adapted from Brunger et al. 2007)

The central part of this enzyme is Zn^{2+} binding motif, HEXXH + E, and is conserved among all BoNTs. The finding is that all the clostridial neurotoxins have similar active site motifs, suggesting common evolutionary origin of these proteins (Fig. 9).

It is notable that the active site of BoNT is located in a very deep and narrow crevice, whereas in thermolysin active site is broad and very near to the surface (Fig. 10). In case of BoNT/A LC, active site crevice travels through half of the enzyme structure (20 Å deep) and is negatively charged (Brunger et al. 2007). Interestingly, translocation domain has a belt, a unique loop in extended conformation (residues 492–545 for BoNT/A and 481–532 for BoNT/B). This belt has a remarkable similarity with residues 141–205 (the Sn2 segment) of SNAP-25 in spite of low sequence similarity of the belts among all the clostridial toxins (BoNT and TeNT; Lacy and Stevens 1999; Chen et al. 2007b, Fig. 10). For BoNT/A, the catalytic Zn^{2+} is bound to the active site by interaction with imidazole ring of His223 and His227 and with the carboxyl side chain of Glu262. The Zn^{2+} is also coordinated by a water molecule, which is activated by Glu224, and believed to

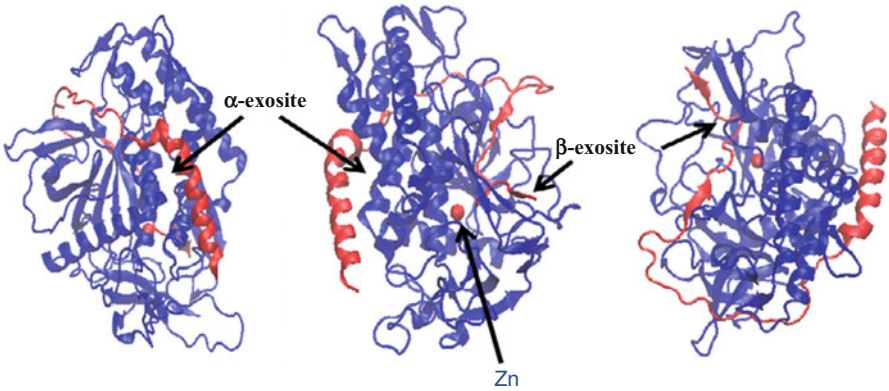

Fig. 11 Crystal structure of BoNT/A LC with its substrate SNAP-25 (Sn2). α-exosite and β-exosite are shown as a primary substrate recognition point. 250 and 370 loops are also interacting with substrate (Breidenbach and Brunger, Nature 2004)

play a critical role in proteolysis. However, Glu262, which is directly coordinating to active site Zn, also plays a pivotal role in the stability of active site (Kukreja et al. 2007). Furthermore, four flexible loops, 50/60 loop, 170 loop, 370 loop, and 250 loop, form the rim of active site cleft and are believed to take part in substrate binding. Rest of the protein is a mixture of α-helix and β-sheets.

A topographical model for the interaction of BoNT with its substrate has been described by Breidenbach and Brunger (2004, Fig. 11) who used enzymatically inactive version of BoNT/A LC (created by two point mutations, E224Q and Y366F) and carboxy-terminal SNARE domain of SNAP-25, residues 141–204 (Sn2), to obtain crystal structure of enzyme-substrate complex. The crystal structure of the complex revealed that apart from active site, this molecule has two exosites: α-exosite and β-exosite. α-exosite is a hydrophobic patch formed at the interface of four light chain-α-helices, residues 102–113 (α-helix 1), 310–321 (α-helix 2), 335–348 (α-helix 3), and 351–358 (α-helix 4). β-exosite is a distorted two strand in the 250 loop of the LC, a peptide segment with residues 242–249. The amino-terminal helical region of Sn2 interacts with the endopeptidase α-exosite, whereas C-terminal of Sn2 interacts with β-exosite. The hydrophilic face of the amphipathic Sn2 helix is oriented towards the solvent, while the hydrophobic side chain buried against the α-exosite. Interaction with β-exosite is primarily mediated by backbone interactions, but Met 202 of Sn2 is also very critical for this interaction. Substrate shorter than residues prior to Met 202 cannot be cleaved by BoNT at a measurable rate (Schmidt and Bostian 1995). β-exosite is very critical for the recognition of BoNT. Notably, residues 183–190 of Sn2 detach from the surface of LC and rejoin the LC at Arg191. There are series of anchoring points that could serve as additional determinants of substrate specificity. Substrate residues 170–172 and 192–193 are two anchoring points, and individual side chains from Arg176, Ile178, Ile181, and Glu183 are also recognized by the endopeptidase. This may be one of the reasons for the requirement of long substrate. The largest

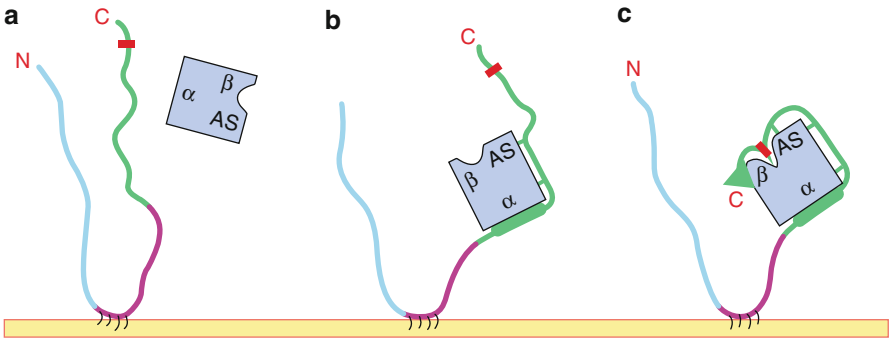

Fig. 12 Schematic model of BoNT/A LC substrate recognition and cleavage (Adapted from Breidenbach and Brunger 2004). (**a**) Sn1 (*cyan*) and Sn2 (*green*) domains of SNAP-25 are attached to presynaptic membrane by palmitoylation (*black*) of cysteine residues of SNAP-25. (**b**) Binding of BoNT/A is initiated by recognition of Sn2 domain of SNAP-25 by α-exosite of protein and few anchoring points (*green* notches). (**c**) After these interaction SNAP-25 binds to β-exosite, inducing conformation change in 370 loop, which leads to cleavage of SNAP-25 by BoNT/A LC

structural changes that are related to substrate binding occur in 250 loop (β-exosite) and 370 loop In BoNT/A; the 250 loop adopts an open conformation which contracts and folds over the 370 loop upon substrate binding at the β-exosite.

The model of active site and exosites of BoNT/A endopeptidase explains the mechanism of SNAP-25 substrate cleavage as follows. SNAP-25 is attached to presynaptic membrane through palmitoylation of cysteine residues. C-terminus and N-terminus are unstructured or flexible in the uncomplexed SNAP-25. SNAP-25 upon binding to BoNT/A probably initiates helix formation which is recognized by α-exosite and anchored points and finally by β-exosite (Figs. 11 and 12).

A major point against this model is that the BoNT/A L chain and SNAP-25 co-crystal structure do not show any significant direct interaction of the α-exosite of the light chain with the S4 motif of SNARE, not considered critical for binding of SNAP-25 (Breidenbach and Brunger 2004), but are critical for the endopeptidase activity (Washbourne et al. 1997). Thus, while the crystal structures provide useful information on the folding patterns of BoNTs, and on the interaction between BoNT/A light chain and SNAP-25, these do not take into consideration the role of dynamic structures in the functioning of the molecule. Nevertheless, this co-crystal structure provides a very good model to understand the behavior of this molecule with its substrate. The crystal structure explains why clostridium neurotoxins can cleave free SNAREs but not the SNARE complex because when SNARE complex is formed, then the residues of SNAP-25 involved in recognition/bindings are not accessible for these interactions. Notably, C-terminus of BoNT/A LC plays important role in catalysis. Finding by Baldwin et al. (2004) suggests that minimum residue needed for optimal catalysis is 1–425. Deletion of C-terminal region beyond 425 has a small effect on substrate binding but drastically reduced the catalytic efficiency. It has been speculated that there is extensive H-bond network in the

C-terminal region which stabilizes the structure of C-terminal loop region as well as active site core (Baldwin et al. 2004).

5.3.3 Static Versus Dynamic Structure: Role of PRIME Conformation

The static crystal structure of botulinum endopeptidase determined by x-ray diffraction studies (Segelke et al. 2004; Breidenbach and Brunger 2004) does not adequately describe the dynamic properties of the enzyme in solution where it represents a manifold of conformational substates. Indeed, crystal packing forces might lead to structural arrangements, which might not be relevant to solution conditions. Conformational fluctuations resulting from collective modes of motion, which involve concerted displacements of many atoms, can push the unbound states of enzymes into conformations closely resembling the bound states, thereby priming them to form complexes with the substrate. Hence, every conformational state of the enzyme that is populated along the reaction pathway presents a unique opportunity for interactions with substrate or drug molecules.

We have recently discovered biologically active molten globule structure of the BoNT/A (Cai and Singh 2001). The catalytic domain of BoNT/A also possesses a novel conformation at the physiological temperature of 37 °C and is named as PRIME (pre-imminent molten globule enzyme) state (Kukreja and Singh 2005). The PRIME state is dynamically flexible and is likely to facilitate specific interactions with its substrate, SNAP-25 for its optimum and selective enzymatic activity. Thus, we suspect that the PRIME conformational state provides a flexible structure which plays a very critical role in the biological function of BoNT/A, especially in its intracellular toxic action.

The schematic model of the temperature-induced PRIME conformational and molten globule states of BoNT/A light chain, as represented in Fig. 13, reflects how these states in BoNT/A light chain may facilitate binding and cleavage of SNAP-25. The intramolecular mobility of PRIME state is significantly higher than that of the native state which can be attributed to increased dynamics and expansion of the protein core, facilitating a maximum specific binding of SNAP-25, leading to its cleavage. The molten globule conformation observed at 50 °C, on the other hand, has a considerably intact secondary structure, loose packing of side chains in the protein core, and partial unfolding of loops. This structure also binds to SNAP-25, although to a lesser degree than the PRIME state, thus showing only about 61 % of the optimum enzymatic activity (Fig. 13).

Our observation of the PRIME conformation of BoNT/A LC with optimum enzymatic activity is the first observation of enzymatically active molten globule or molten globule-like structure (Kukreja and Singh 2005). The role of such a dynamic structure is more significant in view of the extreme specificity of the endopeptidase towards its substrate, SNAP-25, and its tremendous utility in designing specific antidotes against botulism threats. This structure of BoNT/A light chain seems to play a major role in its endopeptidase activity and particularly as it interacts with potential inhibitor candidates. Currently, BoNT/A light chain used in most

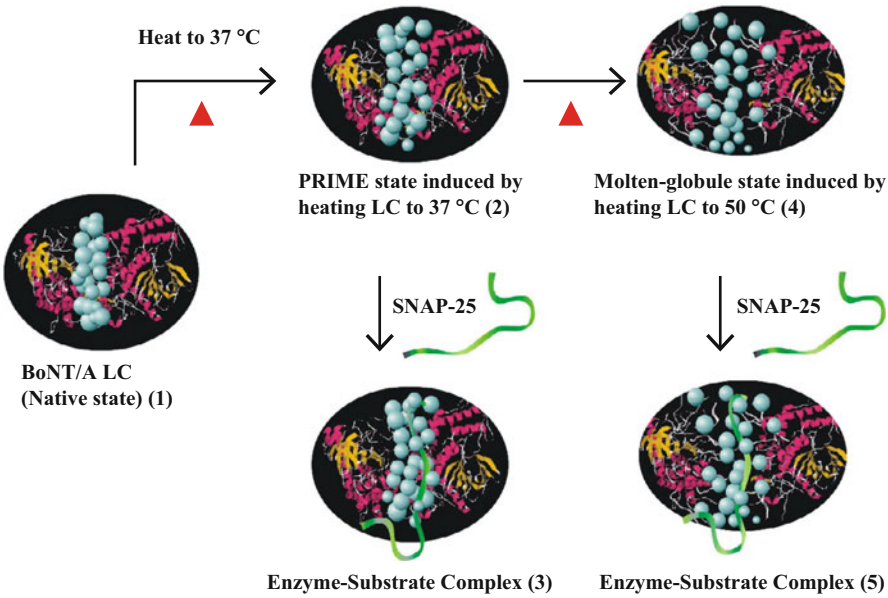

Fig. 13 Schematic model representing the PRIME state and the molten globule state in BoNT/A LC facilitating binding of substrate SNAP-25. The *blue*-colored balls represent the nonpolar side chains which are tightly packed in the native conformation (1). Upon heating to 37 °C, there are significant alterations in the polypeptide folding and the protein core becomes slightly loosened compared to the native state and forms the PRIME state (2), facilitating binding of SNAP-25 to form the enzyme-substrate complex (3). BoNT/A LC exhibits optimum activity in the PRIME state. Further heating of BoNT/A LC to 50 °C leads to the formation of the molten globule (4), facilitating binding of SNAP-25 to form the enzyme-substrate complex (5) although to a lesser degree than the PRIME state, thus exhibiting only about 61 % of the optimum enzyme activity (Kukreja and Singh 2005)

laboratories working on structure and inhibitor development is a truncated form of the 448 residue full-length polypeptide. The forms being used are LC-444, LC-438, LC-425, and LC-424. Although all these truncated forms have enzyme activity, their interactions with potential inhibitors as well as their substrates (peptide substrate and whole protein substrate, SNAP-25) were peculiarly different (Baldwin et al. 2004; Fredenburg et al. 2007), and a dynamic conformational variability could explain these differences. Silvaggi et al. (2008) have in fact observed limited flexibility of active site, although those changes would not necessarily explain differential interaction of BoNT endopeptidases with their respective substrates, because of the involvement of exosites in substrate binding (Breidenbach and Brunger 2004). Burnett et al. (2007) have observed that "Our inability to identify an acceptable binding mode for mpp-RATKML led to a pivotal question: is it possible that the conformation of the x-ray determined BoNT/A LC may not be the bioactive conformation, at least with respect to inhibitor binding?"

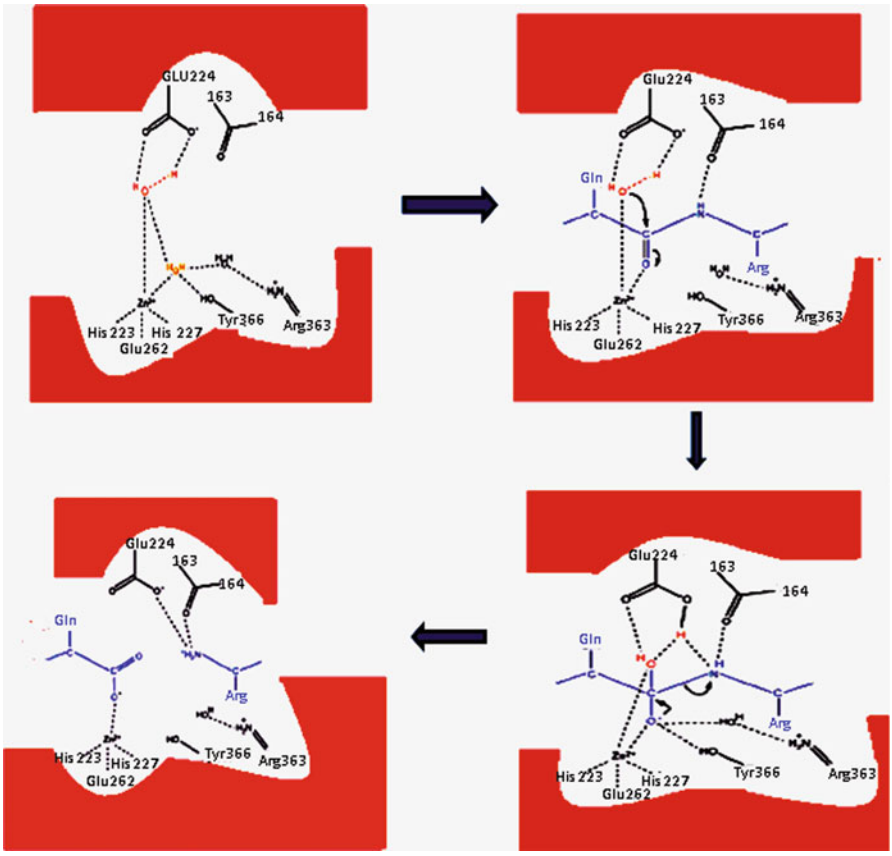

Fig. 14 Schematic representation of proposed BoNT/A LC mechanism. A polarized water molecule, by Glu223 and Zn^{2+} (*top left*), nucleophilically attacks the carbonyl carbon of the scissile bond to form an oxyanion (*top right*). This oxyanion is stabilized by Tyr366 (*bottom right*). Peptide bond of the substrate is cleaved by two proton transfer for activated water molecule mediated by Glu223. This process resulted in a protonated amine (*bottom left*)

Mechanistic details of a flexible structure interacting with its whole substrate have yet to be worked out, but when that is resolved it could reveal transformative role of protein dynamics in the protein-protein interactions involved in such a high specificity in BoNT/A endopeptidase-SNAP-25 interactions.

5.3.4 Chemical Mechanism of Substrate Cleavage

Proposed mechanism of substrate cleavage is depicted in Fig. 14. In the first step water molecule is polarized by Glu224 and Zn^{2+}. Activated oxygen of water mounts a nucleophilic attack on the carbonyl carbon of P1 of scissile bond. Side chain of Tyr366 is responsible for stabilization of the resulting oxyanion, which develops at

P1 carbonyl oxygen atoms. Oxyanion is also stabilized by Arg363 through a water molecule. Peptide bond cleavage is likely achieved by two proton transfer from the attacking water molecule mediated by Glu224 and results in the protonated amine of P1'. Proposed mechanism is slightly different from the mechanism of another Zn-metalloprotease, thermolysin. In thermolysin, Tyr157 and His231 have been proposed to stabilize the transition state (Matthews 1988). No residues have been identified to involve in the stabilization of the transition state of ES (enzyme-substrate) complex of BoNT-catalyzed proteolysis. In fact, the space occupied by these two residues in thermolysin is occupied by Sn2 residues in the case of BoNT/A. This leads to two hypothesis: either there are no residues in the case of BoNT/A to stabilize the evolving oxyanion or that BoNT/A might involve substrate-assisted catalysis.

His223, Glu224, His227, and Glu262 are the important residues in BoNT/A active site, and they are conserved in the other serotypes. Mutation of Glu224 to Asp leads to almost total loss of activity, and removal of His227 abolishes the catalytic activity of BoNT/A. Analysis of TeNT neurotoxin has confirmed that these key residues are identical to BoNT/A. Other residues, such as Glu271 and Tyr375 (in TeNT) and Arg 363 and Tyr365 (in BoNT/A), have been shown to be involved in catalysis, and it has been proposed that these residues may be involved in transition state stabilization.

6 Effects and Consequences of Botulinum Neurotoxin Exposure

As mentioned earlier, botulinum toxins are the most poisonous substance known. The LD50, dose of these toxins in mouse when administered intravenously, subcutaneously, or intraperitoneally, is estimated 1 ng/kg. Tetanus toxin LD50 is 2 ng/kg. All the botulinum toxins are less toxic when exposure is through pulmonary route. Human LD50 is 3 ng/kg (McNally et al. 1994). The effect of botulinum toxin may affect areas away from the injection site and cause serious symptoms including loss of strength and all-over muscle weakness, double vision, blurred vision and drooping eyelids, hoarseness or change or loss of voice (dysphonia), trouble saying words clearly (dysarthria), loss of bladder control, trouble breathing, and trouble swallowing. While all the seven serotypes have similar domain organization and function, and show only immunological distinction (Simpson et al. 2004), duration of their intracellular action (longevity) and potency varies substantially. BoNT/A, BoNT/B, and BoNT/E intoxication is persistent for about 180 days, 90 days, and 30 days, respectively (Adler et al. 2001; Dolly and Aoki 2006; Pickett and Perrow 2011).

Persistence of BoNT/A intoxication is because of stability of its catalytic domain, light chain, inside the neuronal cells. This is supported by intoxication of BoNT/A in spinal cord neurons (Keller et al. 1999) and muscle paralysis experiments (Adler et al. 1996). Notably, neurons and neuroendocrine cells have high level of tyrosine kinases (Brugge et al. 1985; Maher 1988). Therefore, it has been

speculated that intercellular phosphorylation of light chain may contribute to latter's longevity (Montiel et al. 1996). Recently, it has also been proposed that differential ubiquitination of different BoNT light chains may be responsible for differential intercellular stability of BoNT proteins (Tsai et al. 2010). Because of the longevity of BoNT intoxication, and that too due to the survival of toxin inside the cell, an inadvertent consequence is the possibility of the host cellular response to the long-term presence of the toxin.

Microarray analysis reveals that 169 genes were upregulated, while 60 genes were downregulated in epithelial cell (HT-29), whereas 223 genes were upregulated and 18 genes were downregulated in neuronal cell model (SH-SY5Y) exposed to toxin for just 96 h. These modulated genes are associated with neuroinflammatory, ubiquitin-protease degradation, phosphatidylinositol, and calcium signaling in SH-SY5Y pathways (Thirunavukkarasu et al. 2011). Interestingly, these modulated genes are different from those observed upon exposure of HT-29 cells, which are model for gut epithelial cells. Are there gene expression responses related to survival of BoNT intracellularly? Further research work of cells to different serotypes of BoNT will shed more light on the genomic host response and BoNT longevity inside the neuronal cells.

As BoNTs are associated with NAPs, and in most cases of botulism cases or even BoNT as a therapeutic drug, patients are exposed to the BoNT complex, it is important to consider the effect of NAPs as well on biochemical and physiological host response. Immunogenicity of BoNT/A complex is more than that of pure BoNT/A itself. Interestingly, NAPs are more immunogenic than the toxin (Kukreja et al. 2009). HA33, molecule major component of BoNT/A NAPs, exhibits maximum immunogenicity. HA33 is also shown to inhibit apoptosis (Kumar et al. 2012). Apoptosis is also inhibited by BoNT/A in SH-SY5Y cells (Kumar et al. 2012), although BoNT/C is shown to enhance apoptosis in cerebral granular cells (Berliocchi et al. 2005). While these studies indicate that BoNT and NAPs significantly affect cell metabolism and physiology, there are many questions, including variations in responses to different BoNT types, variation in responses of different cell and tissue types, and variation in responses due to different states of cellular physiology, that remain to be addressed with further experiments.

Effect of botulinum toxin on nerve impulse activity and muscle contractions resumes over time as a result of sprouting near to nerve ending, thus renewing the ability of the nerve to cause muscle contraction. Eventually, the new nerve retracts and original nerve ending regains its function (Jankowic 2004; De Paiva et al. 1999, Fig. 15).

Release of neurotransmitter is affected by botulinum neurotoxins due to the cleavage of SNARE proteins, but that is not the only process affected by BoNT. Neuropeptide P, or substance P, involved in neurogenic inflammation and pain disorders is also affected by BoNT (Aoki 2004). There is lack of evidence of direct effect of BoNT on central nervous system. Because of its size, 150 kDa, BoNT cannot penetrate blood–brain barrier (Dressler et al. 2005). Possibility of retrograde transport has been suggested previously; however, no transsynaptic transport has

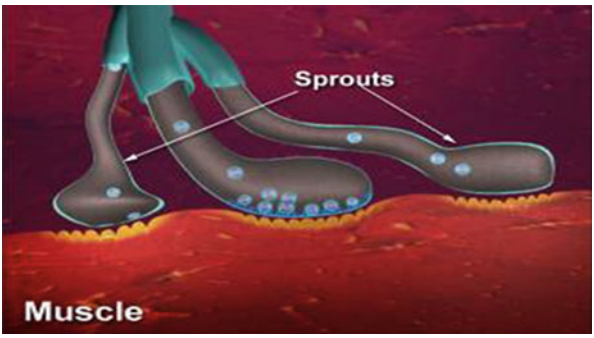

Fig. 15 Nerve sprouting induced by botulinum toxin (http://www.allergan.com/assets/pdf/botox_mechanism_of_action.pdf)

been observed. In vitro studies suggest that BoNT can affect central nervous system (Antonucci et al. 2009; Caleo and Schiavo 2009). BoNT/E has been shown to affect glutamate release and block spikes activity of pyramidal neurons in vivo (Bozzi et al. 2006). A recent study finds that BoNT spreads in CNS tissues (Jamie 2008); however, the injected dose was way too high compared to the doses used in therapeutic application.

7 Medical Applications of Clostridial Neurotoxins

Despite being highly toxic, clostridial neurotoxins are highly effective therapeutic agents. BoNT-based therapeutic products are approved by USFDA which include Botox®, Dysport® (BoNT/A complex), Myobloc®, Neurobloc® (BoNT/B complex), and Xeomin® (purified BoNT/A). These products are very popular cosmetic drugs in the world. USFDA also approved BoNT/A for the treatment of strabismus, blepharospasm, and hemifacial spasm. Since BoNT affects autonomous nervous system, it has been shown for effective treatments in hyperhidrosis, migraine, and myofascial pain. However, like other medical drug, BoNT treatment has some side effects too. Since these therapeutic agents are proteins, they have potential for antibody formation leading to a decrease in effectiveness of treatment. Since cholinergic neurons are important part of cognitive systems, BoNT can have potential role there as well and can have long-term effect on cognition (Lackovic et al. 2009; Ney and Joseph 2007). But this has been observed in relatively low number of patients.

8 Potential Future Applications

Each domain of clostridial toxins has very unique characteristics which can be used for some other medical applications. Retargeting of the endopeptidase domain of clostridial neurotoxin neuronal and nonneuronal cells is successfully performed. Retargeted LH_N (light chain fused with N-terminus of heavy chain) with nerve growth factor (NGF) and wheat germ agglutinin (WGA) has already been reported

(Chaddock et al. 2000a, b). BoNT usefulness in alleviating pain is still under investigation. Mechanism is not very clear and there is no clear dose–response relationship. Retargeting technology has been exemplified using a variety of targeting ligands in a diverse range of cell types (Yeh et al. 2011; Pickett 2010; Somm et al. 2012; e.g., HEK (human embryonic kidney) 293 cells and BMDMs (bone marrow-derived macrophages). Additionally, the ability of BoNT and TeNT to transport large polypeptide makes them a good delivery vehicle. TeNT has been shown to transport DNA and enzymes in neurons (Toivonen et al. 2010; Wang et al. 2012). BoNT/A and BoNT/B have been tested for treatment of Parkinson's, rabies, and Tuorette's disease. One thing for sure is that anticholinergic effect of BoNT can be exploited for medicinal applications associated with physiological system employing cholinergic neurons. Above observations suggest that clostridial neurotoxins have become a valuable research and medicinal tool. Also it seems more uses of BoNT and its derivatives are in the offing. In conclusion, Mother Nature has provided a unique molecule with unique characteristics which can be exploited for other beneficial uses.

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References

Adler, M., McDonald, D. A., Sellin, L. C., & Parker, G. W. (1996). Effect of 3,4-diaminopyridine on rat extensor digitorum longus muscle paralyzed by local injection of botulinum neurotoxin. *Toxicon, 34*(2), 237–249.

Adler, M., Keller, J. E., Sheridan, R. E., & Despande, S. S. (2001). Persistence of botulinum neurotoxin. A demonstrated by sequential administration of serotypes A and E in rat EDL muscle. *Toxicon, 39*, 233–243.

Ahmed, S. A., & Smith, L. A. (2000). Light chain of botulinum A neurotoxin expressed as an inclusion. Body from a synthetic gene is catalytically and functionally active. *Journal of Protein Chemistry, 19*, 475–487.

Ala-Huikku Nurmi, K. E., Pajulahti, H., & Raevuori, M. (1977). The occurrence of *Clostridium botulinum* type E in Finnish trout farms and the prevention of toxin formation in fresh-salted vacuum-packed trout fillets. *Nordisk Veterinaermedicin, 29*, 386–391.

Antonucci, F., Di Garbo, A., Novelli, E., Manno, I., Sartucci, F., Bozzi, Y., & Caleo, M. (2008). Botulinum neurotoxin E reduces CA1 neuron loss and granule cell dispersion, with no effects on chronic seizures, in a mouse model of temporal lobe epilepsy. *Experimental. Neurology, 210*, 388–401.

Antonucci, F., Rossi, C., Gianfranceschi, L., Rossetto, O., & Caleo, M. (2009). Long-distance retrograde effects of botulinum neurotoxin A. *Journal of Neuroscience, 28*, 3689–3696.

Aoki, K. R. (2004). Botulinum toxin: a successful therapeutic protein. *Current Medicinal Chemistry, 11*, 3085–3092.

Aranda, E., Rodriguez, M. M., Asensio, M. A., & Cordoba, J. J. (1997). Detection of *Clostridium botulinum* types A, B, E and F in foods by PCR and DNA probe. *Letters in Applied Microbiology, 25*, 186–190.

Arndt, J. W., Jacobson, M. J., Abola, E. E., Forsyth, C. M., Tepp, W. H., Marks, J. D., Johnson, E. A., & Stevens, R. C. (2006). A structural perspective of the sequence variability within botulinum neurotoxin subtypes A1-A4. *Journal of Molecular Biology, 362*, 733–742.

Ashie, I. N., Smith, J. P., & Simpson, B. K. (1996). Spoilage and shelf-life extension of fresh fish and shellfish. *Critical Reviews in Food Science and Nutrition, 36*, 87–121.

Ashton, A. C., & Dolly, D. O. (1988). Characterization of the inhibitory action of botulinum neurotoxin type A on the release of several transmitters from rat cerebrocortical synaptosomes. *Journal of Neurochemistry, 50*, 1808–1816.

Bade, S., Rummel, A., Reisinger, C., Karnath, T., Ahnert-Hilger, G., Bigalke, H., & Binz, T. (2004). Botulinum neurotoxin type D enables cytosolic delivery of enzymatically active cargo proteins proteins to neurons via unfolded translocation intermediates. *Journal of Neurochemistry, 91*(6), 1461–1472.

Baldassi, L., Hipolito, M., Portugal, M. A., Calil, E. M., Moulin, A. A., & Pires, D. C. (1991). Bovine botulism: Laboratory confirmation of clinical diagnosis in 1986-1989. *Revista de Saúde Pública, 25*, 371–374.

Baldwin, M. R., Bradshaw, M., Johnson, E. A., & Barbieri, J. T. (2004). The C-terminus of botulinum neurotoxin type A light chain contributes to solubility, catalysis, and stability. *Protein Expression and Purification, 37*, 187–195.

Beaumelle, B., Bensammar, L., & Bienvenue, A. (1992). Selective translocation of the A chain of diphtheria toxin across the membrane of purified endosomes. *Journal of Biological Chemistry, 267*, 11525–11531.

Beise, J., Hahnen, J., Andersen- Beckh, B., & Dreyer, F. (1994). Pore formation by tetanus toxin, its chain and fragments in neuronal membrane and evaluation of the underlying motifs in the structure of the toxin molecule. *Naunyn-Schmiedeberg's Archives of Pharmacology, 349*, 66–73.

Berliocchi, L., Eugenio, F., Marcel, L., Volkar, H., David, D., David, R., & Nicotera, P. (2005). Botulinum neurotoxin C initiates two different programs for neurite degeneration and Neuronal apoptosis. *Journal of Cell Biology, 168*, 607–618.

Billon, C. M., McKirgan, C. J., McClure, P. J., & Adair, C. (1997). The effect of temperature on the germination of single spores of *Clostridium botulinum* 62A. *Journal of Applied Microbiology, 82*, 48–56.

Binz, T., & Rummel, A. (2009). Cell entry strategy of clostridial neurotoxins. *Journal of Neurochemistry, 109*, 1584–1595.

Black, D., & Dolly, J. O. (1986). Interaction of ^{125}I-labeled botulinum neurotoxins with nerve terminals: Autoradiographic evidence for its uptake into motor nerves by acceptor-mediated endocytosis. *Journal of Cell Biology, 103*, 535–544.

Boquet, P., Silverman, M. S., Pappanheimer, A. M., & Veron, W. B. (1976). Binding of triton X-100 to diphtheria toxin crossreacting material 45 and their fragments. *Proceedings of the National Academy of Sciences, 73*, 4449–4473.

Bozzi, Y., Constantin, L., Antnucci, F., & Caleo, M. (2006). Action of botulinum neurotoxins in the central nervous system: Antiepileptic effects. *Neurotoxicity Research, 9*, 197–203.

Breidenbach, M. A., & Brunger, A. T. (2004). Substrate recognition strategy for botulinum neurotoxin serotype A. *Nature, 432*, 925–929.

Brugge, J. S., Cotton, P. C., Queral, A. E., Barrett, J. N., Nonner, D., & Keane, R. W. (1985). Neurons expressed high level of a structurally modified, activated form of pp-60c-src. *Nature, 316*, 554–557.

Brunger, A. T., Breidenbach, M. A., Jin, R., Fischer, A., Santos, J. S., & Montal, M. (2007). Botulinum neurotoxin heavy chain belt as an intramolecular chaperone for the light chain. *PLoS Pathogens, 3*, 1191–1194.

Burnett, J. C., Ruthel, G., Stegmann, C. M., Panchal, R. G., Nguyen, T. L., Hermone, A. R., Stafford, R. G., Lane, D. J., Kenny, T. A., McGrath, C. F., Wipf, P., Stahl, A. M., Schmidt, J. J., Gussio, R., Brunger, A. T., & Bavari, S. (2007). Inhibition of metalloprotease botulinum serotype A pseudo-peptide binding mode to a small molecule that is active in primary neurons. *Journal of Biological Chemistry, 282*, 5004–5014.

Burns, G. F., & Williams, H. (1975). *Clostridium botulinum* in Scottish fish farms and farmed trout. *Journal of Hygiene (London), 74*, 1–6.

Cai, S., & Singh, B. R. (2001). Role of the disulfide cleavage induced molten globule state of type a botulinum neurotoxin in its endopeptidase activity. *Biochemistry, 40*, 15327–15333.

Caleo, M., & Schiavo, G. (2009). Central effects of tetanus and botulinum neurotoxins. *Toxicon, 54*, 593–599.

Call, J. E., Cooke, P. H., & Miller, A. J. (1995). In situ characterization of *Clostridium botulinum* neurotoxin synthesis and export. *Journal of Applied Bacteriology, 79*, 257–263.

Campbell, K. D., Collins, M. D., & East, A. K. (1993a). Gene probes for identification of the botulinal neurotoxin gene and specific identification of neurotoxin types B, E, and F. *Journal of Clinical Microbiology, 31*, 2255–2262.

Campbell, K. D., East, A. K., Thompson, D. E., & Collins, M. D. (1993b). Studies on the large subunit ribosomal RNA genes and intergenic spacer regions of non-proteolytic *Clostridium botulinum* types B, E and F. *Research in Microbiology, 144*, 171–180.

Carter, A. T., Paul, C. J., Mason, D. R., Twine, S. M., Alston, M. J., Logan, S. M., Austin, J. W., & Peck, M. W. (2009). Independent evolution of neurotoxin and flagellar genetic loci in proteolytic *Clostridium botulinum*. *BMC Genomics, 10*, 115–120.

Chaddock, J. A., & Acharya, K. R. (2011). Engineering toxins for 21st century therapies. *FEBS Journal, 278*(6), 899–904.

Chaddock, J. A., & Marks, P. M. H. (2006). Clostridial neurotoxins: Structure-function led design of new therapeutics. *Cellular and Molecular Life Sciences, 63*(5), 540–551.

Chaddock, J. A., Purkiss, J. R., Friis, L. M., Broadbridge, J. D., Duggan, M. J., Fooks, S. J., Shone, C. F., Quinn, C. P. A., & Foster, K. A. (2000a). Inhibition of vesicular secretion in both neuronal and non-neuronal cells by a retargeted endopeptidase derivative of *Clostridium botulinum* neurotoxin type A. *Infection and Immunity, 68*, 2587–2600.

Chaddock, J. A., Purkiss, J. R., Duggan, M. J., Shone, C. F., Quinn, C. P. A., & Foster, K. A. (2000b). A conjugate composed of nerve growth factor coupled to a non-toxic derivative of *Clostridium botulinum* neurotoxin type A can inhibit neurotransmitter release in vitro. *Growth Factors, 18*, 147–153.

Chang, T. W. (2011). Sequence analyses & novel antidotes development of Botulinum neurotoxin. Ph.D. Dissertation, UMass Biomedical Engineering and BioTechnology Ph.D. program

Chen, F., Kuziemko, G. M., & Stevens, R. C. (1998). Biophysical characterization of the stability of the 150-kilodalton botulinum toxin, the nontoxic component, and the 900-kilodalton botulinum toxin complex species. *Infection and Immunity, 66*, 2420–2425.

Chen, S., Kim, J. P., & Barbieri, J. T. (2007a). Mechanism of substrate recognition by botulinum neuro-Toxin serotype A. *The Journal of Biological Chemistry, 282*, 9621–9627.

Chen, Y., Korkeala, H., Aarnikunnas, J., & Lindstrom, M. (2007b). Sequencing the botulinum neurotoxin gene and related genes in *Clostridium botulinum* type E strains reveals orfx3 and a novel type E neurotoxin subtype. *Journal of Bacteriology, 189*, 8643–8650.

Collins, M. D., Lawson, P. A., Willema, A., Cordoba, J. J., Fernandez, G. J., Garcia, P., Cai, J., Hippe, H., & Farrow, J. A. (1994). The phylogeny of genus *Clostridium*: proposal of five new genera and eleven new species combinations. *International Journal of Systematic Bacteriology, 44*, 812–826.

Cordoba, J. J., & Collins, M. D. (1995). Studies on the genes encoding botulinum neurotoxin type A of *Clostridium botulinum* from a variety of sources. *Systematic and Applied Microbiology, 44*, 812–826.

Cornille, F., Martin, L., Lenoir, C., Cussac, D., Roques, B. P., & Fournie- Zaluski, M. C. (1997). Cooperative exosite-dependent cleavage of synaptobrevin by tetanus toxin light chain. *Journal of Biological Chemistry, 272*, 3459–3564.

Costantin, L., Bozzi, Y., Richichi, C., Viegi, A., Antonucci, F., Funicello, M., Gobbi, M., Mennini, T., Rossetto, O., Montecucco, C., Maffei, L., Vezzani, A., & Caleo, M. (2005). Antiepileptic effects of botulinum neurotoxin E. *Journal of Neuroscience, 25*, 1943–1951.

Critchley, D. R., Nelson, P. G., Habig, W. H., & Fishman, P. H. (1985). Fate of tetanus toxin bound to the surface of primary neurons in culture: Evidence for rapid internalization. *Journal of Cell Biology, 100*, 1499–1507.

Darios, F., Niranjan, D., Ferrari, E., Zhang, F., Soloviev, M., Rummel, A., Bigalke, H., Suckling, J., Ushkaryov, Y., Naumenko, N., Shakirzyanova, A., Giniatulin, R., Maywood, E., Hastings, M., Binz, T., & Davletov, B. (2010). SNARE tagging allows stepwise assembly of a multimodular medicinal toxin. *Proceedings of the National Academy of Sciences, 107*, 18197–18201.

Dayanithi, G., Stecher, B., Hohne-Zell, B., Yamasaki, S., Binz, T., Weller, U., Niemann, H., & Gratzi, M. (1994). Exploring the functional domain and target of the tetanus toxin light chain in neurohypophysical terminals. *Neuroscience, 58*, 423–431.

De Paiva, A., Meunier, F. A., Molgo, J., Aoki, K. R., & Dolly, O. (1999). Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. *Proceedings of the National Academy of Sciences, 96*, 3200–3205.

Dekleva, M. L., & Das Gupta, B. R. (1989). Nicking of single chain *Clostridium botulinum* type A neurotoxin by an endogenous protease. *Biochemical and Biophysical Research Communications, 162*, 762–772.

Dolly, J. O., & Aoki, K. R. (2006). The structure and model of different botulinum toxins. *European Journal of Neurology, 13*, 1–9.

Dolly, J. O., Black, J., Williams, R. S., & Melling, J. (1984). Acceptors for botulinum neurotoxin reside on motor nerve terminals and mediate its internalization. *Nature, 307*, 457–460.

Dong, M., Richards, D. A., Goodnough, M. C., Tepp, W. H., Johnson, E. A., & Chapman, E. R. (2003). Synaptotagmins I and II mediate entry of botulinum neurotoxin B into cells. *Journal of Cell Biology, 162*, 1293–1303.

Dong, M., Yeh, F., Tepp, W. H., Dean, C., & Johnson, E. A. (2006). SV2 is the protein receptor for botulinum neurotoxin A. *Science, 312*, 592–596.

Dong, M., Liu, H., Tepp, W. H., Johnson, E. A., Janz, R., & Chapman, E. R. (2008). Glycosylated SV2A and SV2B mediate the entry of botulinum neurotoxin E into neurons. *Molecular Biology of the Cell, 19*, 5226–5237.

Dressler, D., Saberi, F. A., & Barbosa, E. R. (2005). Botulinum toxin: mechanisms of action. *Arquivos de Neuro-Psiquiatria, 63*, 180–185.

East, A. K., Bhandari, M., Hielm, S., & Collins, M. D. (1998). Analysis of the botulinum neurotoxin type F genes clusters in proteolytic and nonproteolytic *Clostridium botulinum* and *Clostridium baratii*. *Current Microbiology, 37*, 262–268.

Eleopra, R., Tugnoli, V., Quatrale, R., Rossetto, O., & Montecucco, C. (2004). Different types of botulinum toxin in humans. *Movement Disorders, 19*, S53–S59.

Erbguth, F. J. (1996). Historical note on the therapeutic use of botulinum toxins in neurological disorders. *Journal of Neurology, Neurosurgery, and Psychiatry, 60*, 151–157.

Erbguth, F. J. (1998). Botulinum toxin, a historical note. *Lancet, 351*, 1820–1827.

Erbguth, F. J. (2004). Historical notes on botulism, *Clostridium botulinum*, botulinum toxin, and the idea of the therapeutic use of the toxin. *Movement Disorders, 19*, S2–S6.

Fach, P., Hauser, D., Guillou, J. P., & Popoff, M. R. (1993). Polymerase chain reaction of *Clostridium botulinum* type A strains and detection in food samples. *Journal of Applied Bacteriology, 75*, 234–239.

Fernandez, R. A., Ciccarelli, A. S., Arenas, G. N., & Gimenez, D. F. (1989). Type D and A *Clostridium botulinum* in necropsy samples from bovines with "mal de Aguapey". *Revista Argentina de Microbiología, 21*, 47–53.

Fischer, A., & Montal, M. (2006). Characterization of clostridial botulinum neurotoxin channels in neuroblastoma cells. *Neurotoxicity Research, 9*, 93–100.

Fischer, A., & Montal, M. (2007). Crucial role of the disulfide bridge between botulinum neurotoxin light and heavy chains in protease translocation across membranes. *Journal of Biological Chemistry, 282*, 29604–29611.

Foster, E. M. (1993). Reflections on a half-century of food-borne botulism. In B. R. DasGupta (Ed.), *Botulinum and tetanus neurotoxins* (p. 505). New York: Plenum Press.

Foster, K. A. (2009). Engineered toxins: New therapeutics. *Toxicon, 54*, 587–592.

Fredenburg, R., Wilson, D., Moe, S., Jacobson, A., & Stein, R. (2007). The C-terminal region of the light chain defines its global conformational state: Implications for catalysis and inhibition. In: 44th Annual interagency botulism research coordination committee meeting, Asilomar, CA, 14–17 Oct, 2007. Abstract page 134.

Fu, F. N., Sharma, S. K., & Singh, B. R. (1998). A protease-resistant novel hemagglutinin purified from type A *Clostridium botulinum*. *Journal of Protein Chemistry, 17*, 53–60.

Fu, Z., Chen, C., Barbieri, J. T., Kim, J. J., & Baldwin, M. R. (2009). Glycosylated SV2 and gangliosides as dual receptors for botulinum neurotoxin serotype F. *Biochemistry, 48*, 5631–5641.

Fujita, R., Fujinaga, Y., Inoue, K., Nakajima, H., Kumon, H., & Oguma, K. (1995). Molecular characterization of two forms of nontoxic-nonhemagglutinin components of *Clostridium botulinum* type A progenitor toxins. *FEBS Letters, 376*, 41–44.

Fujinaga, Y., Takeshi, K., Inoue, K., Fujita, R., Ohyama, T., Moriishi, K., & Oguma, K. (1995). Type A and B neurotoxin genes in a *Clostridium botulinum* type AB strain. *Biochemical and Biophysical Research Communications, 213*, 737–745.

Fujinaga, Y., Matsumura, T., Jin, Y., Takegahara, Y., & Sugawara, Y. (2009). A novel function of botulinum toxin-associated proteins: HA proteins disrupt intestinal epithelial barriers to increase toxin absorption. *Toxicon, 54*, 583–686.

Fuji, N., & Kimura, K. (1993). Similarity in nucleotide sequence of the gene encoding nontoxic component of *Clostridium botulinum* type E progenitor toxin. *Journal of General Microbiology, 139*, 79–86.

Gimenez, D. F., & Ciccarelli, A. S. (1970). Studies on strain 84 of *Clostridium botulinum*. *Zentrailbl Bakteriol Orig, 215*, 212–220.

Gu, S., Rumpel, S., Zhou, J., Strotmeier, J., Bigalke, H., Perry, K., Shoemaker, C. B., Rummel, A., & Jin, R. (2012). Botulinum neurotoxin is shielded by NTNHA in an interlocked complex. *Science, 335*, 977–981.

Haagsma, J. (1991). Botulism in cattle, a review. *Tijdschrift voor Diergeneeskunde, 116*, 663–669.

Hatheway, C. L. (1993). Bacteriology and pathology of neurotoxigenic clostrida. In B. R. DasGupta (Ed.), *Botulinum and tetanus neurotoxins* (p. 491). New York: Plenum Press.

Hatheway, C. L., & Ferreira, J. L. (1996). Detection and identification of *Clostridium botulinum* neurotoxins. *Advances in Experimental Medicine and Biology, 391*, 481–498.

Hilger, G. A., & Bigalke, H. (1995). Molecular aspects of tetanus and botulinum neurotoxin poisoning. *Progress in Neurobiology, 46*(1), 83–96.

Hill, K. K., Smith, T. J., Helma, C. H., Ticknor, L. O., Foley, B. T., Svensson, R. T., Brown, J. L., Johnson, E. A., Smith, L. A., Okinaka, R. T., Jackson, P. J., & Marks, J. D. (2007). Genetic diversity among Botulinum Neurotoxin-producing clostridial strains. *Journal of Bacteriology, 189*, 818–832.

Humeau, Y., Doussau, F., Grant, N. J., & Poulain, B. (2000). How botulinum and tetanus neurotoxins block neurotransmitter release. *Biochimie, 82*, 427–446.

Huss, H. H., & Eskildsen, U. (1974). Botulism in farmed trout caused by *Clostridium botulinum* type E: A preliminary report. *Nordisk Veterinaermedicin, 26*, 733–738.

Hutson, R. A., Thonpson, D. E., & Collins, M. D. (1993). Genetic interrelationships of saccharolytic *Clostridium botulinum* types B, E and F and related clostridia as revealed by small-subunit rRNA gene sequences. *FEMS Microbiology Letters, 108*, 103–110.

Innoue, K., Fujinaga, Y., Watanabe, T., Ohyama, T., Takeshi, K., Moriishi, K., Nakajima, H., & Oguma, K. (1996). Molecular composition of *Clostridium botulinum* type A progenitor toxins. *Infection and Immunity, 64*, 1589–1594.

Jamie, T. (2008). Study finds botulinum toxin spreads to CNS tissue in mice. *Neurology Today, 8*, 22–23.

Jankowic, J. (2004). Botulinum toxin in clinical practice. *Journal of Neurology, Neurosurgery, and Psychiatry, 75*, 951–957.

Jeffrey, J. S., Galey, F. D., Meteyer, C. U., Kinde, H., & Rezvani, M. (1994). Type C botulism in turkeys: Determination of the median toxic dose. *Journal of Veterinary Diagnostic Investigation, 6*, 93–95.

Jin, R., Rummel, A., Binz, T., & Brunger, A. T. (2006). Botulinum neurotoxin B recognizes its protein receptor with high affinity and specificity. *Nature, 444*, 1092–1095.

Keller, J. E., Neale, E. A., Oyler, G., & Adler, M. (1999). Persistence of botulinum neurotoxin in cultured spinal cord cells. *FEBS Letters, 456*, 137–152.

Koriazova, L. K., & Montal, M. (2003). Translocation of botulinum neurotoxin light chain protease through the heavy chain channel. *Nature Structural Biology, 10*, 13–18.

Krieglstein, K. G., Das Gupta, B. R., & Henschen, A. H. (1994). Covalent structure of botulinum neurotoxin type A: Location of sulfhydryl groups, and disulphide bridges and identification of C-termini of light and heavy chains. *Journal of Protein Chemistry, 13*, 49–57.

Kukreja, R., & Singh, B. R. (2005). Biologically active novel conformational state of botulinum, the most poisonous poison. *The Journal of Biological Chemistry, 280*, 39346–39352.

Kukreja, R., & Singh, B. R. (2007). Comparative role of neurotoxin associated proteins in the structural stability and endopeptidase activity of botulinum neurotoxin complex types A and E. *Biochemistry, 46*, 14316–14324.

Kukreja, R., Sharma, S., Cai, S., & Singh, B. R. (2007). Role of two active site Glu residues in the molecular action of botulinum neurotoxin endopeptidase. *Biochimica et Biophysica Acta, 1774*, 213–222.

Kukreja, R., Chang, T. W., Cai, S., Lindo, P., Riding, S., Zhou, Y., Ravichandran, E., & Singh, B. R. (2009). Immunological characterization of the subunits of type A botulinum neurotoxin and different components of its associated proteins. *Toxins, 53*(6), 616–24.

Kumar, R., Zhou, Y., Ghosal, K., Cai, S., & Singh, B. R. (2012). Anti-apoptotic activity of Hemagglutinin-33 and botulinum neurotoxin and its implications to therapeutic and countermeasure issues. *Biochemical and Biophysical Research Communications, 417*, 726–731.

Kumaran, D., Easwaramoorthy, S., Furey, W., Navaza, J., Sax, M., & Swaminathan, S. (2009). Domain organization in *Clostridium botulinum* neurotoxin type E is unique: Its implication in faster translocation. *Journal of Molecular Biology, 386*, 233–245.

Kummel, D., Krishnakumar, S. S., Radoff, D. T., Li, F., Claudio, G. G., Pincet, F., Rothman, J. E., & Reinisch, K. M. (2012). Complexin cross-links pre-fusion SNAREs into a zig-zag array: A structure -based model for complexin clamping. *Nature Structural and Molecular Biology, 18*, 927–933.

Lackovic, Z., Rebic, V., & Riederer, P. F. (2009). Single intracerebroventricular injection of botulinum toxin type A produces slow onset and long-term memory impairment in rats. *Journal of Neural Transmission, 116*, 1273–1280.

Lacy, D. B., & Stevens, R. C. (1999). Sequence homology and structural analysis of the clostridial clostridial neurotoxins. *Journal of Molecular Biology, 291*, 1091–1104.

Lund, B. M., & Peck, M. W. (1994). Heat resistance and recovery of spores of non-proteolytic *Clostridium botulinum* in relation to refrigerated, processed foods with an extended shelf-life. *Society for Applied Bacteriology Symposium Series, 23*, 115S–128S.

Maher, P. A. (1988). Nerve-growth factor induces protein-tyrosine phosphorylation. *Proceedings of the National Academy of Sciences, 85*, 6788–6791.

Mahrhold, S., Rummel, A., Bigalke, H., Davletov, B., & Binz, T. (2006). The synaptic vesicle protein 2C mediates the uptake of botulinum neurotoxin A into phrenic nerves. *FEBS Letters, 580*, 2011–2014.

Maksymowych, A. B., Reinhard, M., Malizio, C. J., Goodnough, M. C., Johnson, E. A., & Simpson, L. L. (1999). Pure botulinum neurotoxin is absorbed from the stomach and small intestines produces peripheral neuromuscular blockade. *Infection and Immunity, 67*(9), 4708–4712.

Marks, J. D. (2004). Deciphering antibody properties that lead to potent botulinum neurotoxin neutralization. *Movement Disorders, 19*, S101–S108.

Matsumura, T., Jin, Y., Kabumoto, Y., Takegahara, Y., Oguma, K., Lencer, W. I., & Fujinaga, Y. (2008). The HA proteins of botulinum toxin disrupt intestinal epithelial intercellular junctions to increase toxin absorption. *Cellular Microbiology, 10*, 355–364.

Matthews, B. W. (1988). Structural basis of the action of thermolysin and related zinc peptidases. *Accounts of Chemical Research, 21*, 333–340.

McClure, P. J., Cole, M. B., & Smelt, J. P. (1994). Effects of water activity and pH on growth of *Clostridium botulinum*. *Society for Applied Bacteriology Symposium Series, 23*, 105S–114S.

McNally, R. E., Morrison, M. B., & Berndt, J. E. (1994). *Effectiveness of medical defense interventions against predicted battlefield. levels of botulinum Toxin A* (pp. 1–3). Joppa: Science Applications International Corporation.

Mead, P. S., Slutsker, L., Griffin, P. M., & Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases, 5*, 607–625.

Merrison, A. F., Chidley, K. E., Dunnett, J., & Sieradzan, K. A. (2002). Wound botulism associated with subcutaneous drug use. *BMJ, 325*, 1020–1021.

Minton, N. P. (1995). Molecular genetics of clostridial neurotoxins. *Current Topics in Microbiology and Immunology, 195*, 161–194.

Montal, M. (2010). Botulinum neurotoxin: A marvel of protein design. *Annual Review of Biochemistry, 79*, 591–617.

Montiel, A. V. F., Canaves, J. M., DasGupta, B. R., Wilson, M. C., & Montal, M. (1996). Tyrosine phosphorylation modulates the activity of clostridial neurotoxins. *The Journal of Biological Chemistry, 271*(31), 18322–18325.

Montecucco, C., & Schiavo, G. (1993). Tetanus and botulism neurotoxins: A new group of zinc proteases. *Trends in Biochemical Sciences, 18*, 324–327.

Monteccuco, C., & Schiavo, G. (1994). Mechanism of action of tetanus and botulinum neurotoxins. *Molecular Microbiology, 13*, 1–8.

Montecucco, C., & Schiavo, G. (1995). Structure and function of tetanus and botulinum neurotoxins. *Quarterly Reviews of Biophysics, 28*(4), 423–472.

Montecucco, C., Papini, E., & Schiavo, G. (1994). Bacterial protein toxins penetrate cells via a 4-Step mechanism. *FEBS Letters, 346*, 92–98.

Montecucco, C., Schiavo, G., Papini, E., Rossetto, O., De Bernard, M., Tonello, F., Moll, G. N., & Washbourne, P. E. (1995). *Translocation of bacterial toxins across membranes* (pp. 75–94). Birkhauser Verlogue-Switzerland: Biochemistry of cell membranes.

Moriishi, K., Koura, M., Fujii, N., Fujinaga, Y., Inoue, K., Syuto, B., & Oguma, K. (1996). Molecular cloning of the gene encoding the mosaic neurotoxin, composed of parts of botulinum neurotoxin types C1 and D, and PCR detection of this gene from *Clostridium botulinum* type C organisms. *Applied Environmental Microbiology, 62*, 662–667.

Muraro, L., Tosatto, S., Motterlini, L., Rossetto, O., & Montecucco, C. (2009). The N-terminal half of the receptor domain of botulinum neurotoxin A binds to microdomains of the plasma membrane. *Biochemical and Biophysical Research Communications, 380*, 76–80.

Ney, J. P., & Joseph, K. R. (2007). Neurologic uses of botulinum neurotoxin type A. *Neuropsychiatric Disease and Treatment, 3*, 785–798.

Nishiki, T., Kamata, Y., Nemoto, Y., Omori, A., & Ito, T. (1994). Identification of protein receptor for *Clostridium botulinum* type B neurotoxin in rat brain synaptosomes. *The Journal of Biological Chemistry, 269*, 10498–10503.

Nishiki, T., Tokuyama, Y., Kamata, Y., Nemoto, Y., & Yoshida, A. (1996). The high affinity binding of *Clostridium botulinum* type B neurotoxin to synaptotagmin II associated with gangliosides GT1b/GD1a. *FEBS Letters, 378*, 253–257.

Ortolani, E. L., Brito, L. A., Mori, C. S., Schalch, U., Pacheco, J., & Baldacci, L. (1997). Botulism outbreak associated with poultry litter consumption in three Brazilian cattle herds. *Veterinary and Human Toxicology, 39*, 89–92.

Osen, S. A., Catsicas, M., Staple, J. K., Jones, K. A., Ayala, G., Knowles, J., Grenningloh, G., & Catsicas, S. (1993). Inhibition of axonal growth by SNAP-25 antisense oligonucleotides in vitro and in vivo. *Nature, 364*, 445–448.

Peck, M. W. (2009). Biology and genomic analysis of *Clostridium botulinum*. *Advances in Microbial Physiology, 55*, 183–265.

Pickett, A. (2010). Re-engineering clostridial neurotoxins for the treatment of chronic pain: Current status and future prospects. *BioDrugs, 24*, 173–182.

Pickett, A., & Perrow, K. (2011). Towards new uses of botulinum toxins as a novel therapeutic tool. *Toxins, 3*, 63–81.

Poulain, B., Popoff, M. R., & Molgo, J. (2008). How do the botulinum neurotoxin block neurotransmitter release: From botulism to the molecular mechanism of action. *The Botulinum Journal, 1*, 14–87.

Rainey, F. A., & Stackebrandt, E. (1993). 16S rDNA analysis reveals phylogenetic diversity among the polysacchrolytic clostridia. *FEMS Microbiology Letters, 113*, 125–128.

Raphael, B. H., Choudoir, M. J., Lúquez, C., Fernández, R., & Maslanka, S. E. (2010). Sequence diversity of genes encoding botulinum neurotoxin type F. *Applied and Environmental Microbiology, 76*, 4805–4812.

Roberts, T. A., & Hobbs, G. (1968). Low temperature growth characteristics of clostridia. *Journal of Applied Bacteriology, 31*, 75–88.

Rummel, A., Karnath, T., Henke, T., Bigalke, H., & Binz, T. (2004a). Synaptotagmins I and II act as nerve cell receptors for botulinum neurotoxin G. *The Journal of Biological Chemistry, 279*, 30865–30870.

Rummel, A., Mahrhold, S., Bigalke, H., & Binz, T. (2004b). The HCC-domain of botulinum neurotoxins A and B exhibits a singular ganglioside binding site displaying serotype specific carbohydrate interaction. *Molecular Microbiology, 51*, 631–643.

Rummel, A., Hafner, K., Mahrhold, S., Darashchonal, N., Holt, M., Jahn, R., Beermann, S., Karnath, T., Bigalke, H., & Binz, T. (2009). Botulinum neurotoxins C, E and F bind gangliosides via a conserved binding site prior to stimulation-dependent uptake with botulinum neurotoxin F utilizing the three isoforms of SV2 as second receptor. *Journal of Neurochemistry, 110*, 1942–1954.

Sakaguchi, G. (1983). Clostridial botulinum neurotoxins. *Pharmacology and Therapeutics, 19*, 165–194.

Sakaguchi, G., Sakaguchi, S., Kamata, Y., Tabita, K., Asao, T., & Kozaki, S. (1990). Distinct characters of *Clostridium botulinum* type A strains and their toxin associated with infant botulism in Japan. *International Journal of Food Microbiology, 11*, 231–241.

Schantz, E. J., & Johnson, E. A. (1992). Properties and use of botulinum toxin and other microbial neurotoxins in medicine. *Microbiological Reviews, 56*, 80–99.

Schiavo, G., Rossetto, O., Santucci, A., DasGupta, B. R., & Montecucco, C. (1992a). Botulinum toxins are zinc proteins. *The Journal of Biological Chemistry, 267*, 23479–23483.

Schiavo, G., Poulain, B., Rossetto, O., Benfenati, F., Tauc, L., & Montecucco, C. (1992b). Tetanus toxin is a zinc protein and its inhibition of neurotransmitter release and protease activity depend on zinc. *EMBO Journal, 11*, 3577–3583.

Schiavo, G., Santicci, A., DasGupta, B. R., Mehta, P. P., Jontes, J., Benfenati, F., Wilson, M. C., & Montecucco, C. (1993a). Botulinum neurotoxins serotypes A and E cleave SNAP-25 at distinct COOH-terminal peptide bonds. *FEBS Letters, 335*, 99–103.

Schiavo, G., Poulain, B., Benfenati, F., DasGupta, B. R., & Montecucco, C. (1993b). Novel targets and catalytic activities of bacterial protein toxins. *Trends in Microbiology, 1*, 170–174.

Schiavo, G., Rossetto, O., & Montecucco, C. (1994a). Clostridial neurotoxins as tools to investigate the molecular events of neurotransmitter release. *Seminars in Cell Biology, 5*, 221–229.

Schiavo, G., Rossello, O., & Montecucco, C. (1994b). The molecular mechanism of action of tetanus and botulinum neurotoxins. *Journal of Neurochemistry, 63*, S26–S26.

Schiavo, G., Matteoli, M., & Montecucco, C. (2000). Neurotoxins affecting neuroexocytosis. *Physiological Reviews, 80*, 717–766.

Schmidt, J. J., & Bostian, K. A. (1995). Proteolysis of synthetic peptides by type A botulinum neurotoxin. *Journal of Protein Chemistry, 14*, 703–708.

Segelke, B., Knapp, M., Kadkhodayan, S., Balhorn, R., & Rupp, B. (2004). Crystal structure of *Clostridium botulinum* neurotoxin protease in a product-bound state: Evidence for noncanonical zinc protease activity. *Proceedings of the National Academy of Sciences, 101*, 6888–6893.

Sharma, S. K., & Singh, B. R. (2004). Enhancement of the endopeptidase activity of purified botulinum neurotoxins A and E by an isolated component of the native neurotoxin associated proteins. *Biochemistry, 43*, 4791–4798.

Shone, C. C., & Roberts, A. K. (1994). Peptide substrate specificity and Zn-endopeptidase activity of Botulinum type B neurotoxin. *European Journal of Biochemistry, 225*, 263–270.

Shone, C. C., Quinn, C. P., Wait, R., Hallis, B., Fooks, S. G., & Hanlbleton, P. (1993). Proteolytic cleavage of synthetic fragments of vesicle-associated membrane protein, isoform-2 by botulinum type B neurotoxin. *European Journal of Biochemistry, 217*, 965–971.

Shukla, H. D., & Singh, B. R. (1999). Identification of DnaJ-like chaperone in clostridial botulinum type A. *Journal of Protein Chemistry, 18*, 695–700.

Shukla, H. D., & Singh, B. R. (2009). Hemagglutinin-33 in the neurotoxin complex of type A *Clostridium botulinum* is a heat shock protein. *The Botulinum Journal, 1*(3), 309–317.

Shukla, H. D., Sharma, S. K., & Singh, B. R. (1997). Identification of a hemagglutinin present in the neurotoxin complex of type A *Clostridium botulinum* as a heat shock protein. In 9th General Meeting, American Society for Microbiology, Miami Beach, FL, May 4-8, 1997, p. K-127.

Silvaggi, N., Wilson, D., Tzipori, S., & Allen, K. N. (2008). Catalytic feature of botulinum neurotoxin A light chain revealed by high resolution structure of an inhibitory peptide complex. *Biochemistry, 47*, 5736–5745.

Simpson, L. L. (1986). Molecular pharmacology of botulinum toxin and tetanus toxin. *Annual Review of Pharmacology and Toxicology, 26*, 427–453.

Simpson, L. L. (2004). Identification of the major steps in botulinum toxin action. *Annual Review of Pharmacology and Toxicology, 44*, 167–193.

Simpson, L. L., Maksymowych, A. B., Park, J. B., & Bora, R. S. (2004). The role of the interchain disulfide bond in governing the pharmacological actions of botulinum toxin. *Journal of Pharmacology and Experimental Therapeutics, 306*, 857–864.

Singh, B. R. (1999). Biomedical and toxico-chemical aspects of botulinum neurotoxins (Editorial, 1999). *Journal of Toxicology-Toxin Reviews, 18*, vii–x.

Singh, B. R. (2000). Intimate details of the most poisonous poison. *Nature Structural Biology, 7*, 617–619.

Singh, B. R., & DasGupta, B. R. (1989). Conformational changes associated with the nicking and activation of botulinum neurotoxin type E. *Biophysical Chemistry, 38*, 123–130.

Singh, B. R., Foley, J., & Lafontaine, C. (1995). Physico-chemical and immunological characterization of the type E botulinum neurotoxin binding protein from *Clostridium botulinum*. *Journal of Protein Chemistry, 14*, 7–18.

Singh, B. R., & Zhang, Z. (2004). Novel proteins within the type E botulinum neurotoxin complex. U.S. Patent No. 6,699,966.

Smith, T. J., & Hill, K. K. (2007). Analysis of the neurotoxin complex genes in *Clostridium botulinum* A1-A4 and B1 strains: BoNT/A3,/Ba4 and/B1 clusters are located within plasmids. *PLoS One, 2*, 1271–1276.

Smith, T. J., Lou, J., Geren, I. N., Forsyth, C. M., Tsai, R., Laporte, S. L., Tepp, W. H., Bradshaw, M., Johnson, E. A., Smith, L. A., & Marks, J. D. (2005). Sequence variation within botulinum neurotoxin serotypes impacts antibody binding and neutralization. *Infection and Immunity, 73*(9), 5450–5457.

Somm, E., Bonnet, N., Martinez, A., Marks, P. M. H., Cadd, V. A., Elliot, M., Toulotte, A., Ferrari, S. L., Rizzoli, R., Huppi, P. S., Harper, E., Melmed, S., Jones, R., & Aubert, M. L. (2012). A botulinum Toxin-derived targeted secretion inhibitor downregulates the GH/IGF1 axis. *Journal of Clinical Investigation, 122*, 3295–3306.

Stenmark, P., Dupuy, J., Imamura, A., Kiso, M., & Stevens, R. C. (2008). Crystal structure of botulinum neurotoxin type A in complex with the cell surface co-receptor GT1b – insight into the toxin-neuron interaction. *PLoS Pathogens, 4*, 1–10.

Stringer, S. C., Webb, M. D., & Peck, M. W. (2009). Contrasting effects of heat treatment and incubation temperature on germination and outgrowth of individual spores of nonproteolytic *Clostridium botulinum* bacteria. *Applied and Environmental Microbiology, 75*, 2712–2719.

Sutton, M. A., Wall, N. R., Aakalu, G. N., & Schuman, E. M. (2004). Regulation of dendritic protein-synthesis by miniature synaptic events. *Science, 304*, 1979–1983.

Therre, H. (1999). Botulism in the European Union. *Euro Surveillance, 4*(1), 2–7.

Thirunavukkarasu, N., Ghosal, K. J., Kukreja, R., Zhou, Y., Dombkowski, A., Cai, S., & Singh, B. R. (2011). Microarray analysis of differentially regulated genes in human neuronal and epithelial cell lines upon exposure to type A botulinum neurotoxin. *Biochemical and Biophysical Research Communications, 405*, 684–690.

Thompson, D. E., Hutson, R. A., East, A. K., Allaway, D., Collins, M. D., & Richardson, P. T. (1993). Nucleotide sequence of the gene coding for Clostridium barati type F neurotoxin: Comparison with other clostridial neurotoxins. *FEMS Microbiology Letters, 108*, 175–182.

Toivonen, J. M., Olivan, S., & Osta, R. (2010). Tetanus toxins C-fragment: The courier and the cure? *Toxins, 2*, 2622–2644.

Tsai, Y. C., Maditz, R., LingKuo, C., Fishman, P. S., Shoemaker, C. B., Oyler, G. A., & Weissman, A. M. (2010). Targeting botulinum neurotoxin persistence by the ubiquitin-proteasome system. *Proceedings of the National Academy of Sciences, 107*, 16554–16559.

Vaidyanathan, V. V., Yoshino, K., Jahnz, M., Dorries, C., Bade, S., Nauenburg, S., Niemann, H., & Binz, T. (1999). Proteolysis of SNAP-25 isoforms by botulinum neurotoxin types A, C, and E, domains and amino acid residues controlling the formation of enzyme-substrate complexes and cleavage. *Journal of Neurochemistry, 72*, 327–337.

Van Ermengem, E. (1979). Classics in infectious diseases. A new anaerobic bacillus and its relation to botulism. Originally published as "Ueber einen neuen anaeroben Bacillus und seine Beziehungen zum Botulismus" in Zeitschrift fur Hygiene und Infektionskrankheiten 26: 1-56, 1897. *Reviews of Infectious Diseases, 1*(4), 701–719.

Verderio, C., Pozzi, D., Pravettoni, E., Inverardi, F., Schenk, U., Coco, S., Proux-Gillardeaux, V., Galli, T., Rossetto, O., Frassoni, C., Montecucco, C., & Matteoli, M. (2004). SNAP-25 modulation of calcium dynamics underlies differences in GABAergic and glutamatergic responsiveness to depolarization. *Neuron, 41*, 599–610.

Verderio, C., Grumelli, C., Raiteri, L., Coco, S., Paluzzi, S., Caccin, P., Rossetto, O., Bonanno, G., Montecucco, C., & Matteoli, M. (2007). Traffic of botulinum toxins A and E in excitatory and inhibitory neurons. *Traffic, 8*, 142–153.

Wang, J., Zurawski, T. H., Meng, J., Lawrence, G. W., Aoki, K. R., Wheeler, L., & Dolly, J. O. (2012). Novel chimeras of botulinum and tetanus neurotoxins yield insights into their distinct sites of neuroparalysis. *The FASEB Journal, 26*, 5035–5048.

Washbourne, P., Pellizzari, R., Baldini, G., Wilson, M. C., & Montecucco, C. (1997). Botulinum neuro-toxin types A and E require the SNARE motif in SNAP-25 for proteolysis. *FEBS Letters, 418*, 1–5.

Yeh, F. L., Zhu, Y., Tepp, W. H., Johnson, E. A., Bertics, P. J., & Chapman, E. R. (2011). Retargeted clostridial neurotoxins as novel agents for treating chronic diseases. *Biochemistry, 50*, 10419–10421.

Botulinum Neurotoxins as Therapeutics

Raja Mehanna and Joseph Jankovic

Contents

Abstract

Since first recognized as the cause of food-borne botulism in the early nineteenth century, botulinum toxin was suggested as a potential treatment for involuntary spasms and movements. Multiple double-blind, placebo-controlled, and open-label studies provided evidence that botulinum toxin is a powerful therapeutic tool in a variety of neurologic and other disorders including ophthalmologic, gastrointestinal, urologic, orthopedic, dermatologic, secretory, painful, and cosmetic disorders. We here review the basic mechanisms of botulinum toxin action at the neuromuscular junction and discuss some of its main clinical applications.

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List of Abbreviations

AAN	American Academy of Neurology
Ach	Acetylcholine
BoNT	Botulinum toxin
CD	Cervical dystonia
CD-PROBE	Cervical Dystonia Patient Registry for the Observation of Botulinum Toxin Type A Efficacy Study
EMG	Electromyography
FDA	Food and Drug Administration
nAChRs	Acetylcholine receptors
OMB	Oromandibular dystonia
PD	Parkinson's disease
PREEMPT	Phase III Research Evaluating Migraine Prophylaxis Therapy
SNARE	Soluble *N*-ethylmaleimide sensitive factor attachment protein receptor
TTA	Therapeutics and Technology Assessment
UBI	Unilateral brow injection
VAMP	Vesicle-associated membrane protein

1 Introduction

Since first recognized in 1817 by Christian Andreas Justinus Kerner as the cause of food-borne botulism, botulinum toxin (BoNT) was suggested as a potential treatment for involuntary spasms and movements. However, it was not until 1973 when Alan Scott, M.D., pediatric ophthalmologist and researcher in San Francisco, demonstrated that BoNT injections into the extraocular muscles improved strabismus in monkeys. The first report of clinical application of BoNT was published in 1984, when it was demonstrated to be safe and effective in the treatment of blepharospasm (Frueh et al. 1984). Subsequent multiple double-blind, placebo-controlled, and open-label studies provided evidence that BoNT was a powerful therapeutic tool in a variety of neurologic and other disorders including ophthalmologic, gastrointestinal, urologic, orthopedic, dermatologic, secretory, painful, and cosmetic disorders (Truong and Jost 2006; Jankovic 2009b; Hallett et al. 2013; Naumann et al. 2013) (Table 1).

We will first review the basic mechanisms of BoNT action at the neuromuscular junction and then discuss some of its main clinical applications.

Table 1 Clinical applications of botulinum toxin

Neurologic disorders
Blepharospasm (lid "apraxia")
Oromandibular–facial–lingual dystonia
Laryngeal dystonia (spasmodic dysphonia)
Cervical dystonia (torticollis)
Limb dystonia
Task-specific dystonia (e.g., writer's cramp)
Other axial/focal/segmental dystonias (primary, secondary)
Hemifacial spasm
Limb, head, voice, jaw tremor
Motor and phonic tics
Palatal myoclonus
Nystagmus and oscillopsia
Myokymia
Spasticity (stroke, cerebral palsy, head injury, multiple sclerosis)
Stuttering
Ophthalmologic disorders
Strabismus
Protective ptosis
Pain disorders
Headaches – chronic migraine especially
Tennis elbow and other sports injuries
Chronic anterior knee pain
Lumbosacral strain and back spasms
Radiculopathy with secondary muscle spasm
Myofascial pain syndromes
Gastrointestinal disorders
Sialorrhea
Spasm of the inferior constrictor of the pharynx (cricopharyngeal muscle)
Achalasia (lower esophageal sphincter spasm)
Obesity (distal stomach)
Spasm of the sphincter of Oddi
Anal fissure
Constipation
Anismus
Genito-urologic disorders
Spastic bladder
Detrusor-sphincter dyssynergia
Overactive bladder
Prostatic hypertrophy
Vaginismus

(*continued*)

Table 1 (continued)

Secretory disorders
Hyperlacrimation
Drooling (sialorrhea)
Hyperhidrosis
Gustatory sweating
Cosmetic
Glabellar wrinkles
Brow furrows
Frown lines
"Crow's feet"
Platysma lines
Facial asymmetry
Acne
Others
Diabetic foot ulcers
Pruritus

2 Mechanism of Action of BoNT

2.1 Different and Unique Properties of Various Serotypes of BoNT

The therapeutic value of BoNT is due to its ability to produce local paralysis when injected into a muscle by preventing the release of acetylcholine (Ach) from the presynaptic nerve terminal, also referred to as chemodenervation. There are seven immunologically distinct BoNTs (A–G), with type A being the most studied and most widely used. Synthesized as single-chain polypeptide (molecular weight of 150 kD), each toxin molecule has relatively little potency until it is cleaved by trypsin or bacterial enzymes into a heavy chain (100 kD) and a light chain (50 kD). BoNT exerts its action in a three-step process that involves binding to the receptor to the presynaptic membrane through its heavy chain, internalization of the whole toxin by endocytosis, and enzymatic action of the light chain after it has been cleaved from the heavy chain. The light chain acts as a zinc-dependent protease that selectively cleaves proteins, known as the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins, which are critical for fusion of intraneuronal presynaptic vesicle with the presynaptic membrane, thus preventing the release of Ach. The SNARE target proteins are different for different immunotypes of BoNT. The light chains of both BoNT-A and BoNT-E cleave SNAP-25, but at different sites. The light chains of BoNT-B, -D, and -F cleave synaptobrevin-2, also known as VAMP (vesicle-associated membrane protein), an integral protein of the synaptic vesicle membrane. BoNT-C cleaves both SNAP-25 and syntaxin, another plasma membrane-associated protein. Only types A and B are used clinically.

Table 2 Available types of BoNT in the USA

Generic name	Brand name(manufacturer)	Target
Onabotulinumtoxin A	Botox® (Allergan, Inc., Irvine, CA, USA) or Prosigne® (China)	SNAP 25
Abobotulinumtoxin A	Dysport® (Beaufour Ipsen, France-UK)	SNAP 25
Incobotulinumtoxin A	Xeomin® (Merz Pharmaceuticals GmbH, Frankfurt, Germany)	SNAP 25
Rimabotulinumtoxin B	Myobloc® (US) (WorldMeds, Louisville, KY, USA) or Neurobloc® (Europe)	VAMP (synaptobrevin)

Legend: *BoNT* botulinum toxin, *SNAP* soluble N-ethylmaleimide-sensitive factor attachment protein receptor, *VAMP* vesicle-associated membrane protein

There is considerable evidence that BoNT injected peripherally also influences central nervous system function. By blocking gamma as well as alpha motor neurons, there is denervation of intrafusal muscle fibers. This reduces muscle spindle afferent input to the central nervous system and thereby modifies sensorimotor and proprioceptive pathways (Giladi 1997; Hallett 2000; Rosales and Dressler 2010). These mechanisms may contribute to the therapeutic effects of BoNT in focal dystonias beyond the effects anticipated on the basis of muscle relaxation alone. There is also evidence that BoNT spreads not only to adjacent muscles but also to muscles on the contralateral side of the body (Frick et al. 2012). This is based on a study in which botulinum toxin (2.5 U) was injected into the tibialis muscle of anesthetized rats ($n = 26$) whereas control animals ($n = 25$) received a saline injection. Neuromuscular function, pharmacology, and expression of acetylcholine receptors (nAChRs) were evaluated in the tibialis at 0, 4, and 16 days after injection on the opposite side and in saline-injected controls. Although the mechanism of spread to the contralateral sides is not clear, the investigators demonstrated a decrease in specific twitch tension on the contralateral side and an increased sensitivity to atracurium on the toxin-injected side, despite upregulation of expression of acetylcholine receptors.

The original commercial preparation of BoNT was onabotulinumtoxin A, marketed as Botox® (Allergan, Inc., Irvine, CA, USA). Other forms of BoNT-A clinically available in the USA are abobotulinumtoxin A or Dysport® (Beaufour Ipsen, France-UK) and incobotulinumtoxin A or Xeomin® (Merz Pharmaceuticals GmbH, Frankfurt, Germany) (Albanese 2011). Another type of BoNT-A currently in clinical trials is PureTox® (Johnson & Johnson, USA). In addition, there is a Chinese form of BoNT-A (Prosigne or CBTX-A, Lanzhou Biological Products Institute, China), a Korean preparation (Meditoxin®/Neuronox®, Medy-Tox, South Korea), and many other formulations in experimental clinical trials and in development (Table 2).

Currently, the only preparation of BoNT-B is rimabotulinumtoxin B, known by the brand name Myobloc® (USA) or Neurobloc® (Europe) (US WorldMeds, Louisville, KY, USA). BoNT-B is an antigenically distinct form of BoNT and has unique physical and clinical properties that distinguish it from BoNT-A (Sadick 2003).

It is important to note that the biologic activity, measured in units, is different for the different products. Each preparation should be considered unique in its potency and properties and is measured with proprietary units that are considered to be non-interchangeable (Albanese 2009, 2011). However, through clinical practice when switching from one to another product, also supported by numerous comparison studies, many clinicians have used the following conversion ratios: Dysport® versus Botox® as 2.5 to 1 (Marchetti et al. 2005) and Myobloc/Neurobloc® versus Botox® as 50:1 (Pathak et al. 2006), whereas Xeomin® and Botox® seem to be equivalent in their potency (Jost et al. 2007; Frevert 2010; Dressler et al. 2011).

In Botox® and Dysport®, the BoNT component is formed by botulinum neurotoxin and by nontoxic proteins known as complexing proteins (Dressler and Benecke 2007), whereas the purification and manufacturing process for Xeomin® removes the complexing proteins (Frevert 2009b) giving it the highest specific neurotoxin activity (Frevert 2010).

2.2 Antigenicity and Immunoresistance

In addition to the biologically active toxin, many products include various amounts of nontoxin proteins like hemagglutinins and other proteins that are not required to stabilize the toxin but can have some immune-stimulating activity (Frevert 2009a). Xeomin® and PureTox® are free of such complexing proteins (Frevert 2009b). Some patients stop responding to BoNT injections after sometimes years of benefit. This seems to be secondary to the production of blocking antibodies directed against the heavy chain of the BoNT molecule (Jankovic and Schwartz 1995). Methods used to detect blocking antibodies include the mouse protection assay, the mouse phrenic nerve hemidiaphragm test, and many other tests (Hanna et al. 1999). The most commonly available test is the Western blot assay, but because of its lack of sensitivity and specificity, this test does not reliably predict true immunoresistance (Hanna and Jankovic 1998). A unilateral brow injection (UBI) is a useful clinical test, as inability to frown on the injected side due to weakness of the procerus and corrugator muscles confirms the absence of clinically meaningful immunoresistance (Hanna et al. 1999). The original preparation of Botox® contained 25 ng of neurotoxin complex protein per 100 U, but in 1997, the Food and Drug Administration (FDA) approved a new preparation that contains only 5 ng per 100 U, which has been associated with lower antigenicity (Jankovic et al. 2003). Depending on the technique used to detect blocking antibodies, the risk of antibodies to Botox® has markedly decreased (Mejia et al. 2005) and is now estimated to be about as low as 1.2 % of patients receiving the product repeatedly for up to 4 years (Brin et al. 2008). BoNT-B may be a useful alternative for patients who develop resistance to BoNT-A (Berman et al. 2005), but this preparation may be associated with higher risk of antigenicity (Jankovic et al. 2006). In one study, the effect of BoNT-B was sustained for up to 2.5 years in type A-resistant patients, but the magnitude of response diminished over time (Factor et al. 2005). Although the various neurotoxins are antigenically different, they contain a common subunit structure, and cross-reactive

epitopes may cause cross-neutralization of antibodies (Atassi et al. 2008). Most patients who develop blocking antibodies to one type of BoNT thus have an increased risk of developing blocking antibodies to a different type of BoNT despite a possible initial good response to the new BoNT. While low antigenicity has been predicted with formulations of BoNT without complexing proteins (e.g., Xeomin® and PureTox®), no long-term data are yet available to support this notion.

Although frequently suggested, an association between a large cumulative dose of BoNT and long treatment duration with the development of anti-BoNT antibodies could not be confirmed (Bakheit et al. 2011).

2.3 Adverse Effects

Most of the adverse effects of BoNT are not preventable, but some are clearly associated with wrong dosage, inappropriate selection of muscles, and faulty injection techniques. Clinicians inexperienced with BoNT treatment should consider referral to a specialist experienced with proper use of BoNT. There are many controversies related to BoNT techniques, including the role of electromyography (EMG) in guiding injections, particularly to reach certain muscles (Comella et al. 1992). Although EMG guidance may be appropriate for BoNT treatment of limb dystonia, this does not mean that placement with EMG guidance correlates with better results, since the selection of the muscle involved in the hand dystonia is based on clinical examination and not on EMG (Jankovic 2001). Two class II methodological studies compared EMG versus muscle stimulation for needle localization; one of these studies showed enhanced accuracy of needle placement under EMG guidance (Molloy et al. 2002) while the other was inconclusive (Geenen et al. 1996). A more recent simple blind randomized control study showed that EMG guidance was associated with slightly better clinical outcome and a lower dose of BoNT in the treatment of cervical dystonia (CD), but the control group had a duration of symptoms twice as long as the EMG-guided group, so the results are difficult to interpret (Werdelin et al. 2011).

Side effects of BoNT injection usually result from an excessive weakness in the injected muscles. Local reactions include pain, edema, erythema, ecchymosis, headache, and short-term hyperesthesia (Lu and Lippitz 2009). The toxin can also diffuse to adjacent muscles and result in unwanted weakness. A number of factors seem to influence diffusion of BoNT including injection technique, concentration, and volume (Ramirez-Castaneda et al. 2013). Rarely, the toxin spreads systemically to distant sites resulting in mild generalized muscle weakness, malaise, nausea, fatigue, rash, or flu-like symptoms (Naumann et al. 2006; Baizabal-Carvallo et al. 2011; Dressler 2010a). It has been proposed that systemic distribution may be mediated by capillary or venous uptake and the volume of fluid used for BoNT reconstitution may increase systematic spread (Crowner et al. 2010).

The most common adverse effects encountered when using BoNT for CD include injection-site pain, muscle weakness, dysphagia, dry mouth, and flu-like symptoms (Poewe et al. 1998; Mejia et al. 2005; Naumann et al. 2006). Flu-like

symptoms occur in 1.7–20 % of BoNT-A injections (Baizabal-Carvallo et al. 2011) without marked differences between various serotypes of formulations although head-to-head comparisons are lacking. This side effect, however, seems to be more frequent with BoNT-B, observed in 5–55 % of injections (Baumann et al. 2003; Baumann et al. 2005a, b). The BoNT-related flu-like symptoms are typically mild, develop within 10 days of the injection, and last an average of 2–3 days, but may last up to 14 days and in rare cases render the patient bedridden. The symptom can be usually relieved with over-the-counter antipyretics and analgesics (Baizabal-Carvallo et al. 2011).

Results of a meta-analysis of 36 randomized controlled studies comprised of 1,425 subjects who received treatment with Botox® for various indications reported a rate of mild-to-moderate adverse effects of approximately 25 % in the Botox®-treated group compared with 15 % in the control group ($p < 0.001$). No severe adverse events, however, have been reported (Naumann and Jankovic 2004). In February 2008 the FDA expressed concern about potential severe adverse effects (respiratory compromise and death) associated with the use of onabotulinumtoxin A, based on submitted reports of children treated for cerebral palsy-associated limb spasticity. According to evidence-based clinical guidelines published by the American Academy of Neurology (AAN) in 2010, BoNT is considered to be an effective treatment for spasticity in children and adolescent with cerebral palsy (Delgado et al. 2010). In the 17 studies they reviewed, the most common adverse effects were localized pain, unsteadiness and increased falls, and fatigue. Although excessive weakness was noted in some cases, no deaths were reported.

The Cervical Dystonia Patient Registry for the Observation of Botulinum Toxin Type A Efficacy Study (CD-PROBE) is an ongoing registry aimed at understanding the use of Botox® in the treatment of CD (Jankovic et al. 2011a). Preliminary results from CD-PROBE suggest that repeated injections of Botox® have a low rate of adverse effects and are associated with meaningful and sustained relief of CD pain and improvement in quality of life (Charles et al. 2010). This has been confirmed by other retrospective series (Camargo et al. 2011), and there are other longitudinal observational studies currently under way designed to provide data on long-term clinical use of BoNT (Fernandez et al. 2013a, b).

Patients with post-polio syndrome and Eaton–Lambert syndrome, however, have been reported to have generalized weakness after local BoNT (Erbguth et al. 1993). Other contraindications to the use of BoNT include myasthenia gravis, motor neuron disease, concurrent use of aminoglycoside antibiotics, and pregnancy, although women, inadvertently injected during pregnancy, reported no untoward side effects to the fetus (Jankovic 2010a).

3 Clinical Application

BoNT is currently used in a wide variety of disorders, a few of which have been approved by the FDA (Table 3) or recommended by medical organizations (Table 4).

Table 3 FDA-approved botulinum toxin products

Trade name	Drug name	FDA-approved indications
Botox®	Onabotulinumtoxin A	Cervical dystonia, severe primary axillary hyperhidrosis, strabismus in patients ≥12 years old, blepharospasm in patients ≥12 years of age. Upper limb spasticity in adult patients. Temporary improvement in the appearance of moderate to severe glabellar lines
Dysport®	Abobotulinumtoxin A	Cervical dystonia, temporary improvement in the appearance of moderate to severe glabellar lines
Xeomin®	Incobotulinumtoxin A	Cervical dystonia, blepharospasm
Myobloc®	Rimabotulinumtoxin B	Cervical dystonia

Table 4 Level of evidence for clinical use of BoNT

Level A (established effective)	Blepharospasm Cervical dystonia Spasticity in adults Equinus varus deformity in children with cerebral palsy Axillar hyperhidrosis Anal fissure Idiopathic detrusor overactivity
Level B (possibly effective)	Hemifacial spasm Adductor laryngeal dystonia Adductor spasticity in children with cerebral palsy Focal upper extremity dystonia Upper extremity essential tremor Palmar hyperhidrosis Pain control in children with cerebral palsy and upper extremity spasticity Allergic rhinitis Sialorrhea.
Level C (probably effective)	Gustatory sweating Oromandibular dystonia Laryngeal dystonia.

3.1 Neurological Disorders

Since the first placebo-controlled trials of BoNT in the treatment of CD (Tsui et al. 1985, 1986) and cranial dystonia, including blepharospasm (Jankovic and Orman 1987), hundreds of other studies have been published confirming the efficacy and safety of BoNT in a variety of movement disorders and other neurologic and non-neurologic disorders.

The Therapeutics and Technology Assessment (TTA) Subcommittee of the American Academy of Neurology, in their 2008 evidence-based review (currently being revised), assigned level A recommendation for the use of

BoNT for the treatment of CD and a level B recommendation for blepharospasm (Simpson et al. 2008a, b). This evidence-based review was recently updated by a panel of experts (Hallett et al. 2013). The panel evaluated published data on the four individual commercially available formulations: abobotulinumtoxinA (A/Abo), onabotulinumtoxinA (A/Ona), incobotulinumtoxinA (A/Inco), and rimabotulinumtoxinB (B/Rima) and made the following recommendations: "For the treatment of blepharospasm, the evidence supported a Level A recommendation for BoNT-A, A/Inco, and A/Ona; a Level B recommendation for A/Abo; and a Level U recommendation for B/Rima. For hemifacial spasm, the evidence supported a Level B recommendation for BoNT-A and A/Ona, a Level C recommendation for A/Abo, and a Level U recommendation for A/Inco and B/Rima. For the treatment of oromandibular dystonia, the evidence supported a Level C recommendation for BoNT-A, A/Abo, and A/Ona, and a Level U recommendation for A/Inco and B/Rima. For the treatment of cervical dystonia, the published evidence supported a Level A recommendation for all four BoNT formulations. For limb dystonia, the available evidence supported a Level B recommendation for both A/Abo and A/Ona, but no published studies were identified for A/Inco or B/Rima, resulting in a Level U recommendation for these two formulations. For adductor laryngeal dystonia, evidence supported a Level C recommendation for the use of A/Ona, but a Level U recommendation was warranted for B/Rima, A/Abo, and A/Inco. For the treatment of focal tics, a Level U recommendation was warranted at this time for all four formulations. For the treatment of tremor, the published evidence supported a level B recommendation for A/Ona, but no published studies were identified for A/Abo, A/Inco, or B/Rima, warranting a Level U recommendation for these three formulations".

3.1.1 Blepharospasm

In 1987, Jankovic and Orman reported the results of a double-blind, placebo-controlled trial of onabotulinumtoxin A (Botox®) in 28 patients with cranial-cervical dystonia, including blepharospasm, oromandibular dystonia, and CD, the results of which were in part used by the FDA to approve Botox® in 1989 as a therapeutic agent in patients with strabismus, blepharospasm, and hemifacial spasm (Jankovic and Orman 1987). Subsequently there have been many other studies confirming the safety and efficacy of BoNT-A in the treatment of blepharospasm (Elston 1987; Mejia et al. 2005; Jankovic 2004; Kenney and Jankovic 2008; Gil Polo et al. 2013). Because of the paucity of double-blind, placebo-controlled trials (Roggenkämper et al. 2006), the TTA Subcommittee of the American Academy of Neurology concluded that there is only level B (probably effective) evidence for the efficacy of BoNT-A for the treatment of blepharospasm (Simpson et al. 2008b). This recommendation will likely be changed to level A as a result of more recent, well-designed, controlled trials with various BoNT products.

The onset of the Botox® effect occurs within 24–72 h, peaks at 2–4 weeks, and lasts 3–6 months (Tan 2005). While about a third of all treatment sessions are followed by some side effects (ptosis, blurring of vision or diplopia, tearing, and local hematoma), only 1–2 % affect patient's functioning, and complications

usually improve spontaneously in less than 3 weeks (Elston 1987; Roggenkämper et al. 2006). The relationship between blepharospasm, BoNT, and occurrence of dry eyes in some patients is not well understood.

In a small prospective, randomized, double-blind study on 21 patients with blepharospasm, Prosigne® was found to have similar efficacy, safety, and tolerability than Botox® with a 1:1 dose equivalence (Quagliato et al. 2010a).

Dysport® has also showed sustained efficacy and favorable safety profile with 80 U/eye (Truong et al. 2008a; Truong 2012). The onset of action occurs within 2 weeks of treatment, peaks at 4–8 weeks, and lasts 10–16 weeks (Odergren et al. 1998). In an early double-blind study in patients with blepharospasm who received one injection of Botox® and one injection of Dysport® in two separate treatment sessions, there was similar efficacy and no significant difference in the duration of the treatment effect between the two preparations, although there were significantly ($p < 0.05$) fewer side effects with Botox® (particularly ptosis; $p < 0.01$) (Nüssgens and Roggenkämper 1997). A more recent study however noted that the mean duration of improvement of blepharospasm was significantly longer for Dysport® (Bentivoglio et al. 2009), but confirmed Dysport® had a higher rate of adverse effects. Finally, another study that evaluated the effect of Botox® after switching from Dysport® showed that Botox® was more effective and had a longer duration of effect than Dysport® (Bihari 2005).

The efficacy and safety of Xeomin® in the treatment of blepharospasm was demonstrated by a prospective, double-blind, placebo-controlled, randomized, multicenter study involving 109 patients (Jankovic et al. 2011b). An open-label extension of that study demonstrated maintained efficacy and good tolerability of Xeomin® at 48 weeks, after an average of five injections (Grafe and Hanschmann 2010; Grafe et al. 2010). In that study, the most commonly reported adverse effects were eyelid ptosis, dry eye, and dry mouth. Xeomin® was found to be clinically non-inferior to Botox® in head-to-head studies (Roggenkämper et al. 2006) (Jankovic 2009a; Wabbels et al. 2011). There is no difference between the two preparations in efficacy, safety profile, onset of action, duration, and waning of effect (Jost et al. 2007; Grafe et al. 2009a). Xeomin® is FDA approved for the treatment of blepharospasm (Barnes et al. 2010).

Myobloc® has also been used successfully in the treatment of blepharospasm, especially in Botox®-resistant patients (Dutton et al. 2006), although double-blind controlled studies in these disorders are lacking (Colosimo et al. 2003). However, side effects are more common than typically expected of BoNT-A, particularly pain on injection, ptosis, dry mouth, and dry eyes (Dutton et al. 2006).

Apraxia of eyelid opening, especially when associated with blepharospasm, seems to improve with injection of the pretarsal orbicularis oculi and injection of the pretarsal portion of the eyelid, or pars ciliaris at the lid margin seems critical for the treatment to be effective in that condition (Inoue and Rogers 2007).

3.1.2 Other Cranial Dystonias

Oromandibular dystonia (OMD) is among the most challenging forms of focal dystonia to treat as it rarely improves with medications (Jankovic and Orman 1988;

Klawans and Tanner 1988; Sankhla et al. 1998). Also, there are no surgical treatments, and BoNT therapy can be complicated by swallowing problems. Clenching, trismus, and bruxism are frequent manifestations of oromandibular dystonia. Although most of the data on BoNT in the management of oromandibular dystonia (OMD) come from open-label studies, these provide compelling evidence on the efficacy of both BoNT-A and BoNT-B in patients with OMD (Wan et al. 2005; Jankovic and Orman 1987; Tan and Jankovic 2000; Laskawi and Rohrbach 2001; Alonso-Navarro et al. 2011). Patients with dystonic jaw closure, treated with injections into the masseter and temporalis muscles, generally respond better than those with jaw-opening dystonia. Although most patients with jaw-opening dystonia benefit from injections into the submentalis muscle complex, some may also require injections into the lateral pterygoid muscles (Evidente and Adler 2010). As with blepharospasm, the improvement is usually noted within the first 5 days and persists for about 3–4 months. Early BoNT treatment of oromandibular dystonia, particularly when associated with trismus and bruxism, may prevent dental and other complications, including the temporomandibular joint syndrome.

Frowning without blepharospasm, as a manifestation of upper facial dystonia, often seen in parkinsonian patients, particularly those with progressive supranuclear palsy, can be also effectively treated with BoNT (Hirota et al. 2008).

3.1.3 Laryngeal Dystonia (Spasmodic Dysphonia)

Several studies have established the efficacy and safety of Botox®, Dysport®, and Myobloc® in the treatment of adductor laryngeal dystonia, produced by involuntary contraction of the thyroarytenoid muscle, and this approach is now considered by most to be the treatment of choice for spasmodic dysphonia (Adler et al. 2004b; Wan et al. 2005; Upile et al. 2009). Adverse effects include transient breathy hypophonia, hoarseness, and rarely dysphagia with aspiration (Wan et al. 2005). This approach usually requires a multidisciplinary team, consisting of an otolaryngologist experienced in laryngeal injections and a neurologist knowledgeable about motor disorders of speech and voice. There are three approaches currently used in the BoNT treatment of spasmodic dysphonia: (1) unilateral EMG-guided injection; (2) bilateral approach, injecting with EMG guidance in each vocal fold; and (3) an injection via indirect laryngoscopy without EMG. Irrespective of the technique, most investigators report about 75–95 % improvement in voice symptoms (Jankovic 2010a). However, when Dysport® is used, it seems that unilateral injections are safer than, and as effective as, bilateral injections (Troung et al. 1991; Upile et al. 2009). BoNT produces less consistent benefits when spasmodic dysphonia is accompanied by voice tremor. As for abductor spasmodic dysphonia, one class III prospective, randomized, crossover treatment study involving 15 patients showed no objective benefit of Botox®, whether injected percutaneously or transnasally into the posterior cricoarytenoid muscle (Bielamowicz et al. 2001). However, anecdotal experience from centers that frequently treat patients with spasmodic dysphonia indicates that BoNT-A is an effective treatment even in the abductor form, although the effects are more consistent in patients with adductor form of spasmodic dysphonia (Evidente and Adler 2010).

3.1.4 Cervical Dystonia (CD)

The introduction of BoNT in the treatment of CD has changed the natural history of this disease in that cervical contractures are now much less frequent than they were prior to BoNT (Ramirez-Castaneda and Jankovic 2013). The efficacy and safety of BoNT in the treatment of CD have been demonstrated in several controlled and open trials (Brashear et al. 1999; Brin et al. 1999; Truong et al. 2005, 2010), and the TTA Subcommittee of the American Academy of Neurology concluded that there is level A (effective) evidence for the efficacy of Botox® for the treatment of CD (Simpson et al. 2008b). The most common treatment-related adverse events of BoNT in CD are mild-to-moderate weakness, dysphagia, dry mouth, neck pain, and injection-site pain.

In one of the first clinical trials to evaluate the efficacy of BoNT for the treatment of CD, treatment with Botox® produced significant improvement including reduction in pain. Side effects were few and no significant systemic adverse reactions were noted (Tsui et al. 1986). These results have since been confirmed in numerous short-term (Brin et al. 1987; Gelb et al. 1989; Greene et al. 1990) and long-term studies (Jankovic and Schwartz 1990; Ramirez-Castaneda and Jankovic 2013).

In a small prospective, randomized, double-blind study on 24 patients with CD, Prosigne® was found to have similar efficacy, safety, and tolerability than Botox® with a 1:1 dose equivalence (Quagliato et al. 2010b).

Dysport® was also shown to be significantly more efficacious than placebo at weeks 4, 8, and 12 in a multicenter, double-blind, randomized, placebo-controlled trial on 80 patients with blurred vision and weakness as the only side effects to occur significantly more often with Dysport® (Truong et al. 2005). These results were confirmed by multiple other randomized, double-blind, placebo-controlled trials (Truong et al. 2008b, 2010; Wissel et al. 2001; Truong and Jost 2006). Dysport® has also been shown to be more effective and better tolerated than trihexyphenidyl in the treatment of CD (Brans et al. 1996).

A recent study that evaluated the safety, efficacy, and duration of effect of Botox® after switching from Dysport® in patients with CD showed that Botox® had more efficacy and a longer duration of effect than Dysport® (Bihari 2005). However, another study showed Dysport® to be more effective than Botox® with higher adverse effects, although none of these required withdrawal of therapy or specific management (Ranoux et al. 2002). Finally, a systematic review showed Dysport® to cause more dysphagia than Botox®, possibly as a result of a greater diffusion (Chapman et al. 2007).

Myobloc® has been shown to be more effective than placebo in both type A-responsive and type A-resistant CD (Brashear et al. 1999; Brin et al. 1999; Lew et al. 1997) and may be an alternative in patients who have developed resistance to BoNT-A (Brin et al. 1999). Botox® and Myobloc® were directly compared at 1:40 dose ratio in 139 patients with CD, with no difference in improvement at 4 weeks following injection, but with more frequent dysphagia and dry mouth with Myobloc® (Comella et al. 2005). Another study on 111 patients with CD randomized to receive either Botox® or Myobloc/Neurobloc® at a dosing ratio of 66.7 showed no significant differences in the efficacy or occurrence of

injection-site pain and dysphagia. Dry mouth however was consistently found to be more frequent with Myobloc/Neurobloc® than with Botox® (Pappert et al. 2008). A report of a 44 % of BoNT-B antibody-induced therapy failure in toxin-naïve CD patients after a relatively short exposure may however limit its use (Dressler and Bigalke 2005).

The risk of immunoresistance seems to be higher with Myobloc® than with Botox®. In a prospective, open-label, multicenter study of 333 BoNT-naïve patients with CD, after a median of 9 of Botox® treatments over a mean of 2.5 years (range: 3.2 months to 4.2 years), only 1.2 % tested positive for blocking antibodies (Brin et al. 2008). In one study of 100 patients with CD followed for 42 months over a mean of 5 (up to 12) visits, a third of the patients who were negative for BoNT-B antibodies at baseline became positive for such antibodies at the last visit (Jankovic et al. 2006). Thus, although BoNT-B offers a useful alternative to patients with immunoresistance to BoNT-A, long-term efficacy is limited by the development of blocking antibodies, probably as a result of the cross-reactivity between the two serotypes.

Xeomin® was shown to be an effective and safe treatment of CD in a double-blind, placebo-controlled study of 233 patients, 39 % of which were BoNT naïve (Comella et al. 2011). It was found to be as effective and safe as Botox® in three different clinical trials totaling 816 patients with a 1:1 dose ratio (Jost et al. 2007) and in several other series (Benecke et al. 2005; Dressler 2009). Xeomin® seems to have similar efficacy and safety among treatment-naïve and previously treated patients (Grafe et al. 2009a, b, c; Fernandez et al. 2013a). Repeated treatments with Xeomin® seem effective and well tolerated (Grafe and Hanschmann 2010; Evidente et al. 2013). The clinical effect of Xeomin® begins within 1 week, peaks at approximately 4–6 weeks, and is sustained for about 110 days (Jost et al. 2007). The most frequently reported adverse effects of Xeomin® are mild dysphagia, neck pain, and muscle weakness (Comella et al. 2011). Xeomin® is FDA approved for the treatment of CD (Barnes et al. 2010).

3.1.5 Writer's Cramp, Other Limb Dystonias, and Axial Dystonia

The treatment of hand dystonia with BoNT is more challenging than the other dystonias, because there are more muscles involved in finely coordinated motor function required in the act of writing, dressing, and in various demanding tasks such as playing musical instruments and sport activities. Several open and double-blind controlled trials have concluded that BoNT injections into selected hand and forearm muscles probably provide the most effective relief in patients with various task-specific and occupational dystonias. BoNT stands as the first-line treatment of choice for the majority of focal dystonias (Albanese et al. 2006; Benecke and Dressler 2007; Albanese et al. 2011) and seems to retain its efficacy a decade after initiation of treatment (Lungu et al. 2011). BoNT has also been used to effectively correct abnormal limb postures seen in other movement disorders such as foot dystonia and striatal hand in Parkinson's disease patients (Giladi et al. 1994; Pacchetti et al. 1995), progressive supranuclear palsy, and corticobasal degeneration (Cordivari et al. 2001; Vanek and Jankovic 2001).

A marked improvement in the severity and disability was achieved with EMG-guided injection of BoNT-A in 93 patients with writer's cramp, whereas primary writing tremor was little improved (Marion et al. 2003). In one study, 69 % of 84 musicians reported improvement with EMG-guided Dysport® injections, but only 36 % reported long-term benefit (Schuele et al. 2005). Dysport® was also shown to be effective in a class I randomized, double-blind, placebo-controlled trial in 40 patients with writers' cramp (Kruisdijk et al. 2007) as well as in a class II study (Contarino et al. 2007). On the other hand, Botox® improved focal hand dystonia in 80 % of patients in a class II double-blind, placebo-controlled, crossover study (Cole et al. 1995), with similar results found in another class II study (Tsui et al. 1993).

BoNT has also been used in the treatment of axial postural abnormalities secondary to axial dystonia, with variable success (Jankovic 2010b). One study reported improvement of six of nine patients with lateral axial dystonia (scoliosis) after EMG-guided injection of 500 U of Dysport® into paraspinal muscles at the level of L2–L5 on the side of the trunk flexion (Bonanni et al. 2007). Another study reported improvement of camptocormia in 9 of 11 patients who received 300–600 U of Botox® into the rectus abdominis (Azher and Jankovic 2005). In contrast, ultrasound-guided injection of the iliopsoas muscle with BoNT is not effective in camptocormia (von Coelln et al. 2008).

3.1.6 Hemifacial Spasm

Hemifacial spasm is defined as a neurologic disorder manifested by involuntary, recurrent twitches of the eyelids and other muscles of only one side of the face (Yaltho and Jankovic 2011). The muscular contractions result from an irritative lesion of the ipsilateral facial nerve, most commonly from compression by a vascular loop. While microvascular decompression of the facial nerve has a high success rate, this surgical treatment is associated with certain risks, such as permanent facial paralysis, deafness, stroke, and even death. Therefore, local injections of BoNT into involved facial muscles offer a useful alternative to surgical therapy. Nearly all patients improve; the complications are minimal and transient, and the approach can be individualized by injecting only those muscles – the contractions of which are most disturbing to the patient. Along with blepharospasm, the FDA approved onabotulinumtoxin A injections for hemifacial spasm in 1989.

The average latency from injection to the onset of benefit is 5.4 days, and the total duration of benefit averages 18.4 weeks. Side effects include facial weakness, lid weakness, ptosis, teary or dry eyes, diplopia, and hematoma. The average duration of improvement in hemifacial spasm, 5 months, is longer than in any of the dystonic disorders, and rare patients have achieved long-lasting remissions. Facial myoclonus associated with Rasmussen encephalitis, similar to hemifacial spasm, but pathophysiologically related to focal cortical seizure, has been also reported to improve with BoNT (Jankovic 2010a).

The frequent use of Botox® and Dysport® in the treatment of hemifacial spasm stems mainly from extensive open-label and clinical experience (Gil Polo et al. 2013)

rather than from controlled trials (Jankovic et al. 1990; Jost and Kohl 2001; Kenney and Jankovic 2008). In one class II prospective, blinded study, Botox® was showed to be safe and effective (Yoshimura et al. 1992).

One study that evaluated the effect of Botox® after switching from Dysport® showed that Botox® was more efficacious in treating hemifacial spasm dystonia or hemifacial spasm and had a longer duration of effect than Dysport® (Bihari 2005). However, a class II single-blind, randomized, parallel-design study comparing Botox® and Dysport® at a dose ratio of 1:4 in 91 patients with hemifacial spasm or blepharospasm showed similar clinical efficacy and tolerability of both products (Sampaio et al. 1997). Xeomin® has been shown to be as effective and as safe as Botox®, with a ratio of 1:1 in 17 patients with hemifacial spasm (Dressler 2009). Prosigne® was found to have similar efficacy, safety, and tolerability than Botox® with 1:1 dose equivalence on 36 patients with hemifacial spasm (Quagliato et al. 2010a).

Myobloc® has also been used successfully in the treatment of hemifacial spasm, although double-blind controlled studies in this disorder are lacking (Colosimo et al. 2003; Trosch et al. 2007).

3.1.7 Tremor

Tremor accompanies dystonia in about half of all dystonic patients, and dystonic tremor improves in some patients treated for focal dystonia with BoNT. Chemodenervation with Botox® may ameliorate not only dystonic tremor but also essential tremor involving the hands, as demonstrated by at least two double-blind, placebo-controlled studies (Jankovic et al. 1996; Brin et al. 2001). When wrist extensor injections are avoided, weakness of finger extensors, noted in the initial studies, can be prevented. BoNT can also be considered for rest tremor associated with Parkinson's disease (PD) when antiparkinsonian treatments fail to insure satisfactory relief (Jankovic and Schwartz 1991; Diamond and Jankovic 2006a, b). In an open-label pilot study, Dysport® injection in the masseters markedly improved jaw tremor in 3 PD patients (Schneider et al. 2006).

However, essential tremor and dystonic tremor seem to respond better to BoNT than rest tremors related to PD, and further studies are needed to demonstrate efficacy of BoNT for the latter and to provide insights on how to improve the treatment protocol (Jankovic 2009b).

Although Botox® is clearly a useful treatment in patients with hand tremor, it has also been found effective in the treatment of voice tremor (Adler et al. 2004a) and head tremor (Pahwa et al. 1995). In a small, randomized, crossover trial of onabotulinumtoxin A in 23 patients with disabling tremor in 33 upper limbs related to MS, there was a significant improvement after active treatment compared with that after placebo at 6 and 12 weeks after injection in the Bain score for tremor, writing, Archimedes spiral drawing, drinking from a cup, and in the 9-hole peg test (Van Der Walt et al. 2012). There was, however, no improvement in the quality of life as measured by the Quality of Life in ET Questionnaire (QUEST). Treatment was complicated by transient weakness, noted in 42.2 % of patients treated with BTX, compared to 6.1 % of patients treated with placebo ($p = 0.0005$).

3.1.8 Tics

Motor and phonic tics associated with Tourette syndrome typically improve with antidopaminergic drugs, but when these drugs do not adequately control the tics or are associated with troublesome side effects, BoNT injections into the affected body parts not only may provide satisfactory control of the tics but also may eliminate the premonitory urge. BoNT treatment is particularly useful in the treatment of focal motor tics and phonic tics, including coprolalia. In a placebo-controlled class II study of 18 patients with simple motor tics (Marras et al. 2001), Botox® treatment was associated with a 39 % reduction in the number of tics per minute within 2 weeks after injection against a 6 % increase in the placebo group ($p = 0.004$). In addition, there was a 0.46 reduction in "urge scores" with BoNT, against a 0.49 increase in the placebo group ($p = 0.02$). This preliminary study, however, lacked the power to show significant differences in other measured variables, such as severity score, tic suppression, pain, and patient global impression. However, it measured the results at 2 weeks, when the full effects of Botox® may have not yet been appreciated, and measured the effect of one treatment only, whereas several adjustments in doses and sites of injections over several treatment visits are usually needed in clinical practice. Another class IV open-label study of 15 patients with simple motor tics showed long-term efficacy of Botox® (Rath et al. 2010), with a permanent remission of the treated tic in three patients at 10 years' follow-up. The premonitory urge was similarly reduced.

3.1.9 Spasticity and Other Hypertonic Disorders

In addition to involuntary movement disorders, BoNT has been used effectively to treat spasticity associated with cerebral palsy, multiple sclerosis, or following stroke.

There is extensive data from clinical trials indicating that Botox® is well tolerated and effective in reducing focal upper limb hypertonia following stroke (Childers et al. 2004; Gordon et al. 2004; Turkel et al. 2006; Elia et al. 2009; Simpson et al. 2009). A meta-analysis of seven multicenter, randomized, double-blind, placebo-controlled, parallel-group trials totaling 544 poststroke patients showed that Botox® decreased poststroke spasticity in upper limb-injected muscles proportionally to the dose of BoNT used. Injected muscles included the flexor carpi ulnaris (FCU), flexor carpi radialis (FCR), flexor digitorum superficialis (FDS), flexor digitorum profundus (FDP), and biceps brachii (BB) (Yablon et al. 2011). A multicenter, randomized, double-blind, placebo-controlled study of 120 poststroke patients with lower limb spasticity showed that a onetime Botox® injection yielded significant improvement at 4, 5, and 8 weeks (Kaji et al. 2010). Experienced injectors have recommended the following doses of Botox in the treatment of spasticity: for the lower limb, 3–6 U/kg per muscle; for the upper limb above the elbow, 2–3 U/kg per muscle; and for the upper limb below the elbow and posterior tibialis, 0.5–2 U/kg per muscle (Jankovic 2010a).

A double-blind, placebo-controlled, crossover study also demonstrated improvement in spasticity of thigh adductors in patients with multiple sclerosis after receiving Botox® with subsequent functional gain, specifically easier nursing care, and better comfort when sitting in a wheelchair (Snow et al. 1990).

Dysport® also seems to improve upper limb and, to a lesser extent, lower limb spasticity secondary to stroke (Keam et al. 2011).

Xeomin® was also shown to be effective in reducing poststroke upper limb spasticity in a recent open-label study of 145 patients (Kanovsky et al. 2011).

Based on the review of 11 class I trials for adult upper extremity spasticity, one of them using BoNT-B, and three class I trials for lower limb spasticity, the AAN recommends that BoNT should be offered as a treatment option to reduce muscle tone and improve passive function in adults with spasticity (Simpson et al. 2008a).

Several reviews have assessed the therapeutic effect of BoNT-A, Botox® and Dysport® confounded, for spasticity of the upper or lower limbs secondary to cerebral palsy and the consequent impact on quality of life (Lannin et al. 2006; Naumann et al. 2006; Park and Rha 2006; Coutinho et al. 2011). A meta-analysis of 20 randomized controlled trials of Botox® and/or Dysport® versus placebo or rehabilitation, totaling 882 patients, showed that BoNT-A has a good safety profile during the first months of use, with however more systemic side effects. BoNT-A use was related to respiratory tract infection, bronchitis, pharyngitis, asthma, muscle weakness, urinary incontinence, falls, seizures, fever, and unspecified pain (Albavera-Hernández et al. 2009). Since a dose–response relationship has been observed in the frequency and severity of adverse events of BoNT-A (Naumann et al. 2006), it is possible that children with cerebral palsy have a higher probability of occurrence since they require high doses of toxin relative to their body mass.

In children with cerebral palsy, Botox® (2–8, up to 16 U per kg body weight per muscle) is often found effective when injected in calf muscles to correct equinus deformity and toe walking and in the hamstring muscles to correct crouch and scissor gait, improve sitting and hygiene care, and reduce pain. In a double-blind, controlled study, patients with cerebral palsy receiving 40–80 U of Botox® per muscle experienced a significantly greater improvement in Ashworth score and gait measures than the "low-dose" group (20–40 U per muscle) (Wissel et al. 1999). Multiple studies of Botox® or Dysport® injections into the gastrocnemius showed gait improvement over 1–3 months (Simpson et al. 2008a). One randomized, double-blind, placebo-controlled study injecting Dysport® into the thigh adductors and medial hamstrings of children with adductor spasticity showed significant improvement in knee-to-knee distance as well as adductor muscle tone (Mall et al. 2006). The results of many of the studies are difficult to interpret and compare, because patients with various forms and etiologies of spasticity were enrolled and different methodologies were used to inject and rate the patients. Although some double-blind trials have demonstrated meaningful functional improvement in patients with spasticity, other controlled studies have failed to demonstrate improvement. Many of the published studies suffer methodological problems, particularly in selecting the appropriate outcome measures that may or may not capture the functional goals of the patients. The AAN granted BoNT injections a level A of evidence for the treatment of equinovarus deformity in children with cerebral palsy and a level B for treatment of adductor spasticity and for pain control and in children with cerebral palsy and upper extremity spasticity (Level B) (Simpson et al. 2008a).

Xeomin® was reported useful in treating acquired brain injury-induced upper limb spasticity in a series of 192 patients (Barnes et al. 2010) and lower limb spasticity in one case report (Lippert-Gruner and Svestkova 2011). It has been shown to be as effective and as safe as Botox, with a ratio of 1:1 in a series of 94 patients with spasticity (Dressler 2009).

3.2 Painful Disorders

3.2.1 Headache

No significant difference between Botox® and placebo could be demonstrated in four different studies on patients suffering from episodic migraines, defined as less than 15 days of headache per month (Silberstein et al. 2000; Elkind et al. 2006; Relja et al. 2007; Saper et al. 2007; Naumann et al. 2008). Similarly, out of 4 studies of Botox® for chronic daily headache, none demonstrated a significant effect of Botox® on the primary outcome measure (Mathew et al. 2005). Botox® was also ineffective in treating chronic tension headaches in four randomized, placebo-controlled studies (Naumann et al. 2008). Another study reported that imploding headaches (head seems to be crushed, clamped, or stubbed by external forces) and ocular headaches (eye-popping pain) were more likely to respond to Botox® than exploding headaches (buildup of pressure inside the head) (Jakubowski et al. 2006).

One controlled study demonstrated the effectiveness of fixed-site administration of 100 U of Botox® in the treatment of patients with chronic migraine who specifically did not overuse pain medication (Freitag et al. 2008). However, 150 U of Botox would be needed in patients with chronic migraine and medication overuse (Grazzi, 2013). Chronic migraine is defined as headache occurring $\geq$15 days per month for $\geq$3 months, with headaches occurring on $\geq$8 days being classified as migraine headaches or headaches that respond to migraine-specific medications (Olesen et al. 2006). More recently, pivotal results from the Phase III Research Evaluating Migraine Prophylaxis Therapy (PREEMPT) clinical program involving two double-blinded, placebo-controlled studies established Botox® as a safe, well-tolerated, and effective headache prophylactic treatment for chronic migraine using a combination of fixed-site administration and follow-the-pain approach, at doses ranging from 155 to 195 U administered across seven head and neck muscles every 12 weeks for up to five treatment cycles (Aurora et al. 2010; Blumenfeld et al. 2010; Diener et al. 2010; Dodick et al. 2010). However, these results were not replicated with Dysport® (Chankrachang et al. 2011).

The antinociceptive effect of BoNT in headache was initially thought to be a result of relief of muscle spasms, but in vitro studies have shown that BoNT blocks the peripheral release of pain and inflammatory neurotransmitters such as glutamate, substance P, and calcitonin gene-related peptide (Aoki 2003, 2005; Gazerani et al. 2009). Moreover, it has been inferred from animal studies that BoNT-A exerts its antinociceptive effects through retrograde transport and involvement of the central nervous system (Bach-Rojecky and Lacković 2009).

3.2.2 Chronic Knee Pain

In one randomized, double-blind, placebo-controlled, crossover trial on 24 patients with chronic anterior knee pain associated with quadriceps muscle imbalance, one 500 U injection of Dysport® in the vastus lateralis associated with retraining of the vastus medialis provided marked pain improvement compared to placebo. This intervention permitted patients to restore a more balanced knee extensor control during functional activity (Singer et al. 2011).

3.2.3 Painful Limbs/Moving Extremities

The syndrome of painful limbs/moving extremities describes a clinical condition characterized by involuntary toe or finger movement associated with pain in the affected hand/arm or foot/leg. Botox® injections in the involved muscles have been shown to improve pain and movement in some case reports (Singer and Papapetropoulos 2007; Eisa et al. 2008).

3.2.4 Other Pain Syndromes

BoNT-A seems effective in controlling musculoskeletal pain, such as myofascial pain, low back pain, trigeminal neuralgia, and other chronic pain syndromes (Reilich et al. 2004; Sycha et al. 2004; Bhidayasiri and Truong 2005; Bohluli et al. 2011). However, these conditions represent a diverse group, and the results with BoNT-A have not been universally positive (Waseem et al. 2011; Zhang et al. 2011). A recent meta-analysis failed to confirm any significant improvement of neck pain after BoNT-A injection (Langevin et al. 2011a, b).

3.3 Hyperhidrosis

Several well-designed trials have demonstrated efficacy of intradermal injection of Botox® and Dysport® in axillar (Heckmann et al. 2001; Naumann and Lowe 2001; Doft et al. 2011; Dressler and Adib Saberi 2013) and palmar hyperhidrosis (Lowe et al. 2002; Solish et al. 2007; Ito et al. 2011). A within-subject comparison showed similar effectiveness and tolerance but longer duration of action of Dysport® compared to Botox® (Simonetta et al. 2003). Xeomin® has been shown to be as effective and as safe as Botox®, with a ratio of 1:1 in 64 patients with hyperhidrosis (Dressler 2009). This was confirmed by a double-blinded study on 46 patients complaining of axillar hyperhidrosis with injection of Botox® under one arm and Xeomin® under the other (Dressler 2010b).

Myobloc® has also been shown to improve axillary as well as palmar hyperhidrosis (Baumann et al. 2005a, b) and appears to be as effective as Botox® in the treatment of axillary hyperhidrosis at a conversion factor of 20:1, with however more discomfort on injection (Dressler et al. 2002). In a recent within-subject comparison of 10 patients, Myobloc® was significantly more effective and had a longer duration than Botox®, at a conversion ratio of 50:1 (Frasson et al. 2011). Xeomin® has also been used successfully for the treatment of axillar hyperhidrosis (Dressler 2012). The TTA Subcommittee of the AAN concluded that there is level

A evidence for the recommendation that BoNT should be offered as a treatment option for axillary hyperhidrosis and level B for palmar hyperhidrosis (Naumann et al. 2008).

Gustatory sweating is a unique entity characterized by profuse sweating of the face, scalp, and neck during or immediately after ingestion of food or drink (Naumann et al. 1997). It most often occurs as a complication of surgery to the area of the face near the parotid glands (Nolte et al. 2004). The effect of Botox® on gustatory sweating has been evaluated in one class II and three class III studies totaling 131 patients, with an effect lasting up to a year (Naumann et al. 1997; Laccourreye et al. 1999; Eckardt and Kuettner 2003; Nolte et al. 2004). At present available evidence supports a Level C recommendation for BoNT for the treatment of gustatory sweating.

3.4 Gastrointestinal Conditions

3.4.1 Sialorrhea

Several studies, including randomized placebo-controlled trials (Ondo et al. 2004; Lagalla et al. 2006; Jackson et al. 2009), have provided evidence that Botox®, Dysport®, or Myobloc® injections in the parotid glands with or without injection in the submandibular glands may be the treatment of choice for sialorrhea and drooling associated with PD and other disorders with bulbar dysfunction, such as amyotrophic lateral sclerosis, cerebral palsy, posttraumatic encephalopathy, and bilateral strokes (Nóbrega et al. 2007; Santamato et al. 2008; Molloy 2007; Basciani et al. 2011; Chinnapongse et al. 2011; Moller et al. 2011). Ultrasound-guided injection may improve the efficacy of BoNT treatment for sialorrhea (Dogu et al. 2004). Xeomin® has been shown to be as effective and as safe as Botox®, with a ratio of 1:1 in a small series of patients with sialorrhea (Dressler 2009).

An open-label study of 30 children with neurological disorders and sialorrhea showed no difference in efficacy or side effects between BoNT-A and BoNT-B (Wilken et al. 2008). Other studies provided evidence that BoNT-B may be more effective than BoNT-A in treating sialorrhea as dry mouth is significantly more frequently noted in patients treated for CD with BoNT-B as compared to BoNT-A (Tintner et al. 2005; Jankovic 2009b; Guidubaldi et al. 2011). However, a recent randomized double-blind crossover trial demonstrated similar efficacy but shorter latency and lower cost of Myobloc® compared to Dysport® (Guidubaldi et al. 2011).

The potential adverse effects of using BoNT for sialorrhea include transient dysphagia and xerostomia although dysphagia has not been reported in any study. In fact, a study of oropharyngeal swallowing dynamics, based on swallowing videofluoroscopy, showed no difference before or 30 days after injections of BoNT-A into the parotids in patients with PD (Nóbrega et al. 2009).

3.4.2 Achalasia

Achalasia is manifested by spontaneous and repetitive contractions of the proximal esophagus with failure of the lower esophageal sphincter to relax during swallowing. This leads to dysphagia. Several small studies have demonstrated the beneficial effects of Botox® injections into the lower esophageal sphincter in the treatment of

achalasia, particularly in patients who are not candidates for surgery or balloon dilatation (Pohl and Tutuian 2007; Barnes et al. 2011; Lakhtakia et al. 2011).

3.4.3 Constipation and Fecal Incontinence

Anismus (constipation due to functional obstruction at the pelvic outlet by paradoxical contraction of the striated sphincter muscles during defecation straining) has been suggested to represent a form of focal dystonia and has been considered as a possible etiology of constipation in some patients (Mathers et al. 1988). Several small open-label studies demonstrated the beneficial effects of Botox® anal injections in the treatment of the subtype of constipation secondary to anismus (Albanese et al. 2003; Cadeddu et al. 2005; Hompes et al. 2012). The puborectalis muscle is usually injected under transrectal ultrasonographic guidance.

On the other hand, rectal hypercontractility can lead to fecal incontinence. Intrarectal Dysport® injections have shown some improvements in fecal incontinence in a small series (Bridoux et al. 2011).

3.4.4 Anal Fissure

Botox® and Dysport® injections into the internal anal sphincter seem effective in treating chronic anal fissure with a low rate of transient incontinence secondary to diffusion to the external anal sphincter and without permanent complications. However, the therapeutic response is heterogenous (Brisinda et al. 2002; Maria et al. 2000; Yiannakopoulou 2012).

3.5 Overactive Bladder and Other Urologic Problems

An increasing number of reports provide evidence that Botox® injections into the bladder wall is an effective treatment in increasing the bladder capacity and improving urge and incontinence in patients with overactive bladder associated with neurogenic and idiopathic detrusor overactivity (Patel et al. 2006; Chen and Liao 2011; Deffontaines-Rufin et al. 2011). However, the rate of treatment discontinuation may be as high as 61.3 % at 3 years, secondary the need to intermittent self-catheterization or urinary tract infections (Mohee et al. 2013). A randomized controlled trial comparing anticholinergic versus BoNT for urinary urge incontinence has recently started (Visco et al. 2012). The TTA Subcommittee of the AAN concluded that there is level A evidence for the recommendation that BoNT should be offered as a treatment option for this urinary disorder (Naumann et al. 2008).

Other genitourinary indications for Botox® treatment include voiding dysfunction due to benign prostatic hypertrophy (Chuang and Chancellor 2006; Marchal et al. 2011).

3.6 Allergic Rhinitis

Two class II placebo-controlled studies with a total of 73 patients demonstrated the efficacy of 20–30 units of Botox® in each nasal cavity to manage the symptoms of

allergic rhinitis (Unal et al. 2003; Yang et al. 2008). Botox® was also superior when compared to intranasal triamcinolone (Yang et al. 2008). However, the follow-up was only for 8 weeks, so the durability of effectiveness is unclear.

3.7 Cosmetic

It is beyond the scope of this review to discuss the vast literature on cosmetic use of BoNT, which represents about half of all the uses of BoNT. Botox® and Myobloc® have been used in brow lifts and for treating glabellar lines, horizontal forehead lines, crow's feet, hypertrophic orbicularis, upper nasalis (bunny) lines, repeated nasal flares, melomental folds, perioral rhytides or smoker's lines, mouth frown, peau d'orange skin, mental crease, facial asymmetry with both hyper- and hypofunctional muscle imbalance, necklace lines, and platysmal bands (Glogau 2002; Baumann et al. 2003; Choi et al. 2013a, b; Dessy et al. 2011). Overly aggressive or imprecise treatment in the cervical area can lead to incompetent mouth, weakness of the neck flexors, and dysphagia (Klein 2001). The duration of effect of BoNT for cosmetic indications ranges from 3 to 4 months and up to 6–8 months, depending on the treatment site and injection technique (Carruthers and Carruthers 2001). Myobloc® seems to have a faster onset of action by 1–2 days for all these muscle groups, but a shorter duration and lesser potency of action when compared to Botox® at a conversion factor of 1:20 to 1:100. In addition, intradermal injections with Myobloc® are more painful than with Botox® (Yamauchi and Lowe 2004).

Botox® injected peri-nasally at a dose of 20–25 units for facial tics also improved acne in two patients despite previous failure of several anti-acne agents. Clearing of peri-nasal acne began 1–2 weeks after each treatment and lasted 4–5 months (Diamond and Jankovic 2006a, b). Xeomin® and Dysport® have recently demonstrated efficacy in the treatment of crow's feet (Prager et al. 2011).

4 Conclusion

Since first recognized as the cause of food-borne botulism in the early nineteenth century, BoNT was suggested as a potential treatment for involuntary spasms and movements. Almost 200 years later, its known clinical applications span across numerous neurological and other diseases. The Neurotoxin Institute was established in 2002 as an umbrella organization providing unbiased educational programs and other information about the broad use of BoNT in different specialties (www.neurotoxininstitute.com).

References

Adler, C. H., Bansberg, S. F., Hentz, J. G., Ramig, L. O., Buder, E. H., Witt, K., Edwards, B. W., Krein-Jones, K., & Caviness, J. N. (2004a). Botulinum toxin type A for treating voice tremor. *Archives of Neurology, 61*, 1416–1420.

Adler, C. H., Bansberg, S. F., Krein-Jones, K., & Hentz, J. G. (2004b). Safety and efficacy of botulinum toxin type B (Myobloc) in adductor spasmodic dysphonia. *Movement Disorders, 19*, 1075–1079.

Albanese, A. (2009). Discussion of unique properties of botulinum toxins. *Toxicon, 54*(5), 702–708.

Albanese, A. (2011). Terminology for preparations of botulinum neurotoxins: What a difference a name makes. *Journal of the American Medical Association, 305*, 89–90.

Albanese, A., Brisinda, G., Bentivoglio, A. R., & Maria, G. (2003). Treatment of outlet obstruction constipation in Parkinson's disease with botulinum neurotoxin A. *The American Journal of Gastroenterology, 98*, 1439–1440.

Albanese, A., Barnes, M. P., Bhatia, K. P., Fernandez-Alvarez, E., Filippini, G., Gasser, T., Krauss, J. K., Newton, A., Rektor, I., Savoiardo, M., & Valls-Solè, J. (2006). A systematic review on the diagnosis and treatment of primary (idiopathic) dystonia and dystonia plus syndromes: Report of an EFNS/MDS-ES Task Force. *European Journal of Neurology, 13*, 433–444.

Albanese, A., Asmus, F., Bhatia, K. P., Elia, A. E., Elibol, B., Filippini, G., Gasser, T., Krauss, J. K., Nardocci, N., Newton, A., & Valls-Solé, J. (2011). EFNS guidelines on diagnosis and treatment of primary dystonias. *European Journal of Neurology, 18*, 5–18.

Albavera-Hernández, C., Rodríguez, J. M., & Idrovo, A. J. (2009). Safety of botulinum toxin type A among children with spasticity secondary to cerebral palsy: A systematic review of randomized clinical trials. *Clinical Rehabilitation, 23*(5), 394–407.

Alonso-Navarro, H., Jiménez-Jiménez, F. J., Plaza-Nieto, J. F., Pilo-De la Fuente, B., Navacerrada, F., Arroyo-Solera, M., & Calleja, M. (2011). Treatment of severe bruxism with botulinum toxin type A. *Revista de Neurologia, 53*(2), 73–76.

Aoki, K. R. (2003). Evidence for antinociceptive activity of botulinum toxin type A in pain management. *Headache, 43*(Suppl 1), S9–S15.

Aoki, K. (2005). Review of a proposed mechanism for the antinociceptive action of botulinum toxin type A. *NeuroToxicology, 26*, 785–793.

Atassi, M. Z., Dolimbek, B. Z., Jankovic, J., Steward, L. E., & Aoki, K. R. (2008). Molecular recognition of botulinum neurotoxin B heavy chain by human antibodies from cervical dystonia patients that develop immunoresistance to toxin treatment. *Molecular Immunology, 45*(15), 3878–3888.

Aurora, S. K., Dodick, D. W., Turkel, C. C., DeGryse, R. E., Silberstein, S. D., Lipton, R. B., Diener, H. C., & Brin, M. F. (2010). OnabotulinumtoxinA for treatment of chronic migraine: Results from the double-blind, randomized placebo controlled phase of the PREEMPT 1 trial. *Cephalalgia, 30*, 793–803.

Azher, S. N., & Jankovic, J. (2005). Camptocormia: Pathogenesis, classification, and response to therapy. *Neurology, 65*, 355–359.

Bach-Rojecky, L., & Lacković, Z. (2009). Central origin of the antinociceptive action of botulinum toxin type A. *Pharmacology Biochemistry and Behavior, 94*, 234–238.

Baizabal-Carvallo, J. F., Jankovic, J., & Pappert, E. (2011). Flu-like symptoms following botulinum toxin therapy. *Toxicon, 58*(1), 1–7.

Bakheit, A. M., Liptrot, A., Newton, R., & Pickett, A. M. (2011). The effect of total cumulative dose, number of treatment cycles, interval between injections, and length of treatment on the frequency of occurrence of antibodies to botulinum toxin type A in the treatment of muscle spasticity. *International Journal of Rehabilitation Research, 35*(1), 36–39.

Barnes, M., Schnitzler, A., Medeiros, L., Aguilar, M., Lehnert-Batar, A., & Minnasch, P. (2010). Efficacy and safety of NT 201 for upper limb spasticity of various etiologies–a randomized parallel-group study. *Acta Neurologica Scandinavica, 122*(4), 295–302.

Barnes, M. A., Ho, A. S., Malhotra, P. S., Koltai, P. J., & Messner, A. (2011). The use of botulinum toxin for pediatric cricopharyngeal achalasia. *International Journal of Pediatric Otorhinolaryngology, 75*(9), 1210–1214.

Basciani, M., Di Rienzo, F., Fontana, A., Copetti, M., Pellegrini, F., & Intiso, D. (2011). Botulinum toxin type B for sialorrhoea in children with cerebral palsy: A randomized trial comparing three doses. *Developmental Medicine and Child Neurology, 53*(6), 559–564.

Baumann, L., Slezinger, A., Vujevich, J., Halem, M., Bryde, J., Black, L., & Duncan, R. (2003). A double-blinded, randomized, placebo-controlled pilot study of the safety and efficacy of myobloc (botulinum toxin type B)-purified neurotoxin complex for the treatment of crow's feet: A double-blinded, placebo-controlled trial. *Dermatologic Surgery, 29*, 508–515.

Baumann, L., Slezinger, A., Halem, M., Vujevich, J., Martin, L. K., Black, L., & Bryde, J. (2005a). Pilot study of the safety and efficacy of Myobloc (botulinum toxin type B) for treatment of axillary hyperhidrosis. *International Journal of Dermatology, 44*, 418–424.

Baumann, L., Slezinger, A., Halem, M., Vujevich, J., Mallin, K., Charles, C., Martin, L. K., Black, L., & Bryde, J. (2005b). Double-blind, randomized, placebo-controlled pilot study of the safety and efficacy of myobloc (botulinum toxin type B) for the treatment of palmar hyperhidrosis. *Dermatologic Surgery, 31*, 263–270.

Benecke, R., & Dressler, D. (2007). Botulinum toxin treatment of axial and cervical dystonia. *Disability and Rehabilitation, 29*, 1769–1777.

Benecke, R., Jost, W. H., Kanovsky, P., Ruzicka, E., Comes, G., & Grafe, S. (2005). A new botulinum toxin type A free of complexing proteins for treatment of cervical dystonia. *Neurology, 64*, 1949–1951.

Bentivoglio, A. R., Fasano, A., Ialongo, T., Soleti, F., Lo Fermo, S., & Albanese, A. (2009). Fifteen-year experience in treating blepharospasm with Botox or Dysport: Same toxin, two drugs. *Neurotoxicity Research, 15*, 224–231.

Berman, B., Seeberger, L., & Kumar, R. (2005). Long-term safety, efficacy, dosing, and development of resistance with botulinum toxin type B in cervical dystonia. *Movement Disorders, 20*, 233–237.

Bhidayasiri, R., & Truong, D. D. (2005). Expanding use of botulinum toxin. *Journal of the Neurological Sciences, 235*, 1–9.

Bielamowicz, S., Squire, S., Bidus, K., & Ludlow, C. L. (2001). Assessment of posterior cricoarytenoid botulinum toxin injections in patients with abductor spasmodic dysphonia. *Annals of Otology, Rhinology and Laryngology, 110*, 406–412.

Bihari, K. (2005). Safety, effectiveness, and duration of effect of BOTOX after switching from Dysport for blepharospasm, cervical dystonia, and hemifacial spasm dystonia, and hemifacial spasm. *Current Medical Research and Opinion, 21*, 433–438.

Blumenfeld, A., Silberstein, S. D., Dodick, D. W., Aurora, S. K., Turkel, C. C., & Binder, W. J. (2010). Method of injection of onabotulinumtoxinA for chronic migraine: a safe, well-tolerated, and effective treatment paradigm based on the PREEMPT clinical program. *Headache, 50*(9), 1406–1418.

Bohluli, B., Motamedi, M. H., Bagheri, S. C., Bayat, M., Lassemi, E., Navi, F., & Moharamnejad, N. (2011). Use of botulinum toxin A for drug-refractory trigeminal neuralgia: Preliminary report. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics, 111*(1), 47–50.

Bonanni, L., Thomas, A., Varanese, S., Scorrano, V., & Onofrj, M. (2007). Botulinum toxin treatment of lateral axial dystonia in parkinsonism. *Movement Disorders, 22*, 2097–2103.

Brans, J. W., Lindeboom, R., Snoek, J. W., Zwarts, M. J., van Weerden, T. W., Brunt, E. R., van Hilten, J. J., van der Kamp, W., Prins, M. H., & Speelman, J. D. (1996). Botulinum toxin versus trihexyphenidyl in cervical dystonia: A prospective, randomized, double-blind controlled trial. *Neurology, 46*, 1066–1072.

Brashear, A., Lew, M. F., Dykstra, D. D., Comella, C. L., Factor, S. A., Rodnitzky, R. L., Trosch, R., Singer, C., Brin, M. F., Murray, J. J., Wallace, J. D., Willmer-Hulme, A., & Koller, M. (1999). Safety and efficacy of NeuroBloc (botulinum toxin type B) in type A-responsive cervical dystonia. *Neurology, 53*, 1439–1446.

Bridoux, V., Gourcerol, G., Kianifard, B., Touchais, J. Y., Ducrotte, P., Leroi, A. M., Michot, F., & Tuech, J. J. (2011). Botulinum A toxin as a treatment for overactive rectum with associated fecal incontinence. *Colorectal Disease, 14*(3), 342–348.

Brin, M. F., Fahn, S., Moskowitz, C., Friedman, A., Shale, H. M., Greene, P. E., Blitzer, A., List, T., Lange, D., Lovelace, R. E., et al. (1987). Localized injections of botulinum toxin for the treatment of focal dystonia and hemifacial spasm. *Movement Disorders, 2*, 237–254.

Brin, M. F., Lew, M. F., Adler, C. H., Comella, C. L., Factor, S. A., Jankovic, J., O'Brien, C., Murray, J. J., Wallace, J. D., Willmer-Hulme, A., & Koller, M. (1999). Safety and efficacy of NeuroBloc (botulinum toxin type B) in type A-resistant cervical dystonia. *Neurology, 53*(7), 1431–1438.

Brin, M. F., Lyons, K. E., Doucette, J., Adler, C. H., Caviness, J. N., Comella, C. L., Dubinsky, R. M., Friedman, J. H., Manyam, B. V., Matsumoto, J. Y., Pullman, S. L., Rajput, A. H., Sethi, K. D., Tanner, C., & Koller, W. C. (2001). A randomized, double masked, controlled trial of botulinum toxin type A in essential hand tremor. *Neurology, 56*, 1523–1528.

Brin, M. F., Comella, C. L., Jankovic, J., Lai, F., Naumann, M., & CD-017 BoNTA Study Group. (2008). Long-term treatment with botulinum toxin type A in cervical dystonia has low immunogenicity by mouse protection assay. *Movement Disorders, 23*, 1353–1360.

Brisinda, G., Maria, G., Sganga, G., Bentivoglio, A. R., Albanese, A., & Castagneto, M. (2002). Effectiveness of higher doses of botulinum toxin to induce healing in patients with chronic anal fissures. *Surgery, 131*, 179–184.

Cadeddu, F., Bentivoglio, A. R., Brandara, F., Marniga, G., Brisinda, G., & Maria, G. (2005). Outlet type constipation in Parkinson's disease: Results of botulinum toxin treatment. *Alimentary Pharmacology and Therapeutics, 22*, 997–1003.

Camargo, C. H., Teive, H. A., Becker, N., Munhoz, R. P., & Werneck, L. C. (2011). Botulinum toxin type A and cervical dystonia: A seven-year follow-up. *Arquivos de Neuro-Psiquiatria, 69*(5), 745–750.

Carruthers, A., & Carruthers, J. (2001). Botulinum toxin type A: History and current cosmetic use in the upper face. *Seminars in Cutaneous Medicine and Surgery, 20*, 71–84.

Chankrachang, S., Arayawichanont, A., Poungvarin, N., Nidhinandana, S., Boonkongchuen, P., Towanabut, S., Sithinamsuwan, P., & Kongsaengdao, S. (2011). Prophylactic botulinum type A toxin complex (Dysport(R)) for migraine without aura. *Headache, 51*(1), 52–63.

Chapman, M. A., Barron, R., Tanis, D. C., Gill, C. E., & Charles, P. D. (2007). Comparison of botulinum neurotoxin preparations for the treatment of cervical dystonia. *Clinical Therapeutics, 29*, 1325–1337.

Charles, P. D., Adler, C. H., Comella, C., Jankovic, J., Stacy, M., Kurth, M., et al. (2010). CD-PROBE (cervical dystonia patient registry for observation of BOTOX® efficacy) – preliminary safety data. Presented at: Movement Disorder Society, Buenos Aires. 13–17 June 2010. *Movement Disorders, 25*(Suppl 2), S232.

Chen, G., & Liao, L. (2011). Injections of botulinum toxin A into the detrusor to treat neurogenic detrusor overactivity secondary to spinal cord injury. *International Urology and Nephrology, 43*(3), 655–662.

Childers, M. K., Brashear, A., Jozefczyk, P., Reding, M., Alexander, D., Good, D., Walcott, J. M., Jenkins, S. W., Turkel, C., & Molloy, P. T. (2004). Dose-dependent response to intramuscular botulinum toxin type A for upper-limb spasticity in patients after a stroke. *Archives of Physical Medicine and Rehabilitation, 85*, 1063–1069.

Chinnapongse, R., Gullo, K., Nemeth, P., Zhang, Y., & Griggs, L. (2011). Safety and efficacy of botulinum toxin type B for treatment of sialorrhea in Parkinson's disease: A prospective double-blind trial. *Movement Disorders, 27*(2), 219–226.

Choi, J. W., Youn, C. S., An, H. T., Yoo, J. Y., Na, J. I., Park, K. C., Youn, S. W., & Huh, C. H. (2013a). Combined use of botulinum toxin type A and B for forehead rhytides: A randomized, double-blind, split-face study. *The Journal of Dermatological Treatment, 24*(2), 126–132. PMID: 21801115.

Choi, K. H., Rho, S. H., Lee J. M., Jeon, J.H., Park, S. Y., & Kim, J. (2013b). Botulinum toxin injection of both sides of the face to treat post-paralytic facial synkinesis. *Journal of Plastic Reconstructive and Aesthetic Surgery*. doi:10.1016/j.bjps.2013.04.012.

Chuang, Y. C., & Chancellor, M. B. (2006). The application of botulinum toxin in the prostate. *Journal of Urology, 176*, 2375–2382.

Cole, R., Hallett, M., & Cohen, L. G. (1995). Double-blind trial of botulinum toxin for treatment of focal hand dystonia. *Movement Disorders, 43*, 466–471.

Colosimo, C., Chianese, M., Giovannelli, M., Contarino, M. F., & Bentivoglio, A. R. (2003). Botulinum toxin type B in blepharospasm and hemifacial spasm. *Journal of Neurology, Neurosurgery, and Psychiatry, 74*, 687.

Comella, C. L., Buchman, A. S., Tanner, C. M., Brown-Toms, N. C., & Goetz, C. G. (1992). Botulinum toxin injection for spasmodic torticollis: Increased magnitude of benefit with electromyographic assistance. *Neurology, 42*, 878–882.

Comella, C. L., Jankovic, J., Shannon, K. M., Tsui, J., Swenson, M., Leurgans, S., Fan, W., & Dystonia Study Group. (2005). Comparison of botulinum toxin serotypes A and B for the treatment of cervical dystonia. *Neurology, 65*(9), 1423–1429.

Comella, C. L., Jankovic, J., Truong, D. D., Hanschmann, A., Grafe, S., & U.S. XEOMIN Cervical Dystonia Study Group. (2011). Efficacy and safety of incobotulinumtoxinA (NT 201, XEOMIN®, botulinum neurotoxin type A, without accessory proteins) in patients with cervical dystonia. *Journal of the Neurological Sciences, 308*(1–2), 103–109.

Contarino, M. F., Kruisdijk, J. J., Koster, L., Ongerboer de Visser, B. W., Speelman, J. D., & Koelman, J. H. (2007). Sensory integration in writer's cramp: Comparison with controls and evaluation of botulinum toxin effect. *Clinical Neurophysiology, 118*, 2195–2206.

Cordivari, C., Misra, V. P., Catania, S., & Lees, A. J. (2001). Treatment of dystonic clenched fist with botulinum toxin. *Movement Disorders, 16*, 907–913.

Coutinho dos Santos, L. H., Bufara Rodrigues, D. C., Simões de Assis, T. R., & Bruck, I. (2011). Effective results with botulinum toxin in cerebral palsy. *Pediatric Neurology, 44*(5), 357–363.

Crowner, B. E., Torres-Russotto, D., Carter, A. R., & Racette, B. A. (2010). Systemic weakness after therapeutic injections of botulinum toxin A: A case series and review of the literature. *Clinical Neuropharmacology, 33*, 243–247.

Deffontaines-Rufin, S., Weil, M., Verollet, D., Peyrat, L., & Amarenco, G. (2011). Botulinum toxin A for the treatment of neurogenic detrusor overactivity in multiple sclerosis patients. *International Brazilian Journal of Urology, 37*(5), 642–648.

Delgado, M. R., Hirtz, D., Aisen, M., Ashwal, S., Fehlings, D. L., McLaughlin, J., Morrison, L. A., Shrader, M. W., Tilton, A., & Vargus-Adams, J. (2010). Practice parameter: Pharmacologic treatment of spasticity in children and adolescents with cerebral palsy (an evidence-based review): Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology, 74*(4), 336–343.

Dessy, L. A., Fallico, N., Mazzocchi, M., & Scuderi, N. (2011). Botulinum toxin for glabellar lines: A review of the efficacy and safety of currently available products. *American Journal of Clinical Dermatology, 12*(6), 377–388.

Diamond, A., & Jankovic, J. (2006a). Botulinum toxin in dermatology – beyond wrinkles and sweat. *Journal of Cosmetic Dermatology, 5*, 169.

Diamond, A., & Jankovic, J. (2006b). Treatment of advanced Parkinson's disease. *Expert Review of Neurotherapeutics, 6*, 1181–1197.

Diener, H. C., Dodick, D. W., Aurora, S. K., Turkel, C. C., DeGryse, R. E., Lipton, R. B., Silberstein, S. D., Brin, M. F., & PREEMPT 2 Chronic Migraine Study Group. (2010). Onabotulinumtoxin A for treatment of chronic migraine: Results from the double-blind, randomized, placebo-controlled phase of the PREEMPT 2 trial. *Cephalalgia, 30*, 804–814.

Dodick, D. W., Turkel, C. C., DeGryse, R. E., Aurora, S. K., Silberstein, S. D., Lipton, R. B., Diener, H. C., Brin, M. F., & PREEMPT Chronic Migraine Study Group. (2010). Onabotulinumtoxin A for treatment of chronic migraine: Pooled results from the double-blind, randomized placebo-controlled phases of the PREEMPT clinical program. *Headache, 50*, 921–936.

Doft, M. A., Kasten, J. L., & Ascherman, J. A. (2011). Treatment of axillary hyperhidrosis with botulinum toxin: A single surgeon's experience with 53 consecutive patients. *Aesthetic Plastic Surgery, 35*(6), 1079–1086.

Dogu, O., Apaydin, D., Sevim, S., Talas, D. U., & Aral, M. (2004). Ultrasound guided versus 'blind' intraparotid injections of botulinum toxin-A for the treatment of sialorrhoea in patients with Parkinson's disease. *Clinical Neurology and Neurosurgery, 106*, 93–96.

Dressler, D. (2009). Routine use of Xeomin in patients previously treated with Botox: Long term results. *European Journal of Neurology, 16*(Suppl 2), 2–5.

Dressler, D. (2010a). Botulinum toxin for treatment of dystonia. *European Journal of Neurology, 17*(Suppl 1), 88–96.

Dressler, D. (2010b). Comparing Botox and Xeomin for axillar hyperhidrosis. *Journal of Neural Transmission, 117*(3), 317–319.

Dressler, D. (2012). Five-year experience with incobotulinumtoxinA (Xeomin®): The first botulinum toxin drug free of complexing proteins. *European Journal of Neurology, 19*(3), 385–389.

Dressler, D., & Adib Saberi, F. (2013). Towards a dose optimisation of botulinum toxin therapy for axillary hyperhidrosis: comparison of different Botox® doses. *Journal of Neural Transmission*.

Dressler, D., & Benecke, R. (2007). Pharmacology of therapeutic botulinum toxin preparations. *Disability and Rehabilitation, 29*, 1761–1768.

Dressler, D., & Bigalke, H. (2005). Botulinum toxin type B de novo therapy of cervical dystonia: Frequency of antibody induced therapy failure. *Journal of Neurology, 252*, 904–907.

Dressler, D., Adib Saberi, F., & Benecke, R. (2002). Botulinum toxin type B for treatment of axillar hyperhidrosis. *Journal of Neurology, 249*, 1729–1732.

Dressler, D., Mander, G., & Fink, K. (2011). Measuring the potency labelling of onabotulinumtoxinA (Botox®) and incobotulinumtoxinA (Xeomin ®) in an LD_{50} assay. *Journal of Neural Transmission, 119*(1), 13–15.

Dutton, J. J., White, J. J., & Richard, M. J. (2006). Myobloc for the treatment of benign essential blepharospasm in patients refractory to botox. *Ophthalmic Plastic and Reconstructive Surgery, 22*, 173–177.

Eckardt, A., & Kuettner, C. (2003). Treatment of gustatory sweating (Frey's syndrome) with botulinum toxin A. *Head & Neck, 25*, 624–628.

Eisa, M., Singer, C., Sengun, C., Russel, A., Jabbari, B., & Papapetropoulos, S. (2008). Treatment of painful limbs/moving extremities with botulinum toxin type A injections. *European Neurology, 60*(2), 104–106.

Elia, A. E., Filippini, G., Calandrella, D., & Albanese, A. (2009). Botulinum neurotoxins for post-stroke spasticity in adults: A systematic review. *Movement Disorders, 24*, 801–812.

Elkind, A. H., O'Carroll, P., Blumenfeld, A., DeGryse, R., Dimitrova, R., & BoNTA-024-026-036 Study Group. (2006). A series of three sequential, randomized, controlled studies of repeated treatments with botulinum toxin type A for migraine prophylaxis. *Journal of Pain, 7*, 688–696.

Elston, J. S. (1987). Long-term results of treatment of idiopathic blepharospasm with botulinum toxin injections. *The British Journal of Ophthalmology, 71*, 664–668.

Erbguth, F., Claus, D., Engelhardt, A., & Dressler, D. (1993). Systemic effect of local botulinum toxin injections unmasks subclinical Lambert-Eaton myasthenic syndrome. *Journal of Neurology, Neurosurgery, and Psychiatry, 56*, 1235–1236.

Evidente, V. G., & Adler, C. H. (2010). An update on the neurologic applications of botulinum toxins. *Current Neurology and Neuroscience Reports, 10*(5), 338–344.

Evidente, V. G., Fernandez, H. H., LeDoux, M. S., et al. (2013). Efficacy and safety of repeated incobotulinumtoxinA (Xeomin) injections in subjects with cervical dystonia: a randomized, double-blind extension study. *The Journal of Neural Transmission*.

Factor, S. A., Molho, E. S., Evans, S., & Feustel, P. J. (2005). Efficacy and safety of repeated doses of botulinum toxin type B in type A resistant and responsive cervical dystonia. *Movement Disorders, 20*, 1152–1160.

Fernandez, H. H., Pagan, F., Danisi, F., Greeley, D., Jankovic, J., Verma, A., Sethi, K., & Pappert, E.J., XCiDaBLE study group. (2013a). Prospective study evaluating incobotulinumtoxinA for

cervical dystonia or blepharospasm: interim results from the first 145 subjects with cervical dystonia. Tremor Other Hyperkinet Mov (N Y).

Fernandez, H. H., Pappert, E. J., Comella, C. L., Evidente, V. G., Truong, D. D., Verma, A., & Jankovic, J. (2013b). Efficacy and safety of incobotulinumtoxinA in subjects previously treated with botulinum toxin versus toxin-naïve subjects with cervical dystonia. Tremor Other Hyperkinet Mov (N Y).

Frasson, E., Brigo, F., Acler, M., Didonè, G., Vicentini, S., & Bertolasi, L. (2011). Botulinum toxin type A vs type B for axillary hyperhidrosis in a case series of patients observed for 6 months. *Archives of Dermatology, 147*(1), 122–123.

Freitag, F. G., Diamond, S., Diamond, M., & Urban, G. (2008). Botulinum toxin type A in the treatment of chronic migraine without medication overuse. *Headache, 48*, 201–209.

Frevert, J. (2009a). Xeomin: An innovative new botulinum toxin type A. *European Journal of Neurology, 16*(Suppl 2), 11–13.

Frevert, J. (2009b). Xeomin is free from complexing proteins. *Toxicon, 54*(5), 697–701.

Frevert, J. (2010). Content of botulinum neurotoxin in Botox®/Vistabel®, Dysport®/Azzalure®, and Xeomin®/Bocouture®. *Drugs in R&D, 10*(2), 67–73.

Frick, C. G., Fink, H., Blobner, M., & Martyn, J. (2012). A single injection of botulinum toxin decreases the margin of safety of neurotransmission at local and distant sites. *Anesthesia and Analgesia, 114*(1), 102–119.

Frueh, B. R., Felt, D. P., Wojno, T. H., & Musch, D. C. (1984). Treatment of blepharospasm with botulinum toxin. A preliminary report. *Archives of Ophthalmology, 102*(10), 1464–1468.

Gazerani, P., Pedersen, N. S., Staahl, C., Drewes, A. M., & Arendt-Nielsen, L. (2009). Subcutaneous botulinum toxin type A reduces capsaicin-induced trigeminal pain and vasomotor reactions in human skin. *Pain, 141*, 60–69.

Geenen, C., Consky, E., & Ashby, P. (1996). Localizing muscles for botulinum toxin treatment of focal hand dystonia. *The Canadian Journal of Neurological Sciences, 23*(3), 194–197.

Gelb, D. J., Lowenstein, D. H., & Aminoff, M. J. (1989). Controlled trial of botulinum toxin injections in the treatment of spasmodic torticollis. *Neurology, 39*, 80–84.

Gil Polo, C., Rodríguez Sanz, M. F., Berrocal Izquierdo, N., Castrillo Sanz, A., Gutiérrez Ríos, R., Zamora García, M. I., Mendoza Rodríguez, A., & Duarte García-Luis, J. (2013). Blepharospasm and hemifacial spasm: Long-term treatment with botulinum toxin. *Neurologia, 28*(3), 131–136.

Giladi, N. (1997). The mechanism of action of botulinum toxin type A in focal dystonia is most probably through its dual effect on efferent (motor) and afferent pathways at the injected site. *Journal of the Neurological Sciences, 152*(2), 132–135.

Giladi, N., Meer, J., & Honigman, S. (1994). The use of botulinum toxin to treat "striatal" toes. *Journal of Neurology, Neurosurgery, and Psychiatry, 57*, 659.

Glogau, R. G. (2002). Review of the use of botulinum toxin for hyperhidrosis and cosmetic purposes. *The Clinical Journal of Pain, 18*, S191–S197.

Gordon, M. F., Brashear, A., Elovic, E., Kassicieh, D., Marciniak, C., Liu, J., Turkel, C., & BOTOX Poststroke Spasticity Study Group. (2004). Repeated dosing of botulinum toxin type A for upper limb spasticity following stroke. *Neurology, 63*, 1971–1973.

Grafe, S., Hanschmann, A. (2010). Safety and efficacy of repeated NT 201 (botulinum neurotoxin type A free from complexing proteins) injections of patients with cervical dystonia: a first longterm safety analysis. [P01.270] American Academy of Neurology, Toronto.

Grafe, S., Comella, C., Jankovic, J. (2009a). Efficacy and safety of NT 201, botulinum neurotoxin type A free from complexing proteins, in pre-treated cervical dystonia patients. (Abstract Tu-400).

Grafe, S., Comella, C., Jankovic, J., Truong, D., Hanschmann, A. (2009b). Efficacy and safety of NT 201: Botulinum neurotoxin type A free from complexing proteins, in treatment-naive cervical dystonia patients. (Abstract Tu-401). International Congress of Parkinson's Disease and Movement Disorders, Paris. *Movement Disorders*, S3–S92.

Grafe, S., Comella, C., Sassin, I. (2009c). Clinical efficacy in focal dystonia and overall tolerability of NT 201 (botulinum neurotoxin free from complexing proteins) (Abstract PO7-081). American Academy of Neurology, Seattle, Washington DC, 4. *Neurology*, A34.

Grafe, S., Hanschmann, A., Jankovic, J., Comella, C. (2010). Safety and efficacy of repeated NT 201 (Botulinum neurotoxin type A free from complexing proteins) injections of patients with blepharospasm. In 14th international congress of Parkinson's disease and movement disorders, 13–17 June 2010, Buenos Aires.

Grazzi, L. (2013). Onabotulinum toxin A for treatment of chronic migraine with medication overuse. *Neurological Sciences, 34*(Suppl 1), 27–28.

Greene, P., Kang, U., Fahn, S., Brin, M., Moskowitz, C., & Flaster, E. (1990). Double-blind, placebo controlled trial of botulinum toxin injections for the treatment of spasmodic torticollis. *Neurology, 40*, 1213–1218.

Guidubaldi, A., Fasano, A., Ialongo, T., Piano, C., Pompili, M., Mascianà, R., Siciliani, L., Sabatelli, M., & Bentivoglio, A. R. (2011). Botulinum toxin A versus B in sialorrhea: A prospective, randomized, double-blind, crossover pilot study in patients with amyotrophic lateral sclerosis or Parkinson's disease. *Movement Disorders, 26*, 313–319.

Hallett, M. (2000). How does botulinum toxin work? *Annals of Neurology, 48*(1), 7–8.

Hallett, M., Albanese, A., Dressler, D., Segal, K. R., Simpson, D. M., Truong, D., & Jankovic, J. (2013). Evidence-based review and assessment of botulinum neurotoxin for the treatment of movement disorders. *Toxicon, 67*, 94–114. doi:10.1016/j.toxicon.2012.12.004.

Hanna, P. A., & Jankovic, J. (1998). Mouse bioassay versus Western blot assay for botulinum toxin antibodies: Correlation with clinical response. *Neurology, 50*(6), 1624–1629.

Hanna, P. A., Jankovic, J., & Vincent, A. (1999). Comparison of mouse bioassay and immunoprecipitation assay for botulinum toxin antibodies. *Journal of Neurology, Neurosurgery, and Psychiatry, 66*(5), 612–616.

Heckmann, M., Ceballos-Baumann, A. O., Plewig, G., & Hyperhidrosis Study Group. (2001). Botulinum toxin A for axillary hyperhidrosis (excessive sweating). *The New England Journal of Medicine, 344*, 488–493.

Hirota, N., Hirota, M., & Mezaki, T. (2008). Dystonic frowning without blepharospasm. *Parkinsonism and Related Disorders, 14*(7), 579–580.

Hompes, R., Harmston, C., Wijffels, N., Jones, O. M., Cunningham, C., & Lindsey, I. (2012). Excellent response rate of anismus to botulinum toxin if rectal prolapse misdiagnosed as anismus (pseudoanismus) is excluded. *Colorectal Disease, 14*(2), 224–230.

Inoue, K., & Rogers, J. D. (2007). Botulinum toxin injection into Riolan's muscle: Somatosensory 'trick'. *European Neurology, 58*, 138–141.

Ito, K., Yanagishita, T., Ohshima, Y., Tamada, Y., & Watanabe, D. (2011). Therapeutic effectiveness of botulinum toxin type A based on severity of palmar hyperhidrosis. *The Journal of Dermatology, 38*(9), 859–863.

Jackson, C. E., Gronseth, G., Rosenfeld, J., Barohn, R. J., Dubinsky, R., Simpson, C. B., McVey, A., Kittrell, P. P., King, R., Herbelin, L., & Muscle Study Group. (2009). Randomized double blind study of botulinum toxin type B for sialorrhea in ALS patients. *Muscle & Nerve, 39*, 137–143.

Jakubowski, M., McAllister, P. J., Bajwa, Z. H., Ward, T. N., Smith, P., & Burstein, R. (2006). Exploding vs imploding headache in migraine prophylaxis with botulinum toxin A. *Pain, 125*, 286–295.

Jankovic, J. (2001). Needle EMG guidance for injection of botulinum toxin. Needle EMG guidance is rarely required. *Muscle & Nerve, 24*(11), 1568–1570.

Jankovic, J. (2004). Botulinum toxin in clinical practice. *Journal of Neurology, Neurosurgery, and Psychiatry, 75*, 951–957.

Jankovic, J. (2009a). Clinical efficacy and tolerability of Xeomin in the treatment of blepharospasm. *European Journal of Neurology, 16*(Suppl 2), 14–18.

Jankovic, J. (2009b). Disease-oriented approach to botulinum toxin use. *Toxicon, 54*(5), 614–623.

Jankovic, J. (2010a). Botulinum toxin. In K. Kompoliti & L. Verhagen Metman (Eds.), *Encyclopedia of movement disorders* (p. 1). Oxford: Academic.

Jankovic, J. (2010b). Camptocormia, head drop and other bent spine syndromes: Heterogeneous etiology and pathogenesis of Parkinsonian deformities. *Movement Disorders, 25*(5), 527–528.

Jankovic, J., & Orman, J. (1987). Botulinum A toxin for cranial-cervical dystonia: A double-blind, placebo-controlled study. *Neurology, 37*(4), 616–623.

Jankovic, J., & Orman, J. (1988). Tetrabenazine therapy of dystonia, chorea, tics, and other dyskinesias. *Neurology, 38*, 391–394.

Jankovic, J., & Schwartz, K. (1990). Botulinum toxin injections for cervical dystonia. *Neurology, 40*, 277–280.

Jankovic, J., & Schwartz, K. (1991). Botulinum toxin treatment of tremors. *Neurology, 41*, 1185–1188.

Jankovic, J., & Schwartz, K. (1995). Response and immunoresistance to botulinum toxin injections. *Neurology, 45*, 1743–1746.

Jankovic, J., Schwartz, K., & Donovan, D. T. (1990). Botulinum toxin treatment of cranial-cervical dystonia, spasmodic dysphonia, other focal dystonias and hemifacial spasm. *Journal of Neurology, Neurosurgery, and Psychiatry, 53*, 633–639.

Jankovic, J., Schwartz, K., Clemence, W., Aswad, A., & Mordaunt, J. (1996). A randomized double-blind, placebo controlled study to evaluate botulinum toxin A in essential hand tremor. *Movement Disorders, 11*, 250–256.

Jankovic, J., Vuong, K. D., & Ahsan, J. (2003). Comparison of efficacy and immunogenicity of original versus current botulinum toxin in cervical dystonia. *Neurology, 60*, 1186–1188.

Jankovic, J., Hunter, C., Dolimbek, B. Z., Dolimbek, G. S., Adler, C. H., Brashear, A., Comella, C. L., Gordon, M., Riley, D. E., Sethi, K., Singer, C., Stacy, M., Tarsy, D., & Atassi, M. Z. (2006). Clinico-immunologic aspects of botulinum toxin type B treatment of cervical dystonia. *Neurology, 67*(12), 2233–2235.

Jankovic, J., Adler, C. H., Charles, P. D., Comella, C., Stacy, M., Schwartz, M., Sutch, S. M., Brin, M. F., & Papapetropoulos, S. (2011a). Rationale and design of a prospective study: Cervical Dystonia Patient Registry for Observation of OnaBotulinumtoxinA Efficacy (CD PROBE). *BMC Neurology, 11*, 140.

Jankovic, J., Comella, C., Hanschmann, A., & Grafe, S. (2011b). Efficacy and safety of incobotulinumtoxinA (NT 201, Xeomin) in the treatment of blepharospasm-a randomized trial. *Movement Disorders, 26*(8), 1521–1528.

Jost, W. H., & Kohl, A. (2001). Botulinum toxin: Evidence-based medicine criteria in blepharospasm and hemifacial spasm. *Journal of Neurology, 248*(Suppl 1), 21–24.

Jost, W. H., Blümel, J., & Grafe, S. (2007). Botulinum neurotoxin type A free of complexing proteins (XEOMIN) in focal dystonia. *Drugs, 67*(5), 669–683.

Kaji, R., Osako, Y., Suyama, K., Maeda, T., Uechi, Y., Iwasaki, M., & GSK1358820 Spasticity Study Group. (2010). Botulinum toxin type A in post-stroke lower limb spasticity: A multicenter, double-blind, placebo-controlled trial. *Journal of Neurology, 257*(8), 1330–1337.

Kaňovský, P., Slawek, J., Denes, Z., Platz, T., Comes, G., Grafe, S., & Pulte, I. (2011). Efficacy and safety of treatment with incobotulinum toxin A (botulinum neurotoxin type A free from complexing proteins; NT 201) in post-stroke upper limb spasticity. *Journal of Rehabilitation Medicine, 43*(6), 486–492.

Keam, S. J., Muir, V. J., & Deeks, E. D. (2011). Botulinum toxin A (Dysport®): In dystonias and focal spasticity. *Drugs, 71*(8), 1043–1058.

Kenney, C., & Jankovic, J. (2008). Botulinum toxin in the treatment of blepharospasm and hemifacial spasm. *Journal of Neural Transmission, 115*, 585–591.

Klawans, H. L., & Tanner, C. M. (1988). Cholinergic pharmacology of blepharospasm with oromandibular dystonia (Meige's syndrome). *Advances in Neurology, 49*, 443–450.

Klein, A. W. (2001). Complications and adverse reactions with the use of botulinum toxin. *Seminars in Cutaneous Medicine and Surgery, 20*, 109–120.

Kruisdijk, J. J., Koelman, J. H., Ongerboer de Visser, B. W., de Haan, R. J., & Speelman, J. D. (2007). Botulinum toxin for writer's cramp: A randomized, placebo controlled trial and 1-year follow-up. *Journal of Neurology, Neurosurgery, and Psychiatry, 78*, 264–270.

Laccourreye, O., Muscatello, L., Gutierrez-Fonseca, R., Seckin, S., Brasnu, D., & Bonan, B. (1999). Severe Frey syndrome after parotidectomy: Treatment with botulinum neurotoxin type A. *Ann Otolaryngol Chir Cervicofac, 116*, 137–142.

Lagalla, G., Millevolte, M., Capecci, M., Provinciali, L., & Ceravolo, M. G. (2006). Botulinum toxin type A for drooling in Parkinson's disease: A double blind, randomized, placebo-controlled study. *Movement Disorders, 21*, 704–707.

Lakhtakia, S., Monga, A., Gupta, R., Kalpala, R., Pratap, N., Wee, E., Arjunan, S., & Reddy, D. N. (2011). Achalasia cardia with esophageal varix managed with endoscopic ultrasound-guided botulinum toxin injection. *Indian Journal of Gastroenterology, 30*(6), 277–279.

Langevin, P., Lowcock, J., Weber, J., Nolan, M., Gross, A. R., Peloso, P. M., Roberts, J., Graham, N., Goldsmith, C. H., Burnie, S. J., Haines, T., & Cervical Overview Group. (2011a). Botulinum toxin intramuscular injections for neck pain: A systematic review and metaanalysis. *The Journal of Rheumatology, 38*(2), 203–214. Review.

Langevin, P., Peloso, P. M., Lowcock, J., Nolan, M., Weber, J., Gross, A., Roberts, J., Goldsmith, C. H., Graham, N., Burnie, S. J., & Haines, T. (2011b). Botulinum toxin intramuscular injections for neck pain. *Cochrane Database of Systematic Reviews, 6*(7), CD008626. Review.

Lannin, N., Scheinberg, A., & Clark, K. (2006). AACPDM systematic review of the effectiveness of therapy for children with cerebral palsy after botulinum toxin A injections. *Developmental Medicine and Child Neurology, 48*, 533–539.

Laskawi, R., & Rohrbach, S. (2001). Oromandibular dystonia. Clinical forms, diagnosis and examples of therapy with botulinum toxin. *Laryngo- Rhino-Otologie, 80*, 708–713.

Lew, M. F., Adornato, B. T., Duane, D. D., Dykstra, D. D., Factor, S. A., Massey, J. M., Brin, M. F., Jankovic, J., Rodnitzky, R. L., Singer, C., Swenson, M. R., Tarsy, D., Murray, J. J., Koller, M., & Wallace, J. D. (1997). Botulinum toxin type B: A double-blind, placebo-controlled, safety and efficacy study in cervical dystonia. *Neurology, 49*, 701–707.

Lippert-Gruner, M., & Svestkova, O. (2011). Early use of Xeomin neurotoxin for local anti-spasticity therapy for pes equines after acquired brain injury (ABI). *Brain Injury, 25*(12), 1266–1269.

Lowe, N. J., Yamauchi, P. S., Lask, G. P., Patnaik, R., & Iyer, S. (2002). Efficacy and safety of botulinum toxin type a in the treatment of palmar hyperhidrosis: A double-blind, randomized, placebo-controlled study. *Dermatologic Surgery, 28*, 822–827.

Lu, D. W., & Lippitz, J. (2009). Complications of botulinum neurotoxin. *Disease-a-Month, 55*, 198–211.

Lungu, C., Karp, B. I., Alter, K., Zolbrod, R., & Hallett, M. (2011). Long-term follow-up of botulinum toxin therapy for focal hand dystonia: Outcome at 10 years or more. *Movement Disorders, 26*(4), 750–753.

Mall, V., Heinen, F., Siebel, A., Bertram, C., Hafkemeyer, U., Wissel, J., Berweck, S., Haverkamp, F., Nass, G., Döderlein, L., Breitbach-Faller, N., Schulte-Mattler, W., & Korinthenberg, R. (2006). Treatment of adductor spasticity with BTX-A in children with CP: A randomized, double-blind, placebo-controlled study. *Developmental Medicine and Child Neurology, 48*, 10–13.

Marchal, C., Perez, J. E., Herrera, B., Machuca, F. J., & Redondo, M. (2011). The use of botulinum toxin in benign prostatic hyperplasia. *Neurourology and Urodynamics, 31*(1), 86–92.

Marchetti, A., Magar, R., Findley, L., Larsen, J. P., Pirtosek, Z., Růzicka, E., Jech, R., Sławek, J., & Ahmed, F. (2005). Retrospective evaluation of the dose of Dysport and BOTOX in the management of cervical dystonia and blepharospasm: The REAL DOSE study. *Movement*

Disorders, 20(8), 937–944. Erratum in: Mov Disord. 2005 Aug; 20(8): 1089. Råuzizka, Evzen [corrected to Růzicka, Evzen].

Maria, G., Brisinda, G., Bentivoglio, A. R., Cassetta, E., Gui, D., & Albanese, A. (2000). Influence of botulinum toxin site of injections on healing rate in patients with chronic anal fissure. *The American Journal of Surgery, 179*(1), 46–50.

Marion, M. H., Afors, K., & Sheehy, M. P. (2003). Problems of treating writer's cramp with botulinum toxin injections: Results from 10 years of experience. *Review of Neurology (Paris), 159*(10 Pt 1), 923–927.

Marras, C., Andrews, D., Sime, E., & Lang, A. E. (2001). Botulinum toxin for simple motor tics: A randomized, double-blind, controlled clinical trial. *Neurology, 56*, 605–610.

Mathers, S. E., Kempster, P. A., Swash, M., & Lees, A. J. (1988). Constipation and paradoxical puborectalis contraction in anismus and Parkinson's disease: A dystonic phenomenon? *Journal of Neurology, Neurosurgery, and Psychiatry, 51*, 1503–1507.

Mathew, N. T., Frishberg, B. M., Gawel, M., Dimitrova, R., Gibson, J., Turkel, C., & BOTOX CDH Study Group. (2005). Botulinum toxin type A (BOTOX) for the prophylactic treatment of chronic daily headache: A randomized, double-blind, placebo-controlled trial. *Headache, 45*, 293–307.

Mejia, N. I., Vuong, K. D., & Jankovic, J. (2005). Long-term botulinum toxin efficacy, safety, and immunogenicity. *Movement Disorders, 20*, 592–597.

Mohee, A., Khan, A., Harris, N., & Eardley, I. (2013). Long-term outcome of the use of intravesical botulinum toxin for the treatment of overactive bladder (OAB). *BJU International, 111*(1), 106–113.

Møller, E., Karlsborg, M., Bardow, A., Lykkeaa, J., Nissen, F. H., & Bakke, M. (2011). Treatment of severe drooling with botulinum toxin in amyotrophic lateral sclerosis and Parkinson's disease: Efficacy and possible mechanisms. *Acta Odontologica Scandinavica, 69*(3), 151–157.

Molloy, L. (2007). Treatment of sialorrhoea in patients with Parkinson's disease: Best current evidence. *Current Opinion in Neurology, 20*, 493–498.

Molloy, F. M., Shill, H. A., Kaelin-Lang, A., & Karp, B. I. (2002). Accuracy of muscle localization without EMG: Implications for treatment of limb dystonia. *Neurology, 58*(5), 805–807.

Naumann, M., & Jankovic, J. (2004). Safety of botulinum toxin type A: A systematic review and metaanalysis. *Current Medical Research and Opinion, 20*, 981–990.

Naumann, M., & Lowe, N. J. (2001). Botulinum toxin type A in treatment of bilateral primary axillary hyperhidrosis: Randomised, parallel group, double blind, placebo controlled trial. *British Medical Journal, 323*, 596–599.

Naumann, M., Zellner, M., Toyka, K. V., & Reiners, K. (1997). Treatment of gustatory sweating with botulinum toxin. *Annals of Neurology, 42*, 973–975.

Naumann, M., Albanese, A., Heinen, F., Molenaers, G., & Relja, M. (2006). Safety and efficacy of botulinum toxin type A following long-term use. *European Journal of Neurology, 13*(Suppl 4), 35–40.

Naumann, M., So, Y., Argoff, C. E., Childers, M. K., Dykstra, D. D., Gronseth, G. S., Jabbari, B., Kaufmann, H. C., Schurch, B., Silberstein, S. D., Simpson, D. M., & Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. (2008). Assessment: Botulinum neurotoxin in the treatment of autonomic disorders and pain (an evidence-based review): Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology, 70*, 1707–1714.

Naumann, M., Dressler, D., Hallett, M., Jankovic, J., Schiavo, G., Segal, K. R., & Truong, D. (2013). Evidence-based review and assessment of botulinum neurotoxin for the treatment of secretory disorders. *Toxicon, 67*, 141–152.

Nóbrega, A. C., Rodrigues, B., Torres, A. C., Enzo, A., & Melo, A. (2007). Does botulinum toxin decrease frequency and severity of sialorrhea in Parkinson's disease? *Journal of the Neurological Sciences, 253*, 85–87.

Nóbrega, A. C., Rodrigues, B., & Melo, A. (2009). Does botulinum toxin injection in parotid glands interfere with the swallowing dynamics of Parkinson's disease patients? *Clinical Neurology and Neurosurgery, 111*, 430–432.

Nolte, D., Gollmitzer, I., Loeffelbein, D. J., Hölzle, F., & Wolff, K. D. (2004). Botulinum toxin for treatment of gustatory sweating. A prospective randomized study. *Mund-, Kiefer- und Gesichtschirurgie, 8*, 369–375.

Nüssgens, Z., & Roggenkämper, P. (1997). Comparison of two botulinum-toxin preparations in the treatment of essential blepharospasm. *Graefe's Archive for Clinical and Experimental Ophthalmology, 235*, 197–199.

Odergren, T., Hjaltason, H., Kaakkola, S., Solders, G., Hanko, J., Fehling, C., Marttila, R. J., Lundh, H., Gedin, S., Westergren, I., Richardson, A., Dott, C., & Cohen, H. (1998). A double blind, randomised, parallel group study to investigate the dose equivalence of Dysport and Botox in the treatment of cervical dystonia. *Journal of Neurology, Neurosurgery, and Psychiatry, 64*, 6–12.

Olesen, J., Bousser, M. G., Diener, H. C., Dodick, D., First, M., Goadsby, P. J., Göbel, H., Lainez, M. J., Lance, J. W., Lipton, R. B., Nappi, G., Sakai, F., Schoenen, J., Silberstein, S. D., Steiner, T. J., & Headache Classification Committee. (2006). New appendix criteria open for a broader concept of chronic migraine. *Cephalalgia, 26*, 742–746.

Ondo, W. G., Hunter, C., & Moore, W. (2004). A double-blind placebo controlled trial of botulinum toxin B for sialorrhea in Parkinson's disease. *Neurology, 62*, 37–40.

Pacchetti, C., Albani, G., Martignoni, E., Godi, L., Alfonsi, E., & Nappi, G. (1995). "Off" painful dystonia in Parkinson's disease treated with botulinum toxin. *Movement Disorders, 10*, 333–336.

Pahwa, R., Busenbark, K., Swanson-Hyland, E. F., Dubinsky, R. M., Hubble, J. P., Gray, C., & Koller, W. C. (1995). Botulinum toxin treatment of essential head tremor. *Neurology, 45*, 822–824.

Pappert, E. J., Germanson, T., & Myobloc/Neurobloc European Cervical Dystonia Study Group. (2008). Botulinum toxin type B vs. type A in toxin-naive patients with cervical dystonia: Randomized, double-blind, noninferiority trial. *Movement Disorders, 23*, 510–517.

Park, E. S., & Rha, D. W. (2006). Botulinum toxin type A injection for management of upper limb spasticity in children with cerebral palsy: A literature review. *Yonsei Medical Journal, 47*, 589–603.

Patel, A. K., Patterson, J. M., & Chapple, C. R. (2006). Botulinum toxin injections for neurogenic and idiopathic detrusor overactivity: A critical analysis of results. *European Urology, 50*, 684–709.

Pathak, M. S., Nguyen, H. T., Graham, H. K., & Moore, A. P. (2006). Management of spasticity in adults: Practical application of botulinum toxin. *European Journal of Neurology, 13*(Suppl 1), 42–50.

Poewe, W., Deuschl, G., Nebe, A., Feifel, E., Wissel, J., Benecke, R., Kessler, K. R., Ceballos-Baumann, A. O., Ohly, A., Oertel, W., & Künig, G. (1998). What is the optimal dose of botulinum toxin A in the treatment of cervical dystonia? Results of a double blind, placebo controlled, dose ranging study using Dysport. German Dystonia Study Group. *Journal of Neurology, Neurosurgery, and Psychiatry, 64*, 13–17.

Pohl, D., & Tutuian, R. (2007). Achalasia: An overview of diagnosis and treatment. *Journal of Gastrointestinal and Liver Diseases, 16*, 297–303.

Prager, W., Wissmüller, E., Kollhorst, B., Böer, A., & Zschocke, I. (2011). Treatment of crow's feet with two different botulinum toxin type A preparations in split-face technique. *Hautarzt, 62*(5), 375–379.

Quagliato, E. M., Carelli, E. F., & Viana, M. A. (2010a). Prospective, randomized, double-blind study, comparing botulinum toxins type a botox and prosigne for blepharospasm and hemifacial spasm treatment. *Clinical Neuropharmacology, 33*(1), 27–31.

Quagliato, E. M., Carelli, E. F., & Viana, M. A. (2010b). A prospective, randomized, double-blind study comparing the efficacy and safety of type a botulinum toxins botox and prosigne in the treatment of cervical dystonia. *Clinical Neuropharmacology, 33*(1), 22–26.

Ramirez-Castaneda, J., & Jankovic, J. (2013). Long-term efficacy and safety of botulinum toxin injections in dystonia. *Toxins (Basel), 5*(2), 249–266.

Ramirez-Castaneda, J., Jankovic, J., Comella, C., Dashtipour, K., Fernandez, H. H., & Mari, Z. (2013). Diffusion, spread, dilution, and migration of botulinum toxin. *Movement Disorders, 28*(13), 1775–1783.

Ranoux, D., Gury, C., Fondarai, J., Mas, J. L., & Zuber, M. (2002). Respective potencies of Botox and Dysport: A double blind, randomised, crossover study in cervical dystonia. *Journal of Neurology, Neurosurgery, and Psychiatry, 72*(4), 459–462.

Rath, J. J., Tavy, D. L., Wertenbroek, A. A., van Woerkom, T. C., & de Bruijn, S. F. (2010). Botulinum toxin type A in simple motor tics: Short-term and long-term treatment effects. *Parkinsonism and Related Disorders, 16*(7), 478–481.

Reilich, P., Fheodoroff, K., Kern, U., Mense, S., Seddigh, S., Wissel, J., & Pongratz, D. (2004). Consensus statement: Botulinum toxin in myofascial pain. *Journal of Neurology, 251* (Suppl 1), I36–I38.

Relja, M., Poole, A. C., Schoenen, J., Pascual, J., Lei, X., Thompson, C., & European BoNTA Headache Study Group. (2007). A multicentre, double-blind, randomized, placebo-controlled, parallel group study of multiple treatments of botulinum toxin type A (BoNTA) for the prophylaxis of episodic migraine headaches. *Cephalalgia, 27*, 492–503.

Roggenkämper, P., Jost, W. H., Bihari, K., Comes, G., Grafe, S., & for the NT 201 Blepharospasm Study Team. (2006). Efficacy and safety of a new botulinum toxin type A free of complexing proteins in the treatment of blepharospasm. *Journal of Neural Transmission, 113*, 303–312.

Rosales, R. L., & Dressler, D. (2010). On muscle spindles, dystonia and botulinum toxin. *European Journal of Neurology, 17*(Suppl 1), 71–80.

Sadick, N. (2003). Botulinum toxin type B. *Dermatologic Surgery, 29*, 348–350.

Sampaio, C., Ferreira, J. J., Simões, F., Rosas, M. J., Magalhães, M., Correia, A. P., Bastos-Lima, A., Martins, R., & Castro-Caldas, A. (1997). DYSBOT: A single-blind, randomized parallel study to determine whether any differences can be detected in the efficacy and tolerability of two formulations of botulinum toxin type A – Dysport and Botox – assuming a ratio of 4,1. *Movement Disorders, 12*, 1013–1018.

Sankhla, C., Lai, E. C., & Jankovic, J. (1998). Peripherally induced oromandibular dystonia. *Journal of Neurology, Neurosurgery, and Psychiatry, 65*, 722–728.

Santamato, A., Ianieri, G., Ranieri, M., Megna, M., Panza, F., Fiore, P., & Megna, G. (2008). Botulinum toxin type A in the treatment of sialorrhea in Parkinson's disease. *Journal of the American Geriatrics Society, 56*, 765–767.

Saper, J. R., Mathew, N. T., Loder, E. W., DeGryse, R., VanDenburgh, A. M., & BoNTA-009 Study Group. (2007). A double-blind, randomized, placebo-controlled comparison of botulinum toxin type A injection sites and doses in the prevention of episodic migraine. *Pain Medicine, 8*, 478–485.

Schneider, S. A., Edwards, M. J., Cordivari, C., Macleod, W. N., & Bhatia, K. P. (2006). Botulinum toxin A may be efficacious as treatment for jaw tremor in Parkinson's disease. *Movement Disorders, 21*, 1722–1724.

Schuele, S., Jabusch, H. C., Lederman, R. J., & Altenmüller, E. (2005). Botulinum toxin injections in the treatment of musician's dystonia. *Neurology, 64*(2), 341–343.

Silberstein, S., Mathew, N., Saper, J., & Jenkins, S. (2000). Botulinum toxin type A as a migraine preventive treatment. For the BOTOX® Migraine Clinical Research Group. *Headache, 40*, 445–450.

Simonetta Moreau, M., Cauhepe, C., Magues, J. P., & Senard, J. M. (2003). A double-blind, randomized, comparative study of Dysport vs. Botox in primary palmar hyperhidrosis. *The British Journal of Dermatology, 149*, 1041–1045.

Simpson, D. M., Blitzer, A., Brashear, A., Comella, C., Dubinsky, R., Hallett, M., Jankovic, J., Karp, B., Ludlow, C. L., Miyasaki, J. M., Naumann, M., So, Y., & Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. (2008a). Assessment:

Botulinum neurotoxin for the treatment of movement disorders (an evidence-based review): Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology, 70*, 1699–1706.

Simpson, D. M., Gracies, J. M., Graham, H. K., Miyasaki, J. M., Naumann, M., Russman, B., Simpson, L. L., So, Y., & Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. (2008b). Assessment: Botulinum neurotoxin for the treatment of spasticity (an evidencebased review): Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology, 70*, 1691–1698.

Simpson, D. M., Gracies, J. M., Yablon, S. A., Barbano, R., Brashear, A., & BoNT/TZD Study Team. (2009). Botulinum neurotoxin versus tizanidine in upper limb spasticity: A placebo-controlled study. *Journal of Neurology, Neurosurgery, and Psychiatry, 80*, 380–385.

Singer, C., & Papapetropoulos, S. (2007). A case of painless arms/moving fingers responsive to botulinum toxin A injections. *Parkinsonism and Related Disorders, 13*, 55–56.

Singer, B. J., Silbert, P. L., Song, S., Dunne, J. W., & Singer, K. P. (2011). Treatment of refractory anterior knee pain using botulinum toxin type A (Dysport) injection to the distal vastus lateralis muscle: A randomised placebo controlled crossover trial. *British Journal of Sports Medicine, 45*(8), 640–645.

Snow, B. J., Tsui, J. K., Bhatt, M. H., Varelas, M., Hashimoto, S. A., & Calne, D. B. (1990). Treatment of spasticity with botulinum toxin: A double-blind study. *Annals of Neurology, 28*, 512–515.

Solish, N., Bertucci, V., Dansereau, A., Hong, H. C., Lynde, C., Lupin, M., Smith, K. C., Storwick, G., & Canadian Hyperhidrosis Advisory Committee. (2007). A comprehensive approach to the recognition, diagnosis, and severity-based treatment of focal hyperhidrosis: Recommendations of the Canadian Hyperhidrosis Advisory Committee. *Dermatologic Surgery, 33*, 908–923.

Sycha, T., Kranz, G., Auff, E., & Schnider, P. (2004). Botulinum toxin in the treatment of rare head and neck pain syndromes: A systematic review of the literature. *Journal of Neurology, 251* (Suppl 1), 119–130.

Tan, J. C. (2005). *Practical manual of physical medicine and rehabilitation* (p. 507). Philadelphia: Elsevier/Mosby.

Tan, E. K., & Jankovic, J. (2000). Treating severe bruxism with botulinum toxin. *The Journal of the American Dental Association, 131*, 211–216.

Tintner, R., Gross, R., Winzer, U. F., Smalky, K. A., & Jankovic, J. (2005). Autonomic function after botulinum toxin type A or B: A double-blind, randomized trial. *Neurology, 65*, 765–767.

Trosch, R. M., Adler, C. H., & Pappert, E. J. (2007). Botulinum toxin type B (Myobloc) in subjects with hemifacial spasm: Results from an open-label, dose-escalation safety study. *Movement Disorders, 22*, 1258–1264.

Troung, D. D., Rontal, M., Rolnick, M., Aronson, A. E., & Mistura, K. (1991). Double-blind controlled study of botulinum toxin in adductor spasmodic dysphonia. *Laryngoscope, 101*, 630–634.

Truong, D. (2012). Botulinum toxins in the treatment of primary focal dystonias. *Journal of the Neurological Sciences, 316*(1–2), 9–14.

Truong, D. D., & Jost, W. H. (2006). Botulinum toxin: Clinical use. *Parkinsonism and Related Disorders, 12*, 331–355.

Truong, D., Duane, D. D., Jankovic, J., Singer, C., Seeberger, L. C., Comella, C. L., Lew, M. F., Rodnitzky, R. L., Danisi, F. O., Sutton, J. P., Charles, P. D., Hauser, R. A., & Sheean, G. L. (2005). Efficacy and safety of botulinum type A toxin (Dysport) in cervical dystonia: Results of the first US randomized, double-blind, placebo-controlled study. *Movement Disorders, 20*(7), 783–791.

Truong, D., Comella, C., Fernandez, H. H., Ondo, W. G., & Dysport Benign Essential Blepharospasm Study Group. (2008a). Efficacy and safety of purified botulinum toxin type A (Dysport) for the treatment of benign essential blepharospasm: A randomized, placebo-controlled, phase II trial. *Parkinsonism and Related Disorders, 14950*, 407–414.

Truong, D., Lew, M., & Orlova, O. (2008b). Efficacy of Dysport (botulinum toxin type A) for the treatment of cervical dystonia: A multicenter, randomized, double blind, placebo-controlled phase III study. *Toxicon, 51*, 37–38.

Truong, D., Brodsky, M., Lew, M., Brashear, A., Jankovic, J., Molho, E., Orlova, O., Timerbaeva, S., & Global Dysport Cervical Dystonia Study Group. (2010). Long-term efficacy and safety of botulinum toxin type A (Dysport) in cervical dystonia. *Parkinsonism and Related Disorders, 16*(5), 316–323.

Tsui, J. K., Eisen, A., Mak, E., Carruthers, J., Scott, A., & Calne, D. B. (1985). A pilot study on the use of botulinum toxin in spasmodic torticollis. *The Canadian Journal of Neurological Sciences, 12*, 314–316.

Tsui, J. K., Eisen, A., Stoessl, A. J., Calne, S., & Calne, D. B. (1986). Double-blind study of botulinum toxin in spasmodic torticollis. *Lancet, 2*, 245–247.

Tsui, J. K., Bhatt, M., Calne, S., & Calne, D. B. (1993). Botulinum toxin in the treatment of writer's cramp: A double-blind study. *Neurology, 43*, 183–185.

Turkel, C. C., Bowen, B., Liu, J., & Brin, M. F. (2006). Pooled analysis of the safety of botulinum toxin type A in the treatment of poststroke spasticity. *Archives of Physical Medicine and Rehabilitation, 87*, 786–792.

Unal, M., Sevim, S., Doğu, O., Vayisoğlu, Y., & Kanik, A. (2003). Effect of botulinum toxin type A on nasal symptoms in patients with allergic rhinitis: A double-blind, placebo-controlled clinical trial. *Acta Oto-Laryngologica, 123*(9), 1060–1063.

Upile, T., Elmiyeh, B., Jerjes, W., Prasad, V., Kafas, P., Abiola, J., Youl, B., Epstein, R., Hopper, C., Sudhoff, H., & Rubin, J. (2009). Unilateral versus bilateral thyroarytenoid Botulinum toxin injections in adductor spasmodic dysphonia: A prospective study. *Head and Face Medicine, 5*, 20.

Van Der Walt, A., Sung, S., Spelman, T., et al. (2012). A double-blind, randomized, controlled study of botulinum toxin type A in MS-related tremor. *Neurology, 79*, 92–99.

Vanek, Z., & Jankovic, J. (2001). Dystonia in corticobasal degeneration. *Movement Disorders, 16*(2), 252–257.

Visco, A. G., Brubaker, L., Richter, H. E., Nygaard, I., Paraiso, M. F., Menefee, S. A., Schaffer, J., Wei, J., Chai, T., Janz, N., Spino, C., Meikle, S., & Pelvic Floor Disorders Network. (2012). Anticholinergic versus botulinum toxin A comparison trial for the treatment of bothersome urge urinary incontinence: ABC trial. *Contemporary Clinical Trials, 33*(1), 184–196.

von Coelln, R., Raible, A., Gasser, T., & Asmus, F. (2008). Ultrasound-guided injection of the iliopsoas muscle with botulinum toxin in camptocormia. *Movement Disorders, 23*, 889–892.

Wabbels, B., Reichel, G., Fulford-Smith, A., Wright, N., & Roggenkämper, P. (2011). Double-blind, randomised, parallel group pilot study comparing two botulinum toxin type A products for the treatment of blepharospasm. *Journal of Neural Transmission, 118*, 233–239.

Wan, X. H., Vuong, K. D., & Jankovic, J. (2005). Clinical application of botulinum toxin type B in movement disorders and autonomic symptoms. *Chinese Medical Sciences Journal, 20*, 44–47.

Waseem, Z., Boulias, C., Gordon, A., Ismail, F., Sheean, G., & Furlan, A. D. (2011). Botulinum toxin injections for low-back pain and sciatica. *Cochrane Database of Systematic Reviews, 1*, CD008257.

Werdelin, L., Dalager, T., Fuglsang-Frederiksen, A., Regeur, L., Karlsborg, M., Korbo, L., Munck, O., & Winge, K. (2011). The utility of EMG interference pattern analysis in botulinum toxin treatment of torticollis: A randomised, controlled and blinded study. *Clinical Neurophysiology, 122*(11), 2305–2309.

Wilken, B., Aslami, B., & Backes, H. (2008). Successful treatment of drooling in children with neurological disorders with botulinum toxin A or B. *Neuropediatrics, 39*, 200–204.

Wissel, J., Heinen, F., Schenkel, A., Doll, B., Ebersbach, G., Müller, J., & Poewe, W. (1999). Botulinum toxin A in the management of spastic gait disorders in children and young adults

with cerebral palsy: A randomized, double-blind study of high-dose versus low-dose treatment. *Neuropediatrics, 30*(3), 120–124.

Wissel, J., Kanovsky, P., Ruzicka, E., Bares, M., Hortova, H., Streitova, H., Jech, R., Roth, J., Brenneis, C., Müller, J., Schnider, P., Auff, E., Richardson, A., & Poewe, W. (2001). Efficacy and safety of a standardised 500 unit dose of Dysport (clostridium botulinum toxin type A haemagglutinin complex) in a heterogeneous cervical dystonia population: results of a prospective, multicentre, randomised, double-blind, placebo-controlled, parallel group study. *Journal of Neurology, 248*, 1073–1078.

Yablon, S. A., Brin, M. F., VanDenburgh, A. M., Zhou, J., Garabedian-Ruffalo, S. M., Abu-Shakra, S., & Beddingfield, F. C., 3rd. (2011). Dose response with onabotulinumtoxinA for post-stroke spasticity: A pooled data analysis. *Movement Disorders, 26*(2), 209–215.

Yaltho, T. C., & Jankovic, J. (2011). The many faces of hemifacial spasm: Differential diagnosis of unilateral facial spasms. *Movement Disorders, 26*(9), 1582–1592.

Yamauchi, P. S., & Lowe, N. J. (2004). Botulinum toxin types A and B: Comparison of efficacy, duration, and dose-ranging studies for the treatment of facial rhytides and hyperhidrosis. *Clinics in Dermatology, 22*(1), 34–39.

Yang, T. Y., Jung, Y. G., Kim, Y. H., & Jang, T. Y. (2008). A comparison of the effects of botulinum toxin A and steroid injection on nasal allergy. *Otolaryngology and Head and Neck Surgery, 139*(3), 367–371.

Yiannakopoulou, E. (2012). Botulinum toxin and anal fissure: Efficacy and safety systematic review. *International Journal of Colorectal Disease, 27*(1), 1–9.

Yoshimura, D. M., Aminoff, M. J., Tami, T. A., & Scott, A. B. (1992). Treatment of hemifacial spasm with botulinum toxin. *Muscle and Nerve, 15*, 1045–1049.

Zhang, T., Adatia, A., Zarin, W., Moitri, M., Vijenthira, A., Chu, R., Thabane, L., & Kean, W. (2011). The efficacy of botulinum toxin type A in managing chronic musculoskeletal pain: A systematic review and meta-analysis. *Inflammopharmacology, 19*(1), 21–34.

Neurotoxic Effects, Mechanisms, and Outcome of 192-IgG Saporin

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Contents

Abstract

The first type-selective anti-neuronal active in vivo immunotoxin is 192 IgG-saporin. 192 IgG-saporin selectively destroys cholinergic neurons of basal forebrain that provide cholinergic input to the hippocampus, entire cortical mantle, amygdala, and olfactory bulb. Immunotoxic lesions by 192 IgG-saporin represent a valid animal model of Alzheimer's disease, given the degeneration of basal cholinergic system present in this pathology.

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Selective lesioning of cholinergic innervation by means of intracerebroventricular (i.c.v.) or intraparenchymal (i.pr.) 192 IgG-saporin is able to interfere with experience-dependent plasticity. A number of studies have demonstrated alteration of several structural and biochemical parameters related with neuroplasticity (dendritic spines and branching of pyramidal neurons, adult neurogenesis, levels of neurotrophic factors) in both cortical mantle and hippocampus.

Furthermore, lesions of the cholinergic basal forebrain affect cognitive functions, such as learning, memory, and attention, as well as sleep-waking cycle. The effects of selective immunotoxic lesions have been examined in a variety of behavioral paradigms of learning and memory. The general framework has to take into account the route of injection (i.c.v. or i.pr.), lesion extent, age of lesioning, and kind of behavior analyzed. Namely, cholinergic depletion can elicit specific learning and memory impairments as well as deficits in attentional and discriminative abilities. However, 192 IgG-saporin lesions result in overt behavioral deficits only using high demanding tasks and following high-grade CBF lesions, indicating that the relationship between CBF lesion extent and cognitive impairment is a threshold relationship in which a high degree of neuronal loss can be tolerated without detectable consequences.

Keywords

192 IgG-saporin • Basal forebrain cholinergic system • Cognitive functions • Neurodegenerative disorders • Neuroplasticity

List of Abbreviations

ACh	Acetylcholine
AChE	Acetylcholine esterase
AD	Alzheimer's disease
AF64A	Aziridinium ion of ethylcholine mustard
AMPA	Aminomethylphosphonate
APP	Amyloid precursor protein
BDNF	Brain-derived neurotrophic factor
CBF	Cholinergic basal forebrain
ChAT	Choline acetyltransferase
DBBv	Diagonal band of Broca
DMTP	Delayed matching to position
DMTS	Delayed matching to sample
DNMTP	Delayed nonmatching to position
GABA	Gamma-aminobutyric acid
HAChT	High-affinity choline transport
i.c.v.	Intracerebroventricular
i.pr.	Intraparenchymal
MS	Medial septal nucleus
MWM	Morris Water Maze

NBM	Nucleus basalis magnocellularis
NGF	Nerve growth factor
NMDA	*N*-methyl-D-aspartate
OF	Open field
p75	$p75^{NGFFR}$, nerve growth factor receptor
PA	Passive avoidance
RAM	Radial arm maze
RIPs	Ribosome-inactivating proteins
SI	Substantia innominata

1 Introduction

The cholinergic basal forebrain (CBF) is one of the diffusely projecting systems of the brain. It provides cholinergic input to the hippocampus, entire cortical mantle, amygdala, and olfactory bulb. The physiological function of the CBF is thought to modulate the excitability of cortical and hippocampal neurons. Anatomical and physiological data on the CBF encourage the notion that it modulates some cognitive functions, as learning, memory, and attention, as well as sleep-waking cycle. The evidence for this hypothesis has come from observations that muscarinic antagonists generally impair cognitive function in humans and animals, and cholinomimetics can reverse such impairments or enhance cognition when given alone (Hagan and Morris 1988). Although the precise site within the CNS for the cholinergic drug effects on behavior is uncertain, the CBF is regarded as the probable anatomical substrate for the cognitive effects of cholinergic drugs (Markowska et al. 1995). The discovery that the CBF degenerates in Alzheimer's disease (AD) and that its degree of degeneration correlates with the degree of dementia (Perry et al. 1978) has strongly encouraged interest on the role of the CBF in cognition. Degeneration of the CBF in AD has motivated investigators to attempt to model these pathological features through a variety of experimental lesioning techniques. The approaches employed have included fimbria-fornix transaction (Matsuoka et al. 1991), injection of the toxin aziridinium ion of ethylcholine mustard (AF64A) into the basal forebrain or target areas of cholinergic neurons (Walsh and Opello 1994), and intracerebral injections of excitotoxins (selective toxins for excitatory neurons, such as kainic, ibotenic, *N*-methyl-D-aspartate (NMDA), and quisqualic acids) (Dunnett et al. 1991). The major shortcoming of these works is that each of these techniques lacks selectivity. For example, excitotoxins are able to destroy cell soma without damaging crossing fibers. Yet, their selectivity depends on the injection site: there is great selectivity where the neurons are organized in distinct groups, as for *locus coeruleus*. Differently, since cholinergic neurons of basal forebrain are highly widespread among other cell types, the excitotoxic lesions induce unwanted loss of non-cholinergic neighboring neurons (Dunnett et al. 1991) rendering thus difficult to ascribe the eventual resulting deficits specifically to destruction of the CBF.

In the 1990s, the problem of selective lesions of the CBF has been addressed through the development of the new lesioning techniques that have proved very significant for functional neuroscience research. The power and validity of this approach reside in the nature of the lesion.

2 Immunolesioning Techniques

Immunolesions use monoclonal anti-neuronal antibodies to deliver similar cytotoxin selectivity to only those neurons bearing surface moieties recognized by the antibodies. Immunolesioning can destroy a specific type of neuron locally, or after axonal suicide transport from the injection site, thus combining selectivity for both anatomical region and cell type.

Immunotoxins are composed of monoclonal antibodies conjugated to various ribosome-inactivating proteins (RIPs). The monoclonal antibody component of an immunotoxin binds to specific cell surface molecules to result in endocytosis of the antibody and its coupled RIP. Once internalized, the RIP component inhibits protein synthesis, catalytically inactivating ribosomes and resulting in cell death. If immunotoxin binds to nerve terminals or axons, it is internalized by receptor-mediated endocytosis and then travels by retrograde axonal transport to neuronal cell bodies, where the RIP acts to inhibit protein synthesis. If antigen-bearing soma is present at the injection site, then axonal transport is obviously not necessary to deliver the toxin. However, high local toxin concentrations at the injection site may produce nonspecific cytotoxicity with necrosis of all cell type.

The first immunotoxin that was reported to be effective in making neuronal lesions is OX7-saporin that consisted of the monoclonal antibody OX7 coupled to saporin. Saporin is one RIP often used; derived from the plant *Saponaria officinalis* (soapwort). OX7 recognizes Thy-1, an abundant surface molecule present on all neurons, so the resulting immunotoxin is not selective for any one type of neuron. For a review see Wiley (1992).

3 192 IgG-Saporin

The first type-selective anti-neuronal active in vivo immunotoxin is the 192 IgG-saporin. 192 IgG-saporin consists of the monoclonal antibody 192 IgG which is disulfide coupled to saporin. The antibody component is directed against low-affinity rat nerve growth factor (NGF) receptor ($p75^{NGFFR}$). Targeting p75 serves as an ideal way to selectively lesion the CBF neurons because in the adult rat basal forebrain, only the cholinergic neurons express p75. They possess p75 NGF receptors while neurons containing other neurotransmitters in the region, and even the nearby striatal cholinergic interneurons, do not express detectable levels of same receptors. NGF plays a role in the maintenance of function of the magnocellular CBF neurons, which have NGF receptors (Thomas et al. 1991). When injected, 192 IgG-saporin binds to the surface of p75-bearing neurons and is internalized by

endocytosis. Once in the cytoplasm, the saporin moiety escapes endosomes and enzymatically inactivates the large ribosomal subunit halting protein synthesis and leading ultimately to cell death. For a review see Wiley (1992).

4 Organization of the Basal Forebrain Cholinergic System

Neurons in the basal forebrain provide the major source of cholinergic innervation for the entire neocortex, hippocampus, amygdala, and olfactory bulb. CBF is a continuum of several overlapping nuclei with divergent efferent pathways, such that a single projection field is not exclusive of a particular nucleus. The forebrain and upper brainstem of the rat contain six groups of cholinergic projection neurons designated by Mesulam as Ch1–Ch6 on the basis of cytoarchitectonic criteria and connectivity patterns. The Ch terminology was originally described on the monkey brain and later proposed in extended form in the rat. The Ch1–Ch2 sectors are contained within the medial septal (MS) nucleus and the vertical limb nucleus of the diagonal band of Broca (DBBv), respectively. They provide the major cholinergic projection to the hippocampal formation. The Ch3 sector is mostly contained within the lateral part of the horizontal limb nucleus of the diagonal band and provides the primary cholinergic projection to the olfactory bulb. The Ch4 sector contains the cholinergic neurons of the nucleus basalis magnocellularis (NBM), the substantia innominata (SI), and probably also laterally situated neurons of the vertical limb nucleus as well as medially situated cells of the horizontal limb nucleus. One unifying feature of Ch4 is that its components provide the major cholinergic projection to neocortical targets, as well as to the amygdala. The Ch5–Ch6 regions are located in the pontomesencephalic reticular formation and provide the major cholinergic innervation to the thalamus. For a review see Mesulam et al. (1983).

Developmental studies using biochemical, histochemical, and immunocytochemical techniques have shown that cholinergic neurons in the rodent basal forebrain are generated prenatally according to a distinct spatiotemporal pattern and undergo extensive maturation during the first postnatal month. Cholinergic fibers from Ch1–2 or Ch4 sectors, which start growing as early as embryonic day 15, reach the appropriate target fields in the hippocampus or neocortex at about the time of birth and establish adult-like patterns of innervation in either region within the fourth postnatal week, when the postnatal maturation of cholinergic markers is fairly complete (Leanza et al. 1996).

Several studies addressing the role of cholinergic neurotransmission investigated how to use 192 IgG-saporin, evaluating extent, time course, specificity, and mechanism of action of intraparenchymal or intraventricular injections in the adult CNS.

5 Selectivity and Effectiveness of 192 IgG-Saporin

The selectivity and effectiveness of 192 IgG-saporin for producing lesions of the CBF has now been shown by several research groups. Both intracerebroventricular (i.c.v.)

administration of the 192 IgG-saporin and direct injection into distinctive basal forebrain nuclei induce nearly complete and specific lesions of cholinergic cells but sparing other neuronal systems in the basal forebrain.

192 IgG-saporin i.c.v. injection affects CBF as well as Purkinje cells of the cerebellum (Berger-Sweeney et al. 1994; Heckers et al. 1994) because a subset of these cells starts expressing p75 receptors of NGF during development and continues during adulthood (Pioro and Cuello 1988). Differently, 192 IgG-saporin i.c.v. injections spare the cholinergic neurons of other brain regions, the gamma-aminobutyric acidergic (GABAergic) neurons expressing parvalbumin that are intermingled within the CBF, the calbindin D-28K expressing neurons of the NBM, and the NADPH diaphorase-expressing neurons of the NBM (Heckers et al. 1994; Wiley et al. 1995). 192 IgG-saporin i.c.v. injections also result in loss of cholinergic markers such as choline acetyltransferase (ChAT) activity, acetylcholine esterase (AChE) positive fibers, and high-affinity choline transport (HAChT) in the target fields of the CBF (Wrenn and Wiley 1998). 192 IgG-saporin i.c.v. injections at different time points of neonatal age (from 0 to 30 postnatal day) are used for studying the development of the central cholinergic system (Berger-Sweeney 1998; Leanza et al. 1996).

When injected intraparenchymally (i.pr.) into basal forebrain, 192 IgG-saporin effects are present mainly in the proximity of the injection site, sparing interspersed non-cholinergic neurons (Berger-Sweeney et al. 1994). Notably, i.pr. injections induce a more pronounced gliosis than i.c.v. injections. Delayed loss of cholinergic striatal interneurons after injection into the Ch4 sector has been described (Heckers et al. 1994); however this finding has not been replicated. Striatal cholinergic neurons express the p75 NGF receptor in early development phases, but this expression decreases drastically during adulthood (Pioro and Cuello 1988). The described loss of striatal cholinergic neurons might be due to the lesion-induced re-expression of p75 NGF receptors in the striatum (Heckers et al. 1994). The sphere of CBF-killed cells that results from i.pr. injections can be expanded by increasing the dose. However, at high dose, nonselectivity may become a problem (Wrenn and Wiley, unpublished observation). 192 IgG-saporin i.pr. injections can also be performed in the target fields (hippocampus, cortex) of the CBF. Injections into the hippocampus result in a mainly ipsilateral loss of the Ch1–Ch2 sector cholinergic neurons, an effect blocked by simultaneous injections of colchicines. Similarly, intracortical injections cause a loss of cholinergic fiber density in the cortex and a loss of Ch4 cholinergic neurons (Ohtake et al. 1997).

The selectivity and lethality of 192 IgG-saporin for CBF neurons were further demonstrated by Book et al. (1994) by using a double-labeling approach. In this study, the persistent retrograde tracer Fluoro-Gold was cortically injected 1 week before the i.c.v. injections of 192 IgG-saporin or saline. The Fluoro-Gold pre-labeled both cholinergic and non-cholinergic basal forebrain neurons. After depleting the cholinergic neurons, Ch4 was stained immunohistochemically for ChAT. These authors found that only the neurons that were double-labeled for both Fluoro-Gold and ChAT were lost in the 192 IgG-saporin-treated rats. Thus, 192 IgG-saporin selectively killed the cholinergic neurons of the Ch4 sectors, leaving intact non-cholinergic neurons.

It is important to emphasize that much of the CBF projection to amygdala is not lesioned by i.c.v or i.pr. injections of 192 IgG-saporin. Most cholinergic neurons projecting to the amygdala do not express p75 receptors; therefore, they are unaffected by 192 IgG-saporin (Heckers et al. 1994). The sparing of the cholinergic innervation to the amygdala renders the experimental immunotoxic model significantly different from the AD pathology, representing a methodological limit that has to be taken into account.

In summary, 192 IgG-saporin is the most effective and selective means for producing lesions of the rat CBF. Its advantage over previous techniques is in its ability to selectively kill cholinergic neurons of the basal forebrain and to leave intact intermingled non-cholinergic neurons. Significant drawbacks of 192 IgG-saporin include sparing of the cholinergic innervations to amygdala and destruction of some cerebellar Purkinje neurons.

6 Structural and Biochemical Effects of 192 IgG-Saporin

One of the most interesting functional correlates of cortical cholinergic input is its role in mediating neuroplasticity. Neuroplasticity is a lifelong process that mediates the structural and functional reaction of dendrites, axons, and synapses to experience, aging, and injury.

The selective lesioning of cortical cholinergic innervation by means of 192 IgG-saporin interferes with experience-dependent plasticity in the barrel fields. When all whiskers except for D2 and D3 were trimmed, a pairing between the D2 and D3 barrel fields occurred so the D2 neurons in the cerebral cortex started to show a greater responsivity to the stimulation of D3 than to stimulation of adjacent D1, which had been trimmed. This pairing, indicative of experience-induced synaptic plasticity, is not obtained in rats with selective immunotoxic lesions in the Ch4 (Baskerville et al. 1997). In newborn rat pups, barrel representing intact whiskers failed to show the expected expansion into the territory representing trimmed whiskers in animals with Ch4 lesions (Kilgard and Merzenich 1998). Furthermore, pairing auditory stimuli with electrical stimulation of the Ch4 in adult rats caused a long-lasting reorganization of primary auditory cortex so that the area optimally responsive to the paired tone expanded substantially. This plasticity was not observed following the selective immunotoxic destruction of cholinergic Ch4 neurons. Thus, it appears that cortical cholinergic denervation can undermine the learning-dependent reorganization of cortical representations and perhaps also the ability of the brain to keep itself in good repair in response to injury (Conner et al. 2003).

Several studies have investigated structural modifications of neocortical pyramidal neurons following selective CBF lesions. Injections of 192 IgG-saporin (i.c.v. or i.pr.) induce an increase of apical and basal dendritic spines related to an unaltered dendritic branching in parietal (De Bartolo et al. 2009; Mandolesi et al. 2008) as well as in frontal (Harmon and Wellman 2003; Wellman and Sengelaub 1995) pyramidal neurons during the middle adulthood. The same pattern of changes was found when the selective cholinergic lesion occurred in neonatal age

(De Bartolo et al. 2010). Furthermore, in frontal pyramidal neurons, the lesion-induced increment of dendritic spines has been related to an increased expression of GluR1 subunit of the aminomethylphosphonate (AMPA) receptors (Garret et al. 2006; Kim et al. 2005), suggesting the spines as the morphological substrate for the increase of AMPA receptors induced by the cholinergic lesion. The hypothesis has been advanced that neocortical neurons upregulate AMPA receptors and spine number as a compensatory response to the lesion-induced decreased innervation (Fiala et al. 2002). In fact, the increase in spines would execute a "mechanical" function and prevent lesion-induced atrophy of dendritic branching (Harmon and Wellman 2003). The hypothesis that the spines might provide protection against dendritic atrophy is supported by evidence that in frontal pyramidal neurons of aged rats with forebrain cholinergic lesions, spines did not increase (Harmon and Wellman 2003) and dendritic branching decreased (Works et al. 2004). Nevertheless, an increase in spines does not necessarily imply an increase in functional synapses. A study of slice preparations of hippocampal neurons showed that new spines form even when synapses are inactivated and persist for many hours in the absence of any functional activity (Kirov and Harris 1999). A recent in vivo study (Arellano et al. 2007) reported the existence of non-synaptic spines in the mouse neocortex, indicating that dendritic spines might not be used to form synaptic contacts. A recent review reported that most new spines which appear in adult animals are transient, and only a small percentage of them will ultimately form functional synapses (Alvarez and Sabatini 2007). Moreover, the presence of spatial deficits following forebrain lesions (for details see below) seems to suggest that the newly formed spines of parietal pyramidal neurons are functionally inactive and thus do not directly influence behavior.

The reduced neuroplasticity following CBF-selective lesions is confirmed by many studies investigating the effects of 192 IgG-saporin injections on adult neurogenesis. It has been demonstrated that, 5 weeks after lesion, neurogenesis declined significantly in the granule cell layers of dentate gyrus in hippocampal formation as well as in olfactory bulb. Furthermore, immunotoxic lesions led to increased numbers of apoptotic cells specifically in the subgranular zone, the progenitor regions of dentate gyrus, and within the periglomerular layer of olfactory bulb. So, it has been advanced that the cholinergic system plays a survival-promoting role for neuronal progenitors and immature neurons within regions of adult neurogenesis, similar to effects observed during brain development (Cooper-Kuhn et al. 2004). Moreover, it has been demonstrated that i.c.v. 192 IgG-saporin injections on pnd 7 accelerate the death of newborn cells in the dentate gyrus, but does not change their overall survival rate or phenotypic differentiation (Rennie et al. 2011).

Many effects of selective CBF lesions confirming the reduced neuroplasticity have been described about metabolic markers and are consistent with the view of cholinergic influence over metabolism and amyloid precursor protein (APP) processing in the neocortex and hippocampus. For example, it has been demonstrated that i.c.v. 192 IgG-saporin injections during adulthood causes sustained reductions in glucose utilization in brain regions impacted by cholinergic synapse loss, including frontal cortical and hippocampal regions, relative to glucose use levels (Browne et al. 2001).

Also, neurotrophic factors strongly implicated in neuroplasticity display alterations following selective cholinergic depletion. In consequence of i.c.v. 192 IgG-saporin injections, NGF levels increased in the frontal cortex and hippocampus at 3 and 7 days and decreased in the serum at 7 days from lesion, suggesting that opposite changes in brain and serum occur at early stages of cholinergic depletion (Gelfo et al. 2011). 192 IgG-saporin i.c.v. injections induce a reduction of neurotrophin brain-derived neurotrophic factor (BDNF) levels in the parietal cortex as well as in the serum at later stages (15 days from lesion) (Angelucci et al. 2011). It appears interesting, since BDNF has an important role in survival, differentiation and functionality of neurons undergoing degeneration in pathological conditions such as cholinergic neurons in AD.

7 Behavioral and Cognitive Outcomes of 192 IgG-Saporin Lesions

The effects of selective immunotoxic lesions have been examined in a variety of behavioral paradigms of learning and memory. However, behavioral studies after 192 IgG-saporin lesions provide a quite unexpected picture: massive cholinergic depletion results in little or no impairment in commonly used spatial and not spatial learning and memory tests (Baxter and Chiba 1999; Everitt and Robbins 1997; McGaughy et al. 2000). Differently, deficits have been noted after cholinergic depletion on tasks that focus on attentional and discriminative abilities (Burk et al. 2002; McGaughy et al. 2002; Ricceri 2003). This general framework is further complicated by route of injection (i.c.v. or i.pr.), lesion extent, age of lesioning, and kind of behavior analyzed.

7.1 Mnesic Functions

The i.c.v. injection method has most consistently produced learning and memory deficits, and the most informative studies have used a range of immunotoxin doses which has allowed analyzing correlations between lesion extent and behavioral deficit.

High-dose (4–10 μg) i.c.v. injections of 192 IgG-saporin result in impairment in the acquisition of navigational learning in Morris Water Maze (MWM) and retention memory deficits in passive avoidance (PA) (Berger-Sweeney et al. 1994; Garcia-Alloza et al. 2006; Leanza et al. 1996; Nilsson et al. 1992; Steckler et al. 1995; Traissard et al. 2007; Waite & Thal 1995; Waite et al. 1995, 1999; Walsh et al. 1995; Zhang et al. 1996). Impaired performance in cued versions of MWM task has been also reported (Berger-Sweeney et al. 1994; Walsh et al. 1995). The behavioral changes are paralleled by a greater than 90 % reduction in CBF neurons that result in 80–90 % reduction in ChAT in the hippocampal and cortical target fields. Conversely, rats with less-extensive CBF lesions following i.c.v. (≈55–75 % cholinergic loss) (Cutuli et al. 2009; De Bartolo et al. 2010; Lehmann et al. 2000, 2002; Mandolesi et al. 2008; Nilsson et al. 1988; Waite et al. 1999) or i.pr.

(MS, DBB and/or NBM) immunotoxic injections (≈15–78 % cholinergic loss) were only slightly or not at all behaviorally affected in tasks, as MWM, PA, active avoidance, fear conditioning, and T-maze (Baxter et al. 1995, 1996; Dornan et al. 1996; Frick et al. 2004; Garcia-Alloza et al. 2006; Mandolesi et al. 2008; Torres et al. 1994; Wenk et al. 1994).

192 IgG-saporin i.c.v. lesions also impair spatial learning and working memory. A significant correlation between working memory impairment and lesion extent was observed (Wrenn and Wiley 1998; Wrenn et al. 1999), and this dose-dependent impairment was present only in rats that harbored total CBF lesions greater than 75 % (threshold effect). Several studies reported deficient performances in radial arm maze (RAM) (Cutuli et al. 2009; De Bartolo et al. 2010; Lehmann et al. 2003; Traissard et al. 2007) and in a water version of RAM (Antonini et al. 2009) following 192 IgG-saporin i.c.v. injections. Working memory deficits observed in RAM following CBF depletions are consistent with findings obtained by using T-maze alternation test and delayed matching to position/sample (DMTP/DMTS) and delayed nonmatching to position (DNMTP) tasks (Leanza et al. 1996; Lehmann et al. 2000, 2002; McDonald et al. 1997; Paban et al. 2005a, b; Steckler et al. 1995; Wrenn and Wiley 2001). Once again, i.pr. injections producing small, circumscribed cholinergic depletion (<75 %) did not evoke dramatic working memory deficits (McMahan et al. 1997; Pang et al. 2001) or effect in DNMTP task (Steckler et al. 1995). Galani et al. (2002) observed minor impairments only during initial phases of MWM and RAM tests after NBM injection. Perry et al. (2001) found RAM and MWM long-term (11 months) deficits after NBM and MS 192 IgG-saporin lesion, but not short-term deficits (5 months). The few studies considering perseverative behaviors and procedural strategies put into action during RAM demonstrated pervasive deficits of spatial exploration and cognitive planning following i.c.v. (Cutuli et al. 2009; De Bartolo et al. 2010) and NBM (Mandolesi et al. 2008) 192 IgG-saporin lesions.

The role of damage of cerebellar Purkinje cells has been addressed by including additional control groups with selective cerebellar lesions produced by means of OX7-saporin. Injections of 192 IgG-saporin or OX7-saporin (i.c.v.) result in sensory-motor deficits, compatible with the role of Purkinje cells in hyperactivity, motor coordination, and balance (Gandhi et al. 2000; Waite et al. 1995, 1999). Waite et al. (1999) demonstrated that OX7-saporin lesions mimic the effects on MWM acquisition shown after 192 IgG-saporin i.c.v. injections. Reference memory deficits selectively induced by OX7-saporin injections were also found in MWM and RAM (Gandhi et al. 2000; Traissard et al. 2007), confirming the critical importance of cerebellar damage in the i.c.v. lesioning effects. Deficits in the MWM-cued version are addressed to a non-cholinergic cerebellar Purkinje cell damage (Berger-Sweeney et al. 1994; Walsh et al. 1995). On the contrary, Purkinje cell loss is not responsible for PA retention memory and RAM working memory deficits seen after 192 IgG-saporin i.c.v. injections (Wiley et al. 1995; Wrenn et al. 1999). Namely, a threshold relationship between CBF lesion extent and working memory consequences has been demonstrated, indicating that moderate (about 32 %) Purkinje cell loss alone is insufficient to impair RAM performances (Wrenn and Wiley 2001).

As final notation, whether or not a behavioral deficit is observed depends upon an interplay between lesion extent and task demands. For example, recalling a single platform location in the MWM is less demanding than recalling a list of locations in the RAM. Consequently, when lesion extent was high and tasks were demanding, not all 192 IgG-saporin i.pr. studies have yielded negative results in learning and memory paradigms. In fact, Torres et al. (1994) and Rispoli et al. (2006) found impaired PA retention after injection into the NBM. Working memory deficits were reported in MWM (Baxter et al. 1995) and RAM after injections into the MS and/or NBM (Dornan et al. 1996; Mandolesi et al. 2008; Shen et al. 1996; Walsh et al. 1996). Impaired DNMTP performances were found after injection into the MS and the cingulate cortex (Torres et al. 1994; Walsh et al. 1996). Selective removal of the cholinergic projections to the cortical mantle by NBM injections of 192 IgG-saporin led to impaired social interaction reducing the duration of active social contact (Savage et al. 2011).

7.2 Spatial/Object Novelty Discrimination

Many studies assessed the effects of 192 IgG-saporin lesions on spatial/object recognition. Unlike the MWM, analysis of the open field (OF) with object performances revealed that i.c.v. 192 IgG-saporin lesions significantly affect spatial discrimination, since lesioned rats did not show selective renewed interest in the displaced objects (Cutuli et al. 2009; De Bartolo et al. 2010; Paban et al. 2005a, b). Conversely, after 192 IgG-saporin i.c.v. and i.pr. injections, the lesioned rats detected the presence of a novel object (Cutuli et al. 2009; De Bartolo et al. 2010; Savage et al. 2011; Vnek et al. 1996). A severe object recognition deficit in rats was observed only after injecting 192 IgG-saporin directly into perirhinal cortex (Winters and Bussey 2005).

The lesion-induced absence of spatial change detection is apparently inconsistent with the tuned MWM performances displayed by the 192 IgG-saporin-lesioned animals. It is possible that the MWM task is more affected by stress levels associated with the forced swimming to search for the escape platform (Cutuli et al. 2009; De Bartolo et al. 2010; Ricceri 2003). The stress responses induced by immersion in water might trigger neuromodulatory compensation that masks mild spatial working memory deficits (Everitt and Robbins 1997; McMahan et al. 1997). In fact, tasks involving high levels of stress may lead to stress-related increases of ACh release in the hippocampus (Gilad 1987; Mizoguchi et al. 2001; Mizuno and Kimura 1997). This could compensate for the greater loss of cholinergic functions in relatively stressful (MWM) than in less stressful (spatial/object novelty) tasks.

7.3 Attentional Functions

Many attentional functions have been found to be sensitive to selective cholinergic depletion, and the robustness of these effects varies with the kind of attentional

demands (McGaughy et al. 2000). Tasks requiring decremental attentional processing, such as latent inhibition, have been found to be unaffected by i.pr. cholinergic depletions (Chiba et al. 1995; Doughtery et al. 1996). However, following NBM or posterior parietal cortex 192 IgG-saporin infusions, subjects were impaired in serial conditioning tasks (Bucci et al. 1998; Chiba et al. 1995). 192 IgG-saporin i.c.v.-lesioned rats were impaired in a 5-Choice Serial Reaction Time test with increasing variability of the delay and visual search demands (Waite et al. 1999). Also the cognitive flexibility consisting in efficiently switching between spatially changing response rules was affected by 192 IgG-saporin i.c.v. lesions, as demonstrated by Cutuli et al. (2009) by using a serial learning task in which rats had to reach a food reward, finding out a sequence of correct open doors daily changed.

The extent of neocortical cholinergic depletion steadily correlates with performance in a sustained attention test (McGaughy et al. 1996, 1999; McGaughy and Sarter 1998, 1999). NBM infusions of 192 IgG-saporin produced dramatic, persistent impairment in the ability to detect unpredictably occurring signals, but not in their correct identification of non-signals in a sustained attentional task. The ability to discriminate signals from non-signals early declined with the additional cognitive demand provided by prolonged time on task. Dose-dependent cholinergic cortical denervation after NMB or i.c.v. lesions resulted in consequent behavioral impairments. Similar sensitivity to cortical cholinergic denervation was found when divided attention was tested. In conditional discrimination tests, lesioned animals showed increased response latencies in conditions requiring the simultaneous maintenance of two sets of conditional response rules (Turchi and Sarter 1997; Waite et al. 1999).

7.4 Neonatal Lesions

Selective cholinergic lesions by means of 192 IgG-saporin have been performed also in developing rats. Neonatal 192 IgG-saporin i.c.v. lesions produce a permanent loss in CBF, without inducing the cerebellar damage shown following i.c.v. infusion in adults.

While on the first postnatal day (pnd 1) single 192 IgG-saporin i.c.v. injections induce a very mild cholinergic loss in the cortex (17 % reduction in cortical ChAT activity) accompanied by no reduction in hippocampal ChAT activity and no behavioral alteration (Ricceri 2003; Ricceri et al. 1997, 1999), combined pnd 1 and 3 192 IgG-saporin injections lead to a dramatic reduction of ascending cholinergic projections to both the hippocampus and neocortex and impaired PA learning and MWM (Leanza et al. 1996; Ricceri et al. 2002). After 192 IgG-saporin i.c.v. injections at pnd 4, ChAT activity was markedly reduced in the hippocampus and cortical regions (73–84 %), but rats were unimpaired in MWM (Leanza et al. 1996). At pnd 7 192 IgG-saporin injections provoked consistent reductions in ChAT activity (78–84 % hippocampus, 52–64 % neocortex) accompanied by early and long-term behavioral impairments (Ricceri 2003; Wrenn and Wiley 1998).

In fact, pnd 7-lesioned rats displayed altered ultrasonic vocalizations in maternal separation and fear conditioning paradigms (Ricceri 2003; Ricceri et al. 2007; Scattoni et al. 2005), learning deficits in PA, and reduced exploratory behavior (Ricceri et al. 1997). At adulthood pnd 7-lesioned rats showed no deficits in MWM and delayed spatial alternation T-maze (De Bartolo et al. 2010; Pappas et al. 1996; Ricceri et al. 1999) but altered reactions to spatial novelty (De Bartolo et al. 2010; Ricceri et al. 1999), deficits in social transmission of food preferences (Ricceri et al. 2004), impaired working memory performances in RAM, and impulsive behaviors (De Bartolo et al. 2010; Pappas et al. 2005; Scattoni et al. 2006). In summary, as in adult animals, neonatal 192 IgG-saporin i.c.v. injections result in behavioral impairment only when the resultant CBF lesion is extensive and tasks are highly demanding.

8 Conclusion

The immunotoxin 192 IgG-saporin causes a selective, long-lasting loss of the cholinergic neurons within the CBF and is able to interfere with experience-dependent plasticity. A number of studies have demonstrated alterations of several structural and biochemical parameters related with neuroplasticity (dendritic spines and branching of pyramidal neurons, adult neurogenesis, levels of neurotrophic factors) in both cortical mantle and hippocampus.

The introduction of immunotoxic lesioning methods has proved to be a valuable tool in the investigation of the role of ascending cholinergic systems in cognition. The effects of selective immunotoxic lesions have been examined in a variety of behavioral paradigms. The general framework has to take into account the route of injection (i.c.v. or i.pr.), lesion extent, age of lesioning, and kind of behavior analyzed in the different studies. Overall, cholinergic depletion has been demonstrated to provoke selective learning and memory impairments as well as deficits in attentional and discriminative abilities. However, as demonstrated by the literature reviewed in this chapter, the specificity of the neurotoxic lesioning method is strictly linked to the validity and sensitivity of behavioral methods. Furthermore, 192 IgG-saporin lesions result in overt behavioral deficits only with high-grade CBF lesions, indicating a threshold relationship between CBF lesion extent and cognitive impairment.

References

Alvarez, V. A., & Sabatini, B. L. (2007). Anatomical and physiological plasticity of dendritic spines. *Annual Review of Neuroscience, 30*, 79–97.

Angelucci, F., Gelfo, F., De Bartolo, P., Caltagirone, C., & Petrosini, L. (2011). BDNF concentrations are decreased in serum and parietal cortex in immunotoxin 192 IgG-Saporin rat model of cholinergic degeneration. *Neurochemistry International, 59*, 1–4.

Antonini, V., Prezzavento, O., Coradazzi, M., Marrazzo, A., Ronsisvalle, S., Arena, E., & Leanza, G. (2009). Anti-amnesic properties of (+/−)-PPCC, a novel sigma receptor ligand,

on cognitive dysfunction induced by selective cholinergic lesion in rats. *Journal of Neurochemistry, 109*, 744–754.

Arellano, J. I., Espinosa, A., Fairèn, A., Yuste, R., & DeFelipe, J. (2007). Non-synaptic dendritic spines in neocortex. *Neuroscience, 145*, 464–469.

Baskerville, K. A., Schweitzer, J. B., & Herron, P. (1997). Effects of cholinergic depletion on experience-dependent plasticity in the cortex of the rat. *Neuroscience, 80*, 1159–1169.

Baxter, M. G., & Chiba, A. A. (1999). Cognitive functions of the basal forebrain. *Current Opinion in Neurobiology, 9*, 178–183.

Baxter, M. G., Bucci, D. J., Gorman, L. K., Wiley, R. G., & Gallagher, M. (1995). Selective immunotoxic lesions of basal forebrain cholinergic cells: Effects on learning and memory in rats. *Behavioral Neuroscience, 109*, 714–722.

Baxter, M. G., Bucci, D. J., Sobel, T. J., Williams, M. J., Gorman, L. K., & Gallagher, M. (1996). Intact spatial learning following lesions of basal forebrain cholinergic neurons. *Neuroreport, 31*, 1417–1420.

Berger-Sweeney, J. (1998). The effect of neonatal basal forebrain lesions on cognition: Towards understanding the developmental role of the cholinergic basal forebrain. *Internal Journal of Developmental Neuroscience, 16*, 603–612.

Berger-Sweeney, J., Hechers, S., Mesulam, M. M., Wiley, R. G., Lappi, D. A., & Sharma, M. (1994). Differential effects on spatial navigation of immunotoxin-induced cholinergic lesions of the medial septal area and nucleus basalis. *Journal of Neuroscience, 14*, 4507–4519.

Book, A. A., Wiley, R. G., & Schweitzer, J. B. (1994). 192 IgG-saporin: I. Specific lethality for cholinergic neurons in the basal forebrain of the rat. *Journal of Neuropathology and Experimental Neurology, 53*, 95–102.

Browne, S. E., Lin, L., Mattsson, A., Georgievska, B., & Isacson, O. (2001). Selective antibody-induced cholinergic cell and synapse loss produce sustained hippocampal and cortical hypometabolism with correlated cognitive deficits. *Experimental Neurology, 170*, 36–47.

Bucci, D. J., Holland, P. C., & Gallagher, M. (1998). Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli. *Journal of Neuroscience, 1*, 8038–8046.

Burk, J. A., Herzog, C. D., Porter, M. C., & Sarter, M. (2002). Interactions between aging and cortical cholinergic deafferentation on attention. *Neurobiology of Aging, 23*, 467–477.

Chiba, A. A., Bucci, D. J., Holland, P. C., & Gallagher, M. (1995). Basal forebrain cholinergic lesions disrupt increments but not decrements in conditioned stimulus processing. *Journal of Neuroscience, 15*, 7315–7322.

Conner, J. M., Culberson, A., Packowski, C., Chiba, A. A., & Tuszinski, M. H. (2003). Lesion of the basal forebrain cholinergic system impair task acquisition and abolish cortical plasticity associated with motor skill learning. *Neuron, 38*, 819–829.

Cooper-Kuhn, C. M., Winkler, J., & Kuhn, H. G. (2004). Decreased neurogenesis after cholinergic forebrain lesion in the adult rat. *Journal of Neuroscience Research, 15*, 155–165.

Cutuli, D., Foti, F., Mandolesi, L., De Bartolo, P., Gelfo, F., Federico, F., & Petrosini, L. (2009). Cognitive performances of cholinergically depleted rats following chronic donepezil administration. *Journal of Alzheimer's Disease, 17*, 161–176.

De Bartolo, P., Gelfo, F., Mandolesi, L., Foti, F., Cutuli, D., & Petrosini, L. (2009). Effects of chronic donepezil treatment and cholinergic deafferentation on parietal pyramidal neuron morphology. *Journal of Alzheimer's Disease, 17*, 177–191.

De Bartolo, P., Cutuli, D., Ricceri, L., Gelfo, F., Foti, F., Laricchiuta, D., Scattoni, M. L., Calamandrei, G., & Petrosini, L. (2010). Does age matter? Behavioral and neuro-anatomical effects of neonatal and adult basal forebrain cholinergic lesions. *Journal of Alzheimer's Disease, 20*, 207–227.

Dornan, W. A., McCampbell, A. R., Tinkler, G. P., Hickman, L. J., Bannon, A. W., Decker, M. W., & Gunther, K. L. (1996). Comparison of site-specific injections into the basal forebrain on water maze and radial arm maze performance in the male rat after immunolesioning with 192 IgG saporin. *Behavioral Brain Research, 82*, 93–101.

Doughtery, K. D., Salat, D., & Walsh, T. J. (1996). Intraseptal injection of the cholinergic immunotoxin 192 IgG-saporin fails to disrupt latent inhibition in a conditioned taste aversion paradigm. *Brain Research, 736*, 260–269.

Dunnett, S. B., Everitt, B. J., & Robbins, T. W. (1991). The basal forebrain-cortical cholinergic system: Interpreting the functional consequences of excitotoxic lesions. *Trends in Neurosciences, 14*, 494–501.

Everitt, B. J., & Robbins, T. W. (1997). Central cholinergic systems and cognition. *Annual Review of Psychology, 48*, 649–684.

Fiala, J. C., Spacek, J., & Harris, K. M. (2002). Dendritic spine pathology: Cause or consequence of neurological disorders? *Brain Research Reviews, 39*, 29–54.

Frick, K. M., Kim, J. J., & Baxter, M. G. (2004). Effects of complete immunotoxin lesions of the cholinergic basal forebrain on fear conditioning and spatial learning. *Hippocampus, 14*, 244–254.

Galani, R., Lehmann, O., Bolmont, T., Aloy, E., Bertrand, F., Lazarus, C., Jeltsch, H., & Cassel, J. C. (2002). Selective immunolesions of CH4 cholinergic neurons do not disrupt spatial memory in rats. *Physiological & Behavior, 1*, 75–90.

Gandhi, C. C., Kelly, R. M., Wiley, R. G., & Walsh, T. J. (2000). Impaired acquisition of a Morris water maze task following selective destruction of cerebellar purkinje cells with OX7-saporin. *Behavioral Brain Research, 109*, 37–47.

Garcia-Alloza, M., Zaldua, N., Diez-Ariza, M., Marcos, B., Lasheras, B., Javier Gil-Bea, F., & Ramirez, M. J. (2006). Effect of selective cholinergic denervation on the serotonergic system: Implications for learning and memory. *Journal of Neuropathology and Experimental Neurology, 65*, 1074–1081.

Garret, J. E., Kim, I., Wilson, R. E., & Wellman, C. L. (2006). Effect of *N*-methyl-*d*-aspartate receptor blockade on plasticity of frontal cortex after cholinergic deafferentation in rat. *Neuroscience, 140*, 57–66.

Gelfo, F., Tirassa, P., De Bartolo, P., Caltagirone, C., Petrosini, L., & Angelucci, F. (2011). Brain and serum levels of nerve growth factor in a rat model of Alzheimer's disease. *Journal of Alzheimer's Disease, 25*, 213–217.

Gilad, G. M. (1987). The stress-induced response of the septo-hippocampal cholinergic system. A vectorial outcome of psychoneuroendocrinological interactions. *Psychoneuroendocrinology, 12*, 167–184.

Hagan, J. J., & Morris, R. G. M. (1988). The cholinergic hypothesis of memory: A review of animal experiments. *Handbook of Psychopharmachology, 20*, 237–323.

Harmon, K. M., & Wellman, C. L. (2003). Differential effects of cholinergic lesions on dendritic spines in frontal cortex of young adult and aging rats. *Brain Research, 992*, 60–68.

Heckers, S., Ohtake, T., Wiley, R. G., Lappi, D. A., Geula, C., & Mesulam, M. M. (1994). Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. *Journal of Neuroscience, 14*, 1271–1289.

Kilgard, M. P., & Merzenich, M. M. (1998). Cortical map reorganization enabled by nucleus basalis activity. *Science, 279*, 1714–1718.

Kim, I., Wilson, R. E., & Wellman, C. L. (2005). Aging and cholinergic deafferentation alter GluR1 expression in rat frontal cortex. *Neurobiology of Aging, 26*, 1073–1081.

Kirov, S. A., & Harris, K. M. (1999). Dendrites are more spiny on mature hippocampal neurons when synapses are inactivated. *Nature Neuroscience, 2*, 878–883.

Leanza, G., Nilsson, O. G., Nikkhah, G., Wiley, R. G., & Björklund, A. (1996). Effects of neonatal lesions of the basal forebrain cholinergic system by 192 immunoglobulin G-saporin: Biochemical, behavioural and morphological characterization. *Neuroscience, 74*, 119–141.

Lehmann, O., Jeltsch, H., Lehnardt, O., Pain, L., Lazarus, C., & Cassel, J. C. (2000). Combined lesions of cholinergic and serotonergic neurons in the rat brain using 192 IgG-saporin and 5,7-dihydroxytryptamine: Neurochemical and behavioural characterization. *European Journal of Neuroscience, 12*, 67–79.

Lehmann, O., Jeltsch, H., Lazarus, C., Tritschler, L., Bertrand, F., & Cassel, J. C. (2002). Combined 192 IgG-saporin and 5,7-dihydroxytryptamine lesions in the male rat brain: A neurochemical and behavioral study. *Pharmacology, Biochemistry, and Behavior, 72*, 899–912.

Lehmann, O., Grottick, A. J., Cassel, J. C., & Higgins, G. A. (2003). A double dissociation between serial reaction time and radial maze performance in rats subjected to 192 IgG-saporin lesions of the nucleus basalis and/or the septal region. *European Journal of Neuroscience, 18*, 651–666.

Mandolesi, L., De Bartolo, P., Foti, F., Gelfo, F., Federico, F., Leggio, M. G., & Petrosini, L. (2008). Environmental enrichment provides a cognitive reserve to be spent in the case of brain lesion. *Journal of Alzheimer's Disease, 15*, 11–28.

Markowska, A. L., Olton, D. S., & Givens, B. (1995). Cholinergic manipulations in the medial septal area: Age-related effects on working memory and hippocampal electrophysiology. *Journal of Neuroscience, 15*, 2063–2073.

Matsuoka, N., Maeda, N., Ohkubo, Y., & Yamaguchi, I. (1991). Differential effects of physostigmine and pilocarpine on the spatial memory deficits produced by two septo-hippocampal deafferentations in rats. *Brain Research, 559*, 233–240.

McDonald, M. P., Wenk, G. L., & Crawley, J. N. (1997). Analysis of galanin and the galanin antagonist M40 on delayed non-matching-to-position performance in rats lesioned with the cholinergic immunotoxin 192 IgG-saporin. *Behavioral Neuroscience, 111*, 552–563.

McGaughy, J., & Sarter, M. (1998). Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: Effects of physostigmine and FG 7142. *Behavioral Neuroscience, 112*, 1519–1525.

McGaughy, J., & Sarter, M. (1999). Effects of ovariectomy, 192 IgG-saporin-induced cortical cholinergic deafferentation, and administration of estradiol on sustained attention performance in rats. *Behavioral Neuroscience, 113*, 1–17.

McGaughy, J., Kaiser, T., & Sarter, M. (1996). Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: Selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behavioral Neuroscience, 110*, 247–256.

McGaughy, J., Decker, M. W., & Sarter, M. (1999). Enhancement of sustained attention performance by the nicotinic acetylcholine receptor agonist ABT-418 in intact but not basal forebrain-lesioned rats. *Psychopharmacology, 119*, 175–182.

McGaughy, J., Everitt, B. J., Robbins, T. W., & Sarter, M. (2000). The role of cortical cholinergic afferent projections in cognition: Impact of new selective immunotoxins. *Behavioural Brain Research, 115*, 251–263.

McGaughy, J., Dalley, J. W., Morrison, C. H., Everitt, B. J., & Robbins, T. W. (2002). Selective behavioral and neurochemical effects of cholinergic lesions produced by intrabasalis infusions of 192 IgG-saporin on attentional performance in a five-choice serial reaction time task. *Journal of Neuroscience, 22*, 1905–1913.

McMahan, R. W., Sobel, T. J., & Baxter, M. G. (1997). Selective immunolesions of hippocampal cholinergic input fail to impair spatial working memory. *Hippocampus, 7*, 130–136.

Mesulam, M. M., Mufson, E. J., Wainer, B. H., & Levey, A. I. (1983). Central cholinergic pathways in the rat: An overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience, 10*, 1185–1201.

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., & Tabira, T. (2001). Effect of chronic stress on cholinergic transmission in rat hippocampus. *Brain Research, 915*, 108–111.

Mizuno, T., & Kimura, F. (1997). Attenuated stress response of hippocampal acetylcholine release and adrenocortical secretion in aged rats. *Neuroscience Letters, 222*, 49–52.

Nilsson, O. G., Strecker, R. E., Daszuta, A., & Björklund, A. (1988). Combined cholinergic and serotonergic denervation of the forebrain produces severe deficits in a spatial learning task in the rat. *Brain Research, 453*, 235–246.

Nilsson, O.G., Leanza, G., Rosenblad, C., Lappi, D.A., Wiley, R.G., & Björklund, A. (1992). Spatial learning impairments in rats with selective immunolesion of the forebrain cholinergic system. *Neuroreport, 3,* 1005–1008.

Ohtake, T., Heckers, S., Wiley, R. G., Lappi, D. A., Mesulam, M. M., & Geula, C. (1997). Retrograde degeneration and colchicine protection of basal forebrain cholinergic neurons following hippocampal injections of an immunotoxin against the P75 nerve growth factor receptor. *Neuroscience, 78*, 123–133.

Paban, V., Chambon, C., Jaffard, M., & Alescio-Lautier, B. (2005a). Behavioral effects of basal forebrain cholinergic lesions in young adult and aging rats. *Behavioral Neuroscience, 119*, 933–945.

Paban, V., Jaffard, M., Chambon, C., Malafosse, M., & Alescio-Lautier, B. (2005b). Time course of behavioral changes following basal forebrain cholinergic damage in rats: Environmental enrichment as a therapeutic intervention. *Neuroscience, 132*, 13–32.

Pang, K. C., Nocera, R., Secor, A. J., & Yoder, R. M. (2001). GABAergic septohippocampal neurons are not necessary for spatial memory. *Hippocampus, 11*, 814–827.

Pappas, B. A., Davidson, C. M., Fortin, T., Nallathamby, S., Park, G. A., Mohr, E., & Wiley, R. G. (1996). 192 IgG-saporin lesion of basal forebrain cholinergic neurons in neonatal rats. *Brain Research. Developmental Brain Research, 96*, 52–61.

Pappas, B. A., Payne, K. B., Fortin, T., & Sherren, N. (2005). Neonatal lesion of forebrain cholinergic neurons: Further characterization of behavioral effects and permanency. *Neuroscience, 133*, 485–492.

Perry, E. K., Tomlinson, B. E., Blessed, G., Bergmann, K., Gibson, P. H., & Perry, R. H. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *British Medical Journal, 2*, 1457–1479.

Perry, T., Hodges, H., & Gray, J. A. (2001). Behavioural, histological and immunocytochemical consequences following 192 IgG-saporin immunolesions of the basal forebrain cholinergic system. *Brain Research Bulletin, 54*, 29–48.

Pioro, E. P., & Cuello, A. C. (1988). Purkinje cells of adult rat cerebellum express nerve growth factor receptor immunoreactivity: Light microscopy observations. *Brain Research, 455*, 182–186.

Rennie, K., Fréchette, M., & Pappas, B. A. (2011). The effects of neonatal forebrain cholinergic lesion on adult hippocampal neurogenesis. *Brain Research, 1373*, 79–90.

Ricceri, L. (2003). Behavioral patterns under cholinergic control during development: Lessons learned from the selective immunotoxin 192 IgG saporin. *Neuroscience and Biobehavioral Reviews, 27*, 377–384.

Ricceri, L., Minghetti, L., Moles, A., Popoli, P., Confaloni, A., De Simone, R., Piscopo, P., Scattoni, M.L., Di Luca, M., Calamandrei, G. (2004). Cognitive and neurological deficits induced by early and prolonged basal forebrain cholinergic hypofunction in rats. *Experimental Neurology, 189,* 162–172.

Ricceri, L., Cutuli, D., Venerosi, A., Scattoni, M.L., Calamandrei, G. (2007). Neonatal basal forebrain cholinergic hypofunction affects ultrasonic vocalizations and fear conditioning responses in preweaning rats. *Behavioral Brain Research, 183,* 111–117.

Ricceri, L., Calamandrei, G., & Berger-Sweeney, J. (1997). Different effects of postnatal day 1 versus 7 192 immunoglobulin G-saporin lesions on learning, exploratory behaviors, and neurochemistry in juvenile rats. *Behavioral Neuroscience, 111*, 1292–1302.

Ricceri, L., Usiello, A., Valanzano, A., Calamandrei, G., Frick, K., & Berger-Sweeney, J. (1999). Neonatal 192 IgG-saporin lesions of basal forebrain cholinergic neurons selectively impair response to spatial novelty in adult rats. *Behavioral Neuroscience, 113*, 1204–1215.

Ricceri, L., Hohmann, C., & Berger-Sweeney, J. (2002). Early neonatal 192 IgG saporin induces learning impairments and disrupts cortical morphogenesis in rats. *Brain Research, 954*, 160–172.

Rispoli, V., Marra, R., Costa, N., Scipione, L., Rotiroti, D., De Vita, D., Liberatore, F., & Carelli, V. (2006). Choline pivaloyl ester strengthened the benefit effects of Tacrine and Galantamine on electroencephalographic and cognitive performances in nucleus basalis magnocellularis-lesioned and aged rats. *Pharmacology, Biochemistry, and Behavior, 84*, 453–467.

Savage, S., Kehr, J., Olson, L., & Mattsson, A. (2011). Impaired social interaction and enhanced sensitivity to phencyclidine-induced deficits in novel object recognition in rats with cortical cholinergic denervation. *Neuroscience, 195*, 60–69.

Scattoni, M. L., Puopolo, M., Calamandrei, G., & Ricceri, L. (2005). Basal forebrain cholinergic lesions in 7-day-old rats alter ultrasound vocalisations and homing behaviour. *Behavioural Brain Research, 161*, 169–172.

Scattoni, M. L., Adriani, W., Calamandrei, G., Laviola, G., & Ricceri, L. (2006). Long-term effects of neonatal basal forebrain cholinergic lesions on radial maze learning and impulsivity in rats. *Behavioural Pharmacology, 17*, 517–524.

Shen, J., Barnes, C. A., Wenk, G. L., & McNaughton, B. L. (1996). Differential effects of selective immunotoxic lesions of medial septal cholinergic cells on spatial working and reference memory. *Behavioral Neuroscience, 110*, 1181–1186.

Steckler, T., Keith, A. B., Wiley, R. G., & Sahgal, A. (1995). Cholinergic lesions by 192 IgG-saporin and short-term recognition memory: Role of the septo-hippocampal projection. *Neuroscience, 66*, 101–114.

Thomas, L. B., Book, A. A., & Schweitzer, J. B. (1991). Immunohistochemical detection of a monoclonal antibody directed against the NGF receptor in basal forebrain neurons following intraventricular injection. *Journal of Neuroscience Methods, 37*, 37–45.

Torres, E. M., Perry, T. A., Blockland, A., Wilkinson, L. S., Wiley, R. G., Lappi, D. A., & Dunnet, S. B. (1994). Behavioural, histochemical and biochemical consequences of selective immunolesions in discrete regions of the basal forebrain cholinergic system. *Neuroscience, 63*, 95–122.

Traissard, N., Herbeaux, K., Cosquer, B., Jeltsch, H., Ferry, B., Galani, R., Pernon, A., Majchrzak, M., & Cassel, J. C. (2007). Combined damage to entorhinal cortex and cholinergic basal forebrain neurons, two early neurodegenerative features accompanying Alzheimer's disease: Effects on locomotor activity and memory functions in rats. *Neuropsychopharmacology, 32*, 851–871.

Turchi, J., & Sarter, M. (1997). Cortical acetylcholine and processing capacity: Effects of cortical cholinergic deafferentation on crossmodal divided attention in rats. *Cognitive Brain Research, 6*, 147–158.

Vnek, N., Kromer, L. F., Wiley, R. G., & Rothblat, L. A. (1996). The basal forebrain cholinergic system and object memory in the rat. *Brain Research, 710*, 265–270.

Waite, J. J., & Thal, L. J. (1996). Lesions of the cholinergic nuclei in the rat basal forebrain: Excitotoxins vs. an immunotoxin. *Life Sciences, 58*, 1947–1953.

Waite, J. J., Chen, A. D., Wardlow, M. L., Wiley, R. G., Lappi, D. A., & Thal, L. J. (1995). 192 Immunoglobulin G-saporin produces graded behavioral and biochemical changes accompanying the loss of cholinergic neurons of the basal forebrain and cerebellar purkinje cells. *Neuroscience, 65*, 463–476.

Waite, J. J., Wardlow, M. L., & Power, A. E. (1999). Deficit in selective and divided attention associated with cholinergic basal forebrain immunotoxic lesion produced by 192-saporin; motoric/sensory deficit associated with Purkinje cell immunotoxic lesion produced by OX7-saporin. *Neurobiology of Learning and Memory, 71*, 325–352.

Walsh, T. J., & Opello, K. D. (1994). The use of AF64A (ethylcholine aziridium ion) to model Alzheimer's disease. In *Toxi-induced models of neurological disorders* (pp. 259–279). New York: Plenum press.

Walsh, T. J., Kelly, R. M., Dougherty, K. D., Stackman, R. W., Wiley, R. G., & Kutscher, C. L. (1995). Behavioral and neurobiological alterations induced by the immunotoxin 192-IgG-saporin: Cholinergic and non-cholinergic effects following i.c.v. injection. *Brain Research, 702*, 233–245.

Walsh, T. J., Herzog, C. D., Gandhi, C., Stackman, R. W., & Wiley, R. G. (1996). Injection of IgG 192-saporin into the medial septum produces cholinergic hypofunction and dose-dependent working memory deficits. *Brain Research, 726*, 69–79.

Wellman, C. L., & Sengelaub, D. R. (1995). Alterations in dendritic morphology of frontal cortical neurons after basal forebrain lesions in adult and aged rats. *Brain Research, 669*, 48–58.

Wenk, G. L., Stoehr, J. D., Quintana, G., Mobley, S., & Wiley, R. G. (1994). Behavioral, biochemical, histological, and electrophysiological effects of 192 IgG-saporin injections into the basal forebrain of rats. *Journal of Neuroscience, 14*, 5986–5995.

Wiley, R. G. (1992). Neural lesioning with ribosome-inactivating proteins: Suicide transport and immunolesioning. *Trends in Neurosciences, 15*, 285–290.

Wiley, R. G., Berbos, T. G., Deckwerth, T. L., Johnson, E. M., Jr., & Lappi, D. A. (1995). Destruction of the cholinergic basal forebrain using immunotoxin to rat NGF receptor: Modeling the cholinergic degeneration of Alzheimer's disease. *Journal of the Neurological Sciences, 128*, 157–166.

Winters, B. D., & Bussey, T. J. (2005). Removal of cholinergic input to perirhinal cortex disrupts object recognition but not spatial working memory in the rat. *European Journal of Neuroscience, 21*, 2263–2270.

Works, S. J., Wilson, R. E., & Wellman, C. L. (2004). Age-dependent effect of cholinergic lesion on dendritic morphology in rat frontal cortex. *Neurobiology of Aging, 25*, 963–974.

Wrenn, C. C., & Wiley, R. G. (1998). The behavioral functions of the cholinergic basal forebrain: lessons from 192 IgG-saporin. *International Journal of Developmental Neuroscience, 16*, 595–602.

Wrenn, C. C., & Wiley, R. G. (2001). Lack of effect of moderate Purkinje cell loss on working memory. *Neuroscience, 107*, 433–445.

Wrenn, C. C., Lappi, D. A., & Wiley, R. G. (1999). Threshold relationship between lesion extent of the cholinergic basal forebrain in the rat and working memory impairment in the radial maze. *Brain Research, 847*, 284–298.

Zhang, Z. J., Berbos, T. G., Wrenn, C. C., & Wiley, R. G. (1996). Loss of nucleus basalis magnocellularis, but not septal, cholinergic neurons correlates with passive avoidance impairment in rats treated with 192-saporin. *Neuroscience Letters, 203*, 214–218.

TRPV1 Activators ("Vanilloids") as Neurotoxins

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Contents

Abstract

A distinct subset of primary sensory neurons is distinguished by their unique sensitivity to capsaicin, the pungent ingredient in hot chili peppers. The initial excitation by capsaicin of these neurons is followed by a long-lasting, but fully reversible, refractory state (traditionally termed as desensitization) or under certain conditions, like neonatal treatment, frank neurotoxicity. This neurotoxic action was extensively used to identify capsaicin-sensitive neuronal pathways and to explore their physiological function. In 1997, a specific receptor for capsaicin and related compounds (collectively referred to as vanilloids) was identified as transient receptor potential cation

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channel subfamily V member 1 (TRPV1), a multifunctional channel involved in thermosensation (heat) and taste perception (e.g., peppers and vinegar). Importantly, TRPV1 also functions as a molecular integrator for a broad range of seemingly unrelated noxious stimuli including venoms from spiders and jellyfish. Indeed, TRPV1 is thought to be a major transducer of the thermal hyperalgesia that follows inflammation and/or tissue injury. Ablation of sensory neurons by vanilloids is, however, not only a research tool but also has a clear therapeutic potential. Currently, site-specific resiniferatoxin (an ultrapotent capsaicin analog) injections are being evaluated as "molecular scalpels" to achieve permanent analgesia in cancer patients with chronic, intractable pain. In this chapter, we review our knowledge of the molecular mechanisms underlying vanilloid-induced neurotoxicity, which includes both TRPV1-mediated and independent signalling pathways.

Keywords

Capsaicin • Resiniferatoxin • The capsaicin (vanilloid) receptor TRPV1 • Vanilloids

List of Abbreviations

CCK-B	Cholecystokinin receptor-B
CGRP	Calcitonin gene-related peptide
CNS	Central nervous system
CTB	Cholera toxin subunit B
DRG neuron	Dorsal root ganglion neuron
ETC1	Electron transport chain complex 1
ETC3	Electron transport chain complex 3
ICK-peptides	Inhibitory cystine knot peptides
ISH	In situ hybridization
MPT	Membrane permeability transition
NADA	*N*-arachidonoyl-dopamine
NNOS	Neuronal nitric oxide synthase
OLDA	*N*-oleoyldopamine
PALDA	*N*-palmitoyldopamine
PCR	Polymerase chain reaction
PKC	Protein kinase C
PMOR	Plasma membrane NADH oxidoreductase
ROPA	Resiniferonol 9,13,14-orthophenylacetate
ROS	Reactive oxygen species
RTX	Resiniferatoxin
SP	Substance P
STEARDA	*N*-stearoyldopamine
TG-neurons	Trigeminal ganglion neurons

TiTX	Tinyatoxin
TRPV1	Transient receptor potential cation channel subfamily V member 1

1 Introduction

Natural products provide a window of opportunity to identify new targets for pharmacological intervention. Capsaicin, the active principle in hot chili peppers (*Capsicum annum*), is a prime example. Connoisseurs of hot spicy food are intimately familiar with the predominant pharmacological actions of capsaicin from personal experience: it induces profuse perspiration (known as gustatory sweating) as well as a hot, burning sensation that dissipates upon repeated challenge (desensitization). Evolutionary selective pressure seems to have maximized the pungency of capsaicin. It was speculated that the compound's pungency is able to deter ambulatory animals from eating chili pepper fruits, favoring those plants whose seeds were dispersed widely by birds. Indeed, the avian TRPV1 receptor is not activated by capsaicin, and hence birds are undeterred from ingesting chili pepper fruits and can excrete the pepper seeds large distances away. This forms the basis of the development of hot pepper-flavored "squirrel-free" bird feed. It is still a mystery, however, why the same pungency that repels squirrels is perceived as pleasurable by many human beings.

Although topical capsaicin has been used in folk medicine to relieve pain for centuries, it took the genius of the late Miklós (Nicholas) Jancsó to ask the obvious question: how can an irritant molecule paradoxically cause analgesia? Jancsó noticed that the initial transient irritation by capsaicin was followed by a long-lasting refractory state in which the neurons not only did not respond to a repeated capsaicin challenge but were also insensitive to various unrelated stimuli, and he termed this state as "desensitization" (Jancsó and Jancsó 1949; Jancsó-Gábor et al. 1970). Per definition, capsaicin-mediated desensitization is reversible. However, following neonatal treatment or when sufficiently high doses are used in adult animals, capsaicin induces neuronal death and causes permanent analgesia (Jancsó et al. 1984). This forms the experimental foundation for the use of site-specific resiniferatoxin (an ultrapotent capsaicin analog) injections to achieve permanent relief of otherwise intractable pain in cancer patients. Furthermore, chemical ablation of sensitive neurons by capsaicin turned out to be an invaluable tool to dissect capsaicin-sensitive pathways and to explore their physiological function. Generally speaking, these neurons comprise a functional subset of peptidergic primary sensory neurons with unmyelinated (C-type) fibers that respond to noxious stimuli and initiate the cascade of neurogenic inflammation.

The molecular mechanisms underlying capsaicin-mediated desensitization are poorly understood, and the line between desensitization and neurotoxicity is most likely ill defined and arbitrary. The sensitization of TRPV1 by different kinases and involvement of different pathways also remain unclear (Vellani et al. 2006;

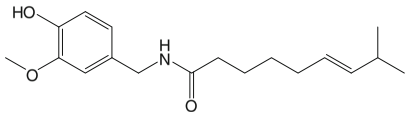
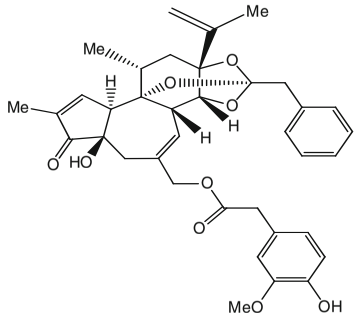
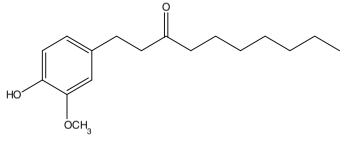
Otten et al. 1983; Negri et al. 2006). The cloned capsaicin (vanilloid) receptor TRPV1 is an inwardly rectifying cation channel with limited selectivity for Ca^{2+}, and Ca^{2+}-overload is a well-established cause of neurotoxicity (Chard et al. 1995; Caterina et al. 1997; Sugimoto et al. 1998; Czaja et al. 2008). Indeed, neurochemical calcium and other ions such as cobalt stains were historically used to visualize sensitive neurons in capsaicin-treated animals (Winter 1987). At the ultrastructural level, neuronal calcium staining is coupled to swollen mitochondria, but it is still hotly debated if this reflects a buffering of cytoplasmic Ca^{2+} by the mitochondria (a direct TRPV1-mediated effect) or an indirect, TRPV1-independent interference of capsaicin with the mitochondrial electron transport chains (ETCs) (Szolcsányi et al. 1971).

The first vanilloids to be identified were pungent phytochemical compounds that typically contain a vanillyl (4-hydroxy-3-methoxybenzyl) moiety essential for bioactivity (hence the name "vanilloid") and are present in nature, mainly as plant resources (Table 1). Prime examples include capsaicin (the pungent ingredient in chili peppers), piperine (responsible for the piquancy of black pepper), resiniferatoxin (isolated from the latex of the cactus-like plant *Euphorbia resinifera*), curcumin (from *Curcuma longa*), eugenol (in clove), and zingerone (from ginger). Subsequently, vanilloids (so-called endovanilloids) were isolated from animal tissues (e.g., *N*-arachidonoyl-dopamine, NADA, isolated from rat brain) or were obtained via chemical synthesis to explore structure-activity relations (Huang et al. 2002). Few more examples are *N*-oleoyldopamine (OLDA), *N*-palmitoyldopamine (PALDA), and *N*-stearoyldopamine (STEARDA) (Chu et al. 2003). Indeed, the existence of a specific capsaicin receptor was first postulated based on the fairly strict structural requirements for capsaicin-like bioactivity.

2 Capsaicin-Sensitive Neurons

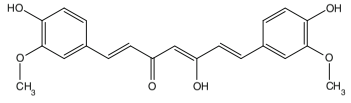
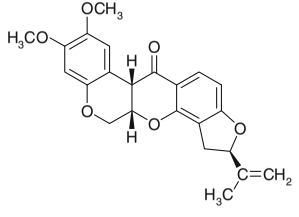
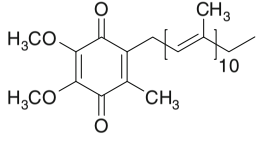
In culture, capsaicin kills ~35 % of dorsal root ganglion (DRG) neurons. It was postulated that capsaicin destroys these neurons due to influx of excess Ca^{2+} (Chard et al. 1995). Indeed, capsaicin causes a drastic increase in basal intracellular Ca^{2+} concentration when applied to the cultured DRG neuron. This in vitro toxicity correlates with the observation that application of capsaicin in vivo for prolonged periods can cause irreversible toxic effect leading to loss of pain-sensing neurons (Holzer 1991; Maggi 1991). The DRG neurons abolished by capsaicin are small in size, possess either unmyelinated C- or thinly myelinated Aδ–fibers, and show large inward intracellular Ca^{2+} currents in response to capsaicin. Both Ca^{2+} current and the neurotoxic effect of capsaicin were prevented by pre-treating the cells with ruthenium red, currently recognized as a general, nonspecific blocker of TRP channels. The neurotoxic action of capsaicin can also be prevented by chelating extracellular Ca^{2+} and/or by blocking Ca^{2+}-activated proteases like calcineurin (Docherty et al. 1996). Taken together, these results imply that capsaicin exerts its neurotoxic effects predominantly due to

Table 1 Example of some vanilloids

Vanilloids	Chemical structure	Molecular formula	Origin	Properties and action	Reference
Capsaicin		$C_{18}H_{27}NO_3$	*Capsicum* genus	Analgesic, anticancer, antiobesity, anti-inflammatory, antioxidant activates TRPV1 at 10–8 to 10–6 M	Caterina et al. 1997; Luo et al. 2011; Knotkova et al. 2008.
Resiniferatoxin		$C_{37}H_{40}O_9$	*Euphorbia resinifera*	Inhibits inflammatory hyperalgesia, activates TRPV1 at 10–10 to 10–8 M	Neubert et al. 2008; Caterina et al. 1997.
Paradol		$C_{17}H_{26}O_3$	*Aframomum melegueta*	Antioxidative, antitumor, activates TRPA1 and TRPV1	Chung et al. 2001; Riera et al. 2009.

(*continued*)

Table 1 (continued)

Vanilloids	Chemical structure	Molecular formula	Origin	Properties and action	Reference
Curcumin		$C_{21}H_{20}O_6$	*Zingiberaceae* family	Used for pain relief, anticarcinogenic and food additive	Kuptniratsaikul et al. 2009; Aggarwal and Shishodia 2004.
Rotenone		$C_{23}H_{22}O_6$	*Lonchocarpus nicou*	Used as pesticide, insecticide, piscicide, and neurotoxin	Swarnkar et al. 2012; Cabeza-Arvelaiz and Schiestl 2012
Coenzyme Q		$C_{59}H_{90}O_4$	Most eukaryotic cells	Antioxidant	Littarru and Tiano 2007; Hoppe et al. 1999

consequences of both increased Ca^{2+} concentration inside the cell and cascades of cellular signalling events (Chard et al. 1995).

Capsaicin-sensitive sensory neurons are bipolar neurons with somata in dorsal root and trigeminal ganglia. The peripheral fibers innervate the skin as well as the viscera, whereas the central axons enter the central nervous system (CNS) where they form synapse at second-order neurons in the dorsal horn of the spinal cord and the spinal nucleus of the trigeminal tract, respectively. The vagal nerve also supplies capsaicin-sensitive innervations to some visceral organs: the cell bodies of these neurons are in the nodose ganglia, and they centrally project to the area postrema. The presence of TRPV1 in these neuronal pathways is firmly established by a combination of [^{3}H]-labeled resiniferatoxin (RTX) autoradiography, TRPV1 immunostaining, and other molecular methods (Szallasi and Blumberg 1999). The existence of TRPV1-expressing brain nuclei is, however, controversial.

In 1988, using a silver impregnation method, Ritter and Dinh described unexpected neuronal degeneration in several discrete forebrain and hindbrain areas of adult rats following i.p. capsaicin administration (50 or 90 mg/kg) (Ritter and Dinh. 1988). Subsequently, the presence of TRPV1 receptors was reported in corresponding (and also in additional) rat brain nuclei (and also in other parts) and the cortex in [^{3}H]RTX-binding experiments as well as by polymerase chain reaction (PCR), in situ hybridization (ISH), Western blot analysis, and immunostaining studies targeting to detect TRPV1 at RNA and protein level (Acs et al. 1996; Tóth et al. 2005; Doyle et al. 2002; Valtschanoff et al. 2001; Caterina et al. 1997; Goswami et al. 2010; Sasamura et al. 1998). Recordings obtained from rat brain slices implied that these TRPV1 receptors were functional and might be involved in learning and memory formation. Indeed, TRPV1 has been linked to the pathogenesis of Alzheimer disease (Micale et al. 2010; Pákáski et al. 2009). An additional important role for brain TRPV1 in anxiety and fear was postulated based on studies in TRPV1 knockout animals (Marsch et al. 2007). Most recently, however, only weak and minimal TRPV1 expression was found in rodent brain using a sensitive and selective reporter-mouse model (Tóth et al. 2005). This discrepancy is puzzling. Furthermore, relevant to the topic of this review, it clearly questions the value of capsaicin-induced toxicity to dissect "capsaicin sensitive" (i.e., TRPV1-expressing) pathways.

3 Plant-Derived Vanilloid Toxins

Though there are handful examples of vanilloids from plant origin, in this chapter, only vanilloids, namely, capsinoid and resiniferanoids, will be discussed in details.

Capsinoids: Capsaicin, the archetypal vanilloid, is responsible for the piquancy of hot chili peppers (Buck and Burks 1986). Capsaicin and related compounds are collectively called capsinoids. Naturally occurring capsinoids include capsiate, dihydrocapsiate, and nordihydrocapsiate. Like capsaicin, capsinoids activate TRPV1 (Iida et al. 2003), yet, they were reported to be sweet-tasting. Of synthetic capsinoids,

in the past olvanil and nuvanil had attracted attention as "improved" (candidate molecules that can be given per os to achieve desensitization) in preclinical models of chronic pain. The synthetic capsaicin, civamide (*cis*-capsaicin), is under clinical development by Winston Pharmaceuticals for indications like cluster headache.

Resiniferanoids: Resiniferatoxin (RTX) is a naturally occurring ultrapotent capsaicin analog isolated from the dried latex of the cactus-like perennial *Euphorbia resinifera*, a native of the Anti-Atlas Mountains in Morocco (Appendino and Szallasi 1997). The closely related tinyatoxin (TiTX) is present in the soap of *Euphorbia poissonii*, a succulent plant in Nigeria, which is traditionally used as a pesticide. TiTX is somewhat (approximately threefold) less potent than RTX. Although a method for the full synthesis of RTX is available, the molecule is still isolated from its natural host or obtained semi-synthetically from its parent diterpene, resiniferonol 9,13,14-orthophenylacetate (ROPA). Attempts to recapitulate the ultra potency of RTX by synthesizing phorbol-based vanilloids (phorbol is inexpensive and its chemistry is well known) met limited success. Of note, ROPA is a potent activator of protein kinase C (PKC) and acts as a tumor promoter (Frey et al. 2004). At nanomolar concentrations RTX activates TRPV1, but does not activate PKC, and does not promote the formation of tumor either in the mouse skin model of two-stage carcinogenesis (Driedger and Blumberg 1980). RTX, however, did show unexpected activity against various cancer cell lines in a most likely TRPV1-independent manner (discussed later in details).

4 Venoms as Toxins for Vanilloid Receptors

Venomous creatures such as spiders, scorpions, snakes, and snails contain a wealth of peptide toxins which can activate or block different ion channels including vanilloid receptors and produce shock, paralysis, or death (Escoubas and Rash 2004; Miller 1995; Terlau and Olivera 2004). Often such venoms and toxins and their activity on ion channels define the pray-predator relationship and exert selection pressure of certain species in specific environmental niches (Fry et al. 2006). Some venoms contain toxins which act on somatosensory neurons and provoke a noxious sensation, primarily in mammals (Siemens et al. 2006; Bohlen and Julius 2012). Somewhat unexpectedly, bites or stings by such venomous creatures were shown to activate the TRPV1 receptor and produce inflammatory pain (Siemens et al. 2006; Cuypers et al. 2006).

Venom from a *Tarantula* sp., *Psalmopoeus cambridgei*, commonly found in the West Indies, has three inhibitory cystine knot (ICK) peptides present in one peptide (Siemens et al. 2006). Previously, it was shown that venoms and toxins from spiders, snakes, and scorpions mainly target three members of the TRP channel family, namely, TRPV1, TRPA1, and TRPM8 (Siemens et al. 2006). By reverse phase chromatographic technique, the *Tarantula* venom was purified and fractionated further. When testing the bioactive fractions on HEK-293 cells heterogeneously expressing different TRP channels, it was demonstrated that the toxin was selective to TRPV1 (Siemens et al. 2006). Edman sequencing revealed the

presence of three closely related peptides named vanillotoxin 1, vanillotoxin 2, and vanillotoxin 3, all of which can act as agonists for TRPV1. Similar to the ICK peptide, these vanillotoxins have six cysteine residues in each toxin sequence (Zhu et al. 2003). Recently, another tarantula, namely, *Ornithoctonus huwena*, from China has been reported to produce a toxin that functions as a TRPV1 agonist (Bohlen et al. 2010). Interestingly, this toxin is similar to the vanillotoxin 1–3. This toxin is named double knot toxin because it has two ISK motifs repeated in tandem (Bohlen et al. 2010).

For the sake of completeness, it should be mentioned that there are venoms which contain inhibitory components against TRPV1. For example, extract from a nematocyte (sea anemone *Heteractis crispa*) contains a peptide, named APHC1, which blocks TRPV1 (Andreev et al. 2008). Furthermore, venom from a funnel web spider (*Agelenopsis aperta*) has two toxin components (AG489 and AG505) which inhibit TRPV1 (Kitaguchi and Swartz 2005).

5 The Diversity of Vanilloid Actions

The spectrum of vanilloid actions depends not only on the pharmacophore but also on the species and the route of administration. One has to carefully consider all these factors before selecting the appropriate compound for the studies.

Pharmacophore: RTX functions as an ultrapotent capsaicin analog with a peculiar spectrum of pharmacological activities. For example, in the rat, RTX is several thousandfold more potent than capsaicin in inhibiting the xylene-induced neurogenic inflammatory response or in causing twitch inhibition in the vas deferens (Wardle et al. 1996). By contrast, RTX is only similar in potency to capsaicin in evoking the eye-wiping response upon intraocular instillation. RTX also has unique actions: unlike capsaicin which repeatedly evokes the pulmonary chemo-reflex with no evidence of desensitization, RTX desensitizes this reflex without causing any detectable prior activation. Interestingly, RTX and capsaicin congeners showed distinct structure-activity relations for receptor binding and Ca^{2+} uptake, respectively. Resiniferanoids displayed high affinity in the binding assay and low activity in the Ca^{2+} uptake assay, whereas capsinoids showed the opposite pattern. Based on these observations, the existence of two types of vanilloid receptor was postulated, an R-type (presumably metabotropic) vanilloid receptor responsible for desensitization and a C-type (most likely) ionotropic receptor mediating irritation. This hypothesis, however, turned out to be not true: following the cloning of TRPV1, it was recognized that the same receptor mediates both R-type binding and C-type calcium responses (Caterina et al. 1997). Pharmacokinetics appears to play an important role in determining RTX-like and capsaicin-like activities. In most cases, capsaicin-evoked Ca^{2+} current is rapid in onset, large in magnitude, and short in duration. By contrast, RTX induces sustained, slowly developing and long-lasting currents. This observation might explain why RTX treatment shows a better desensitization-to-excitation ratio than capsaicin for most end-points examined.

Species Differences: Evolutionary, heat, and capsaicin sensitivity seems to have developed over a time in different species. However, different species reveal differences in responsiveness against heat and capsaicin (Nagy and Rang 2000; Phillips et al. 2004; Jordt and Julius 2002; Sardar et al. 2012). Even mammalian species show striking differences in the way they react to capsaicin challenge (Gavva et al. 2004). Reptiles, such as snakes, seem to be insensitive to capsaicin as trigeminal ganglion (TG) neurons from snakes do not respond to capsaicin (Gracheva et al. 2010). Birds do not respond to capsaicin. In keeping with this, chicken DRG neurons lack specific [^{3}H]RTX-binding sites, and the treatment of DRG neurons from chicken with capsaicin does not results in Ca^{2+} influx (Jordt and Julius 2002; Goswami et al. 2007). Chicken, however, do possess TRPV1 but it is resistant to capsaicin due to a point mutation at the position of S512 which confers capsaicin sensitivity in mammals (Jordt and Julius 2002). Within mammals, rats and rabbits are a good example for species-related differences in capsaicin sensitivity (Gavva et al. 2004). In rats, application of 1 % capsaicin to the saphenous nerve resulted in a 35 % decrease in the C:Aδ fiber ratio (Pini et al. 1990), while the same concentration of capsaicin administration to saphenous nerve of rabbits did not change the C: Aδ fiber ratio (Lynn and Shakhanbeh 1988). This implies the existence of low density and/or low capsaicin-affinity TRPV1 receptors in the rabbit. Indeed, no high-affinity RTX binding was detected in rabbit DRG neuron membranes (Gavva et al. 2004). An intriguing species-related difference in RTX actions was reported in the cat. Unlike in rats where, as described above, RTX desensitizes the pulmonary chemoreflex without prior activation, in the cat, RTX evokes the full reflex triad (Pórszász and Szolcsányi 1991–1992; Sculptoreanu et al. 2005). Bat has two isoforms of TRPV1 of which one is responsive to infrared (Gracheva et al. 2011). However, both isoforms are responsive to capsaicin and high temperature.

Routes of Administration: The site of administration of vanilloids can be an important factor in determining neurotoxicity. This is due to the fact that intrathecal, perineural, intraganglionic, subcutaneous, intraperitoneal, and topical application of vanilloids exposes different parts of sensory neurons to differential vanilloid concentrations. For example, intraganglionic injections expose the cell bodies of sensitive neurons to high vanilloid concentrations, leaving the peripheral terminals intact. By contrast, topical vanilloid administration mainly targets the peripheral nerve terminals with no detectable effect on perikarya. As an added complication, different parts of the primary afferent neurons (central terminals, cell bodies, axons, and peripheral terminals) have their own range of sensitivity to vanilloid agonists. For example, peripheral sensory axons have different sensitivity to capsaicin, with the most vulnerable part being the subepidermal part of the axon (Chung et al. 1990). Intrathecal or intraganglionic administration of RTX selectively deletes TRPV1-expressing neurons and confers irreversible thermal, inflammatory, and cancer pain relief (Brown et al. 2005; Karai et al. 2004; Tender et al. 2005). By contrast, peripheral administration of a single dose of RTX produces a long-lasting but reversible analgesia by ablating nociceptive nerve terminals (Karai et al. 2004; Kissin 2008; Neubert et al. 2003; Goswami et al. 2007). This effect could

last from few days to few weeks and is preceded by a rapid loss of specific [^{3}H]RTX-binding sites which is already apparent 1 h after RTX treatment. Similarly, a long-lasting (up to 1 month) but fully reversible loss of TRPV1-like immunoreactivity was observed in skin biopsies taken from volunteers following exposure to a high-concentration capsaicin patch (Qutenza) (Inoue et al. 2002). Taken together, these results imply that it is impossible to extrapolate vanilloid actions from one species to another, especially if the route of administration is also changed.

6 Vanilloid Interaction with Receptors

Among all TRPV channels, TRPV1 represents the best characterized channel in terms of the vanilloid-mediated activation. It has been demonstrated that the temperature gating of TRPV1 is actually a voltage-dependent process (Voets et al. 2005). In a similar manner, vanilloids also modulate the voltage dependency of the TRP channel in ambient temperature and thus eliminates the requirement for high temperatures. In a simplified manner, it can be said that at molecular level, interaction of vanilloids with TRPV1 results in a conformational change which reduces the requirements for high temperature and thus is suitable for channel opening. However, information regarding the species-specific interaction of vanilloids to TRPV1 remains insufficient and discrepancies in the vanilloid-binding sites remain ambiguous.

Initially, it was demonstrated that capsaicin and capsazepine are competitive inhibitors of RTX binding on membrane fraction suggesting that these compounds probably bind at the same region (Szallasi et al. 1993). Capsaicin was originally believed to activate TRPV1 by binding to its extracellular loops. However, use of a membrane impermeable analog of capsaicin, i.e., DA-5018.HCl, reveals opposite effect on the TRPV1 present in the inside-out patched membrane. This compound activates TRPV1 when applied outside of the pipet, but failed to activate TRPV1 when applied from inside of the patch. This result confirms that capsaicin, being lipophilic in nature, can cross the plasma membrane and thus can bind to an intracellular/intramembranous region of TRPV1 (Jung et al. 1999). Indeed, it was demonstrated that capsaicin binds to the TM regions of TRPV1. The capsaicin-binding region was mapped down to a critical residue (Y511) located at the region between the 1st intracellular loop joining TM2 and TM3 regions of rat TRPV1 (Jordt and Julius 2002). The same position is responsible for capsaicin sensitivity too. In agreement with the involvement of that residues in capsaicin sensitivity, two mutants, namely, TRPV1-Y511A and TRPV1-S512Y (rat TRPV1), abolished capsaicin-mediated activation, while TRPV1-Y511A mutant failed to respond to anandamide also (an endogenous vanilloid that activates TRPV1) (Jordt and Julius 2002). Functional studies with the capsaicin-insensitive orthologs of TRPV1 (avian and rabbit) have demonstrated that capsaicin-mediated gating requires a small region of the TRPV1 which includes residues at positions 511 and 512 (Jordt and Julius 2002; Gavva et al. 2004). Interestingly, the mutant, namely, TRPV1-S512Y, converted the activity of the antagonist 5'I-RTX into an intrinsic agonist, albeit

with a lower potency than its parent compound, RTX (Sutton et al. 2005). Notably, these two residues are present between transmembrane domain 3 (TM3) and the first intracellular loop region. Based on these observations, it was proposed that gating of this channel may involve a sequential movement of a paddle structure. According to this concept, the TM3 and TM4 region of the channel is predicted to form a gating paddle (Chou et al. 2004), with residues such as M547 (Rat) and L547 (human) forming a part of the key agonist-binding site which is accessible from the intracellular interface (Johnson et al. 2006). However, this suggests that the critical agonist-binding region is not buried deep within the transmembrane region as suggested by the traditional homology models (Jordt and Julius 2002; Gavva et al. 2004; Phillips et al. 2004). This concept is also supported by the recent observation made in voltage-dependent potassium channel KvAP where X-ray crystal structure confirmed that amino acids at position 512 and 547 are located in close proximity (Jiang et al. 2003). In agreement with the joint action of TM3 and TM4 in agonist recognition, residue M/L547 located at the TM4 mediates significant species differences in resiniferatoxin (RTX) sensitivity, and the S512 is critical for discriminating between pH and capsaicin gating of TRPV1 (Jordt and Julius 2002).

However, a number of studies indicated the involvement of additional residues located within TM4, the putative S5–S6 pore region, and also at the N- and C-termini of TRPV1 on vanilloid-mediated activities and capsaicin responsiveness (Welch et al. 2000; Vlachova et al. 2003; Gavva et al. 2004; Phillips et al. 2004; Jung et al. 2002). For example, involvement of residues (R114 and E761) located in the N- and C-cytosolic tails of rat TRPV1 respectively has influence on the RTX-binding and RTX-mediated response to TRPV1 (Jung et al. 2002). Similarly, a number of critical residues located within the TM3 and TM4 (also considered as voltage sensor) regions have been shown to be responsible for major species-specific differences in vanilloid activity (Chou et al. 2004; Gavva et al. 2004; Phillips et al. 2004). In agreement with the involvement of TM3 and TM4 in the capsaicin activity in different species, M547 and T550 located in TM3 and TM4 region of TRPV1 (rat as well as human) confer vanilloid sensitivity, [^{3}H]RTX-binding and capsazepine-binding, parameters which are different in rabbit TRPV1 (Gavva et al. 2004). Changing the single residue at 550 in rabbit TRPV1 to the corresponding residue found in rat and human TRPV1 (I550T) was sufficient to confer gain of function for activation by capsaicin (Gavva et al. 2004). Furthermore, TRPV1 (rat as well as in human) mutants, namely, TRPV1-T550I and TRPV1-Y511A, demonstrate a loss of sensitivity to capsaicin (Gavva et al. 2004). A single mutation embedded in the TM4 region of TRPV1 (human), namely, TRPV1-L547M, produced a 30-fold increase in sensitivity to [^{3}H]RTX, whereas the reverse mutation in the rat isoform caused a decrease in sensitivity of equal amplitude (Chou et al. 2004). These critical residues affect ligand recognition to some extent and also affect channel function in response to ligand binding (Gavva et al. 2004).

Three amino acid residues located near the pore region of the rat TRPV1 are also involved in the capsaicin responsiveness. This is confirmed by the TRPV1 mutants,

namely, TRPV1-E636Q, TRPV1-D646N, and TRPV1-E648Q mutants, which affects capsaicin-mediated gating but not the heat or proton-mediated activation of TRPV1 (Welch et al. 2000). In addition, mutation of three residues located in TM6 (NML676FAP) also abolished capsaicin-mediated activation with little effect on ligand binding to rat TRPV1 (Kuzhikandathil et al. 2001). Similarly, another mutant, namely, TRPV1-L547M (Human) located at the 4th TM region, causes a decrease in capsaicin potency (Johnson et al. 2006).

7 Vanilloid-Induced Messenger Plasticity

It was postulated that vanilloids do not affect non-nociceptive neurons and/or mechanosensitive nociceptive neurons (Karai et al. 2004). This is important because, unlike local anaesthetics which target ubiquitous sodium channels in all axons, vanilloids selectively block heat-sensitive TRPV1-positive nociceptors and thus leave other sensory modalities intact, rendering these molecules a better choice for pain relief. In the rat spinal cord, RTX treatment ameliorated the so-called "wind-up" phenomenon after repeated peripheral C-fiber stimulation that is believed to correspond to central sensitization (Xu et al. 1997). This effect on the "wind-up" response correlated to the time course of the thermal hypoalgesia in the hot plate test (Xu et al. 1997). Unexpectedly, RTX treatment also resulted in reduced sensitivity to mechanical stimulation (Pan et al. 2003). The mechanical hypoalgesia was, however, transient (a few days) compared to the thermal hypoalgesia (several weeks). Since TRPV1-expressing C-fiber neurons do not respond to mechanical stimuli (and mechanosensitive A neurons do not express TRPV1), it is unclear how RTX treatment elevates the threshold for mechanical stimulation. One might wonder, however, if this is somehow part of the "messenger plasticity" that follows vanilloid administration.

Paradoxically, RTX treatment can cause mechanical allodynia. Pan et al. injected 200 μg/kg RTX i.p. to rats and observed rapid increase in the paw withdrawal latency to a heat stimulus, while profound tactile allodynia developed in 3 weeks (Pan et al. 2003). This unexpected increase in mechanical sensitivity lasted for at least 6 weeks (Pan et al. 2003). In the RTX-treated rats, IB4-labeled unmyelinated C-fiber terminals in the dorsal horn were significantly reduced, and cholera toxin subunit B (CTB)-labeled myelinated fiber terminals appeared to sprout into lamina II of the spinal dorsal horn (Pan et al. 2003). Electron microscopic examination of the sciatic nerve also revealed significant loss of unmyelinated fibers and extensive ultrastructural damage of myelinated fibers in RTX-treated rats (Pan et al. 2003). Immunofluorescence labeling showed diminished TRPV1-like immunoreactivity in DRG neurons and the spinal dorsal horn following RTX administration. This study suggests that systemic (i.p.) RTX administration diminishes the thermal pain sensitivity by depletion of unmyelinated afferent fibers. At the same time, RTX damages the myelinated afferent fibers and causes their abnormal sprouting in lamina II of the spinal dorsal horn. The latter anatomical change might be the reason behind delayed tactile allodynia (Pan et al. 2003).

As discussed above, desensitization to vanilloids is reversible and thus contrasts to irreversible neurotoxicity. For example, it has been reported that a single topical application of RTX to the rat cornea reduces the capsaicin-evoked eye-wiping response in a dose-dependent manner for 3–5 days, while the normal nociceptive responses return by 5–7 days (Bates et al. 2010). Importantly, RTX administration did not impair epithelial wound healing and blink reflex or cause detectable histological damage to the cornea (Bates et al. 2010). Immunohistochemistry experiments revealed that RTX treatment caused a temporary loss of calcitonin gene-related peptide (CGRP) expressing nociceptive fibers: the majority of fibers reappeared within 12 days and full recovery was attained within 4 months (Bates et al. 2010). Systemic (s.c.) RTX treatment also inhibits the capsaicin-induced eye-wiping response and depletes CGRP-like immuoreactivity in the dorsal horn of the spinal cord: these effects last much longer (months) than those observed after topical administration (less than 2 weeks) but are fully reversible. Subcutaneous RTX, however, causes skin ulcerations (that can be severe in some animals) in the head (mostly in the facial skin and around the ears), the cause of which is unclear. It was suggested that RTX may cause paradoxical itch and the ulcers are secondary to the scratching behavior of the animals. This effect of RTX is somewhat different than other vanilloids such as capsaicin and curcumin which reveal anticarcinogenic and antitumor activity (Surh et al. 1995; Park and Surh 1997; Limtrakul et al. 1997; Jang et al. 1989; Huang et al. 1997; Tanaka et al. 2002). Also, high-concentration RTX (leaking from the injection site) may damage the epithelium, either directly (there are reports that keratinocytes may express functional TRPV1 receptors) or indirectly (Li et al. 2007). Finally, intraganglionic RTX injection (into the trigeminal ganglion) was reported to abolish eye wiping in response to capsaicin in an irreversible fashion; this was associated with a loss of TRPV1/CGRP-positive neurons (Karai et al. 2004).

The effect of RTX-induced denervation on TRPV1 expression in surrounding tissues has been recently examined. Surgical removal of both the sciatic and saphenous nerves from rat right hind legs and a parallel setup administration of RTX to the rat leg subcutaneously were performed (Kun et al. 2012). Two weeks after administration, the dorsal and plantar paw skin samples of hind legs, as well as the oral mucosa, were excised. Neither chemical nor surgical denervation influenced the level of TRPV1 receptor mRNA and protein expression in non-neural cells of either skin regions or mucosa (Kun et al. 2012). This indicates that RTX pretreatment is cytotoxic only to TRPV1-positive neurons and does not affect surrounding non-neural tissues.

As discussed above, vanilloid treatment depletes the proinflammatory neuropeptides substance-P (SP) and CGRP from capsaicin-sensitive sensory neurons. It was proposed that capsaicin blocks centripetal intra-axonal transport and thereby starves the cell bodies of nerve growth factor (NGF) which is produced in the periphery and is important for neuropeptide synthesis. Indeed, RTX treatment causes a marked decrease in pre-protachykinin mRNA levels encoding SP (Szallasi et al. 1999). Subsequently, it was discovered that vanilloid-induced changes in neuropeptide levels are, in fact, bidirectional: while SP and CGRP are

downregulated after capsaicin or RTX treatment, the expression of an endogenous analgesic peptide galanin is increased. Moreover, increased neuronal nitric oxide synthase (nNOS) and cholecystokinin receptor-B (CCK-B) levels were observed in the RTX-treated animals (Burliński et al. 2011). Collectively, these changes were termed "vanilloid-induced messenger plasticity" (Szallasi and Blumberg 1999). It was postulated that vanilloid treatment alters the phenotype of sensory neurons from proinflammatory to analgesic/anti-inflammatory. Thus, vanilloid-induced messenger plasticity may represent a major mechanism of desensitization.

8 Vanilloids and Mitochondrial Dysfunction

In cultured DRG neurons, there is a clear separation between concentrations at which capsaicin activates TRPV1 (ED_{50}<100 nM) and at which it becomes neurotoxic (30–100 μM) (Chard et al. 1995; Wood et al. 1988). Moreover, capsaicin was reported to kill a variety of non-neuronal cells such as in human B-cells, mouse myeloid cell lines, and Jurkat T-cells as well as squamous cell carcinoma cell lines (Wolvetang et al. 1996; Macho et al. 1999; Lee and Surh. 1998; Hail and Lotan. 2002). Capsaicin-induced apoptosis was also described in rat thymocytes (Amantini et al. 2004). It is still hotly debated if these non-neuronal cells express functional TRPV1. For example, in dendritic cells, both the presence and absence of TRPV1 were reported (O'Connell et al. 2005; Tóth et al. 2009).

The effect of capsaicin on cell death seems to be either specific (TRPV1 mediated) or nonspecific (not mediated by TPV1) in nature. Capsaicin can cause apoptosis or necrosis depending on the dose applied. In cultured rat DRG cells, capsaicin causes apoptosis by increasing the intracellular Ca^{2+} concentration, enhancing mitochondrial Ca^{2+} accumulation, dissipation of the inner transmembrane potential ($\Delta\psi_m$), activation of Ca^{2+}-sensitive proteases, and DNA fragmentation (Dedov et al. 2001). Capsaicin evokes similar signalling events in transformed and mitogen-activated T-cells (Macho et al. 1999) and in human and rat glioblastoma cells (Bíró et al. 1998; Lee et al. 2000).

The intercellular Ca^{2+} homeostasis is maintained by Ca^{2+}-binding proteins present in cytoplasm, endoplasmic reticulum (ER), and mitochondria (Kostyuk and Verkhratsky 1994; Svichar et al 1997; Verkhratsky and Petersen 1998). The elevated intracellular Ca^{2+} triggered by capsaicin leads to activation of Ca^{2+}-dependent enzymes such as different phospholipases, proteases, and endonucleases that can cause apoptosis in neuronal cells as well as non-neuronal cells (McConkey and Orrenius 1996; Wood et al. 1988). Though in general mitochondria are able to sequester intracellular Ca^{2+}, excess Ca^{2+}-influx into mitochondria causes membrane permeability transition (MPT) pore in mitochondrial membrane (Wong et al. 2012). This is considered as prototypical inducing factor. This MPT allows water and other small molecules to infiltrate inside the mitochondrial matrix which leads to osmotic swelling of mitochondria and may cause physical rupture of mitochondrial membrane (Green and Reed 1998; Bernardi 1992; Crompton 1999; Kroemer and Reed 2000).

However, several reports suggest that vanilloids may exert effects which are independent of Ca^{2+}-influx and TRPV1 receptors. This is due to the fact that capsaicin has significant effects in biological systems that are much lower in the phylogenetic tree and do not contain TRPV1. For example, capsaicin acts as inhibitor for organisms such as *Paracoccus denitrificans*, *Escherichia coli*, and *Thermus thermophilus* HB-8 where it affects ubiquinone reduction by NADH (Yagi 1990). Not only pure capsaicin, other vanilloids such as dihydrocapsaicin and RTX can also act as inhibitor of NADH oxidase (Wolvetang et al. 1996). Indeed, these inhibitors are able to induce apoptosis in human B-cell and mouse myeloid cell line (Wolvetang et al. 1996). In contrast, it was shown that in organisms which do not have the energy transducing site, such as in *Saccharomyces cerevisiae* mitochondria and *Bacillus subtilis* membranes, capsaicin does not inhibit NADH-ubiquinone reductase, suggesting that mitochondria can be a potential target of capsaicin action (Yagi 1990). In addition to mitochondria, recently it has been shown that in hippocampal astrocytes capsaicin can act as an inhibitor of tyrosyl tRNA synthetase and by inhibiting this enzyme, it induces cell death (Cochereau et al. 1996, 1997).

The TRPV1-independent functions of capsaicin mostly indicate the deleterious effect of capsaicin on mitochondria. Earlier research suggests that after the systemic application of capsaicin in the Aβ-type sensory neuron of adult rat as well as neonatal rat leads to mitochondrial swelling and results in the formation of atypical hollow mitochondria (Joó et al. 1969; Szolcsányi et al. 1975; Jancsó et al. 1977; Jancsó and Király 1981; Szöke et al. 1998, 2002). However, the real molecular mechanism behind the formation of hollow mitochondria is not clear. In addition to the TRPV1 receptor-mediated effects, it seems that capsaicin can also exert receptor-independent effects on the mitochondria. Due to its structure, capsaicin can act as analog of coenzyme Q, a lipophilic mobile electron carrier present in plasma membrane and involved in maintaining the redox potential of membrane. Indeed, it has been reported that preincubation of human lymphoblastoid cells with coenzyme Q prevents capsaicin-induced apoptosis (Wolvetang et al. 1996; Macho et al. 2000). It suggests that capsaicin competes for coenzyme Q and alters the redox potential of plasma membrane. Apart from that it has been also reported in transformed and activated T-cells; capsaicin inhibits the plasma membrane NADH oxidoreductase (PMOR), an enzyme that transfers electrons from cytoplasmic NADH to external electron acceptors such as oxygen via coenzyme Q (ubiquinone) (Morré et al. 1995, 1996; Wolvetang et al. 1996; Macho et al. 2000). Capsaicin can also inhibit the NADH:coenzyme Q oxidoreductase (complex I) activity of the mitochondrial electron transport system (Shimomura et al. 1989; Yagi 1990) which causes alteration on the mitochondrial membrane structure and its function (Aranda et al. 1995; Tsuchiya 2001). In vitro experiments in transformed cells as well as in activated T-cells suggest that capsaicin treatment enhances the generation of reactive oxygen species (Macho et al. 1998, 1999; Garle et al. 2000) and depolarization of mitochondrial membrane (Dedov et al. 2001) and apoptosis. Capsaicin suppresses the growth of cancer cells by NF-kB inactivation, reactive oxygen species (ROS) generations, cell-cycle arrest, and modulating EGFR/HER-2

pathways (Hail 2003; Kang et al. 2003; Lee et al. 2004; Min et al. 2004; Surh 2002; Thoennissen et al. 2010). Similarly, RTX was also reported to inhibit the NADH oxidase located in plasma membranes, to generate reactive oxygen species, and to induce apoptosis in transformed cells (Wolvetang et al. 1996; Macho et al. 1999, 2000; Garle et al. 2000). The exact molecular mechanism by which capsaicin causes oxidative stress and apoptosis remains rudimentary (Pramanik et al. 2011).

9 Conclusion

Historically, capsaicin and RTX proved to be invaluable tools in defining a fundamental subdivision of the peripheral nervous system (Appendino and Szallasi 1997; Buck and Burks 1986). Capsaicin-sensitive neurons are peptidergic nociceptive (primary sensory) neurons that express the vanilloid (capsaicin) receptor TRPV1. The activation of these neurons not only transmits painful stimuli to the CNS but also initiates the neurogenic inflammatory response. In turn, neurogenic inflammation was postulated to play a pivotal role in the pathogenesis of diverse diseases states, ranging from migraine through asthma to irritable bowel disease.

What makes capsaicin unique among naturally occurring irritant agents is that the initial stimulation of these neurons is followed by a lasting but fully reversible refractory state (traditionally termed desensitization) or irreversible neurotoxicity. Desensitization to capsaicin has a clear therapeutic potential. Indeed, capsaicin-containing creams and patches have clinical application for decades for indications like diabetic polyneuropathy. Irreversible neurotoxicity can also be exploited for therapeutic purposes. Indeed, at present site-specific RTX injections are undergoing clinical trials to achieve permanent analgesia in cancer patients with chronic, intractable pain secondary to metastatic disease.

Despite the tremendous progress that has been made after the identification and molecular cloning of TRPV1 in understanding capsaicin mechanisms, several outstanding questions remain unsolved. Most important, the exact molecular mechanisms underlying reversible desensitization versus irreversible toxicity are yet to be established. At high concentrations, the specificity of vanilloids for TRPV1 is lost, and the distinction between specific (TRPV1-mediated) and nonspecific (non-TRPV1-mediated) vanilloid actions becomes problematic. As an added complication, the existence of functional TRPV1 receptors in cells other than capsaicin-sensitive primary sensory neurons remains controversial. For example, TRPV1 knockout mice show altered behavior (e.g., reduced fear response) and capsaicin causes extensive neurodegenerative changes in the rat brain; yet, reporter mice show essentially no TRPV1 expression in the brain (Cavanaugh et al. 2011; Marsch et al. 2007; Tóth et al. 2005; Roberts et al. 2004; Starowicz et al. 2008; Goswami et al. 2010). In this context it is worth to mention that involvement of TRPV1 in the regulation of spine morphology and synaptic transmission has been demonstrated (Goswami et al. 2010). Another puzzling example is the keratinocytes present in skin. Keratinocytes were reported to express TRPV1 (both mRNA and protein) and

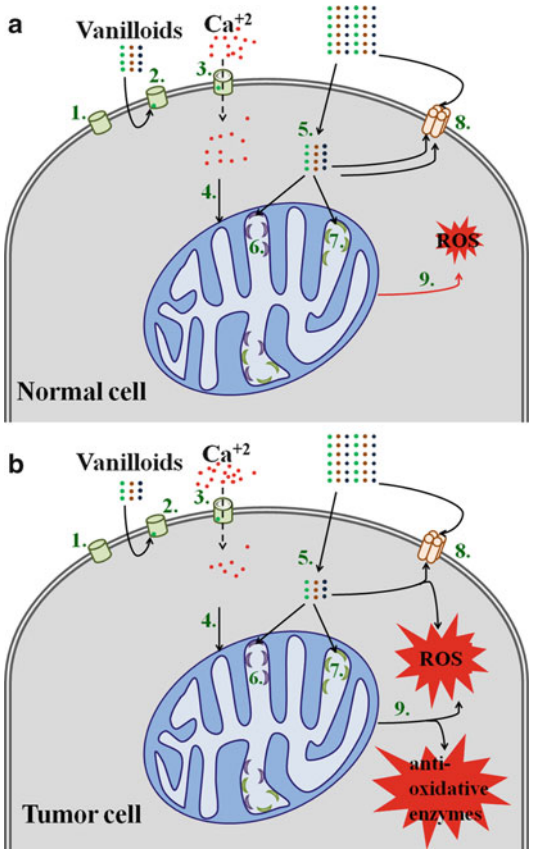


Fig. 1 TRPV1-dependent and TRPV1-independent vanilloid actions in normal cell (**a**) and tumor cell (**b**). TRPV1 present in the plasma membrane can be activated by different vanilloids with different affinities and binding kinetics (*Steps 1–2*). Potent vanilloids such as capsaicin or RTX activate TRPV1 at very low concentration and cause Ca^{2+} influx (*Step 3*). Vanilloids at much higher concentrations can cross plasma membrane and can also act on electron transport chain complex 1 (*ETC1*) and electron transport chain complex 3 (*ETC3*) (*Steps 5–7*). Vanilloids can also act on the coenzyme Q and PMOR (*Steps 8–9*). All these factors result in production of ROS (*Step 10*) relevant for neurotoxicity

respond to capsaicin with Ca^{2+}-uptake (Southall et al. 2003; Lee et al. 2008; Pecze et al. 2008). Yet, capsaicin evokes no responses in the rat skin after skin denervation (Fig. 1).

The mechanisms responsible for the marked species-related differences in vanilloid actions are only partially understood. It is now clear that birds do not respond to capsaicin because the avian TRPV1 receptor lacks the functional vanilloid-binding motif of mammalian TRPV1 receptors. However, it remains a mystery why the pulmonary chemoreflex is differentially regulated by RTX in rats (desensitization without prior excitation) and cats (repeatable excitation with no desensitization). However, much more and detailed studies are needed to characterize vanilloids for therapeutic applications in near future.

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References

Acs, G., Palkovits, M., & Blumberg, P. M. (1996). Specific binding of [3H]resiniferatoxin by human and rat preoptic area, locus ceruleus, medial hypothalamus, reticular formation and ventral thalamus membrane preparations. *Life Sciences, 59*, 1899–1908.

Aggarwal, B. B., & Shishodia, S. (2004). Suppression of the nuclear factor-kappaB activation pathway by spice-derived phytochemicals: Reasoning for seasoning. *Annals of the New York Academy of Sciences, 1030*, 434–441.

Amantini, C., Mosca, M., Lucciarini, R., Perfumi, M., Morrone, S., Piccoli, M., & Santoni, G. (2004). Distinct thymocyte subsets express the vanilloid receptor VR1 that mediates capsaicin-induced apoptotic cell death. *Cell Death and Differentiation, 11*, 1342–1356.

Andreev, Y. A., Kozlov, S. A., Koshelev, S. G., Ivanova, E. A., Monastyrnaya, M. M., Kozlovskaya, E. P., & Grishin, E. V. (2008). Analgesic compound from sea anemone *Heteractis crispa* is the first polypeptide inhibitor of vanilloid receptor 1 (TRPV1). *The Journal of Biological Chemistry, 283*, 23914–23921.

Appendino, G., & Szallasi, A. (1997). Euphorbium: Modern research on its active principle, resiniferatoxin, revives an ancient medicine. *Life Sciences, 60*, 681–696.

Aranda, F. J., Villalaín, J., & Gómez-Fernández, J. C. (1995). Capsaicin affects the structure and phase organization of phospholipid membranes. *Biochimica et Biophysica Acta, 1234*, 225–234.

Bates, B. D., Mitchell, K., Keller, J. M., Chan, C. C., Swaim, W. D., Yaskovich, R., Mannes, A. J., & Iadarola, M. J. (2010). Prolonged analgesic response of cornea to topical resiniferatoxin, a potent TRPV1 agonist. *Pain, 149*, 522–528.

Bernardi, P. (1992). Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. Evidence that the pore can be opened by membrane depolarization. *The Journal of Biological Chemistry, 267*, 8834–8839.

Bíró, T., Brodie, C., Modarres, S., Lewin, N. E., Acs, P., & Blumberg, P. M. (1998). Specific vanilloid responses in C6 rat glioma cells. *Brain Research. Molecular Brain Research, 56*, 89–98.

Bohlen, C. J., & Julius, D. (2012). Receptor-targeting mechanisms of pain-causing toxins: How ow? *Toxicon, 60*, 254–264.

Bohlen, C. J., Priel, A., Zhou, S., King, D., Siemens, J., & Julius, D. (2010). A bivalent tarantula toxin activates the capsaicin receptor, TRPV1, by targeting the outer pore domain. *Cell, 141*, 834–845.

Brown, D. C., Iadarola, M. J., Perkowski, S. Z., Erin, H., Shofer, F., Laszlo, K. J., Olah, Z., & Mannes, A. J. (2005). Physiologic and antinociceptive effects of intrathecal resiniferatoxin in a canine bone cancer model. *Anesthesiology, 103*, 1052–1059.

Buck, S. H., & Burks, T. F. (1986). The neuropharmacology of capsaicin: Review of some recent observations. *Pharmacological Reviews, 38*, 179–226.

Burliński, P. J., Gonkowski, S., & Całka, J. (2011). Tetrodotoxin- and resiniferatoxin-induced changes in paracervical ganglion ChAT- and nNOS-IR neurons supplying the urinary bladder in female pigs. *Acta Veterinaria Hungarica, 59*, 455–463.

Cabeza-Arvelaiz, Y., & Schiestl, R. H. (2012). Transcriptome analysis of a rotenone model of parkinsonism reveals complex I-tied and -untied toxicity mechanisms common to neurodegenerative diseases. *PloS One, 7*, e44700.

Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., & Julius, D. (1997). The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature, 389*, 816–824.

Cavanaugh, D. J., Chesler, A. T., Jackson, A. C., Sigal, Y. M., Yamanaka, H., Grant, R., O'Donnell, D., Nicoll, R. A., Shah, N. M., Julius, D., & Basbaum, A. I. (2011). Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. *The Journal of Neuroscience, 31*, 5067–5077.

Chard, P. S., Bleakman, D., Savidge, J. R., & Miller, R. J. (1995). Capsaicin-induced neurotoxicity in cultured dorsal root ganglion neurons: Involvement of calcium-activated proteases. *Neuroscience, 65*, 1099–1108.

Chou, M. Z., Mtui, T., Gao, Y. D., Kohler, M., & Middleton, R. E. (2004). Resiniferatoxin binds to the capsaicin receptor (TRPV1) near the extracellular side of the S4 transmembrane domain. *Biochemistry, 43*, 2501–2511.

Chu, C. J., Huang, S. M., De Petrocellis, L., Bisogno, T., Ewing, S. A., Miller, J. D., Zipkin, R. E., Daddario, N., Appendino, G., Di Marzo, V., & Walker, J. M. (2003). N-oleoyldopamine, a novel endogenous capsaicin-like lipid that produces hyperalgesia. *The Journal of Biological Chemistry, 278*, 13633–13639.

Chung, K., Klein, C. M., & Coggeshall, R. E. (1990). The receptive part of the primary afferent axon is most vulnerable to systemic capsaicin in adult rats. *Brain Research, 511*, 222–226.

Chung, W. Y., Jung, Y. J., Surh, Y. J., Lee, S. S., & Park, K. K. (2001). Antioxidative and antitumor promoting effects of [6]-paradol and its homologs. *Mutation Research, 496*, 199–206.

Cochereau, C., Sanchez, D., Bourhaoui, A., & Creppy, E. E. (1996). Capsaicin, a structural analog of tyrosine, inhibits the aminoacylation of tRNA(Tyr). *Toxicology and Applied Pharmacology, 141*, 133–137.

Cochereau, C., Sanchez, D., & Creppy, E. E. (1997). Tyrosine prevents capsaicin-induced protein synthesis inhibition in cultured cells. *Toxicology, 117*, 133–139.

Crompton, M. (1999). The mitochondrial permeability transition pore and its role in cell death. *The Biochemical Journal, 341*, 233–249.

Cuypers, E., Yanagihara, A., Karlsson, E., & Tytgat, J. (2006). Jellyfish and other cnidarian envenomations cause pain by affecting TRPV1 channels. *FEBS Letters, 580*, 5728–5732.

Czaja, K., Burns, G. A., & Ritter, R. C. (2008). Capsaicin-induced neuronal death and proliferation of the primary sensory neurons located in the nodose ganglia of adult rats. *Neuroscience, 154*, 621–630.

Dedov, V. N., Mandadi, S., Armati, P. J., & Verkhratsky, A. (2001). Capsaicin-induced depolarisation of mitochondria in dorsal root ganglion neurons is enhanced by vanilloid receptors. *Neuroscience, 103*, 219–226.

Docherty, R. J., Yeats, J. C., Bevan, S., & Boddeke, H. W. (1996). Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. *Pflügers Archiv, 431*, 828–837.

Doyle, M. W., Bailey, T. W., Jin, Y. H., & Andresen, M. C. (2002). Vanilloid receptors presynaptically modulate cranial visceral afferent synaptic transmission in nucleus tractus solitarius. *The Journal of Neuroscience, 22*, 8222–8229.

Driedger, P. E., & Blumberg, P. M. (1980). Different biological targets for resiniferatoxin and phorbol 12 myristate 13-acetate. *Cancer Research, 40*, 1400–1404.

Escoubas, P., & Rash, L. (2004). Tarantulas: eight-legged pharmacists and combinatorial chemists. *Toxicon, 43*, 555–574.

Frey, M. R., Clark, J. A., Bateman, N. W., Kazanietz, M. G., Black, A. R., & Black, J. D. (2004). Cell cycle- and protein kinase C-specific effects of resiniferatoxin and resiniferonol 9,13,14-ortho-phenylacetate in intestinal epithelial cells. *Biochemical Pharmacology, 67*, 1873–1886.

Fry, B. G., Vidal, N., Norman, J. A., Vonk, F. J., Scheib, H., Ramjan, S. F., Kuruppu, S., Fung, K., Hedges, S. B., Richardson, M. K., Hodgson, W. C., Ignjatovic, V., Summerhayes, R., & Kochva, E. (2006). Early evolution of the venom system in lizards and snakes. *Nature, 439*, 584–588.

Garle, M. J., Knight, A., Downing, A. T., Jassi, K. L., Clothier, R. H., & Fry, J. R. (2000). Stimulation of dichlorofluorescin oxidation by capsaicin and analogues in RAW 264 monocyte/macrophages: Lack of involvement of the vanilloid receptor. *Biochemical Pharmacology, 59*, 563–572.

Gavva, N. R., Klionsky, L., Qu, Y., Shi, L., Tamir, R., Edenson, S., Zhang, T. J., Viswanadhan, V. N., Toth, A., Pearce, L. V., Vanderah, T. W., Porreca, F., Blumberg, P. M., Lile, J., Sun, Y., Wild, K., Louis, J. C., & Treanor, J. J. (2004). Molecular determinants of vanilloid sensitivity in TRPV1. *The Journal of Biological Chemistry, 279*, 20283–20295.

Goswami, C., Schmidt, H., & Hucho, F. (2007). TRPV1 at nerve endings regulates growth cone morphology and movement through cytoskeleton reorganization. *The FEBS Journal, 274*, 760–772.

Goswami, C., Rademacher, N., Smalla, K. H., Kalscheuer, V., Ropers, H. H., Gundelfinger, E. D., & Hucho, T. (2010). TRPV1 acts as a synaptic protein and regulates vesicle recycling. *Journal of Cell Science, 123*, 2045–2057.

Gracheva, E. O., Ingolia, N. T., Kelly, Y. M., Cordero-Morales, J. F., Hollopeter, G., Chesler, A. T., Sánchez, E. E., Perez, J. C., Weissman, J. S., & Julius, D. (2010). Molecular basis of infrared detection by snakes. *Nature, 464*, 1006–1011.

Gracheva, E. O., Cordero-Morales, J. F., González-Carcacía, J. A., Ingolia, N. T., Manno, C., Aranguren, C. I., Weissman, J. S., & Julius, D. (2011). Ganglion-specific splicing of TRPV1 underlies infrared sensation in vampire bats. *Nature, 476*, 88–91.

Green, D. R., & Reed, J. C. (1998). Mitochondria and apoptosis. *Science, 281*, 1309–1312.

Hail, N., Jr., & Lotan, R. (2002). Examining the role of mitochondrial respiration in vanilloid-induced apoptosis. *Journal of the National Cancer Institute, 94*, 1281–1292.

Hail, N., Jr. (2003). Mechanisms of vanilloid-induced apoptosis. *Apoptosis, 8*, 251–262.

Holzer, P. (1991). Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacological Reviews, 43*, 143–201.

Hoppe, U., Bergemann, J., Diembeck, W., Ennen, J., Gohla, S., Harris, I., Jacob, J., Kielholz, J., Mei, W., Pollet, D., Schachtschabel, D., Sauermann, G., Schreiner, V., Stäb, F., & Steckel, F. (1999). Coenzyme Q10, a cutaneous antioxidant and energizer. *BioFactors, 9*, 371–378.

Huang, M. T., Ma, W., Yen, P., Xie, J. G., Han, J., Frenkel, K., Grunberger, D., & Conney, A. H. (1997). Inhibitory effects of topical application of low doses of curcumin on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion and oxidized DNA bases in mouse epidermis. *Carcinogenesis, 18*, 83–88.

Huang, S. M., Bisogno, T., Trevisani, M., Al-Hayani, A., De Petrocellis, L., Fezza, F., Tognetto, M., Petros, T. J., Krey, J. F., Chu, C. J., Miller, J. D., Davies, S. N., Geppetti, P., Walker, J. M., & Di Marzo, V. (2002). An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proceedings of the National Academy of Sciences USA, 99*, 8400–8405.

Iida, T., Moriyama, T., Kobata, K., Morita, A., Murayama, N., Hashizume, S., Fushiki, T., Yazawa, S., Watanabe, T., & Tominaga, M. (2003). TRPV1 activation and induction of nociceptive response by a non-pungent capsaicin-like compound, capsiate. *Neuropharmacology, 44*, 958–967.

Inoue, K., Koizumi, S., Fuziwara, S., Denda, S., Inoue, K., & Denda, M. (2002). Functional vanilloid receptors in cultured normal human epidermal keratinocytes. *Biochemical and Biophysical Research Communications, 291*, 124–129.

Jancsó, N., & Jancsó, A. (1949). Desensitization of sensory nerve endings (in Hungarian). *Kísérletes Orvostudomány, 2*(Suppl), 15.

Jancsó, G., & Király, E. (1981). Sensory neurotoxins: Chemically induced selective destruction of primary sensory neurons. *Brain Research, 210*, 83–89.

Jancsó, G., Kiraly, E., & Jancsó-Gábor, A. (1977). Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. *Nature, 270*, 741–743.

Jancsó, G., Karcsú, S., Király, E., Szebeni, A., Tóth, L., Bácsy, E., Joó, F., & Párducz, A. (1984). Neurotoxin induced nerve cell degeneration: Possible involvement of calcium. *Brain Research, 295*, 211–216.

Jancsó-Gábor, A., Szolcsányi, J., & Jancsó, N. (1970). Stimulation and desensitization of the hypothalamic heat-sensitive structures by capsaicin in rats. *The Journal of Physiology, 208*, 449–459.

Jang, J. J., Kim, S. H., & Yun, T. K. (1989). Inhibitory effect of capsaicin on mouse lung tumor development. *In Vivo, 3*, 49–53.

Jiang, Y., Ruta, V., Chen, J., Lee, A., & MacKinnon, R. (2003). The principle of gating charge movement in a voltage-dependent K^+ channel. *Nature (London), 423*, 42–48.

Johnson, D. M., Garrett, E. M., Rutter, R., Bonnert, T. P., Gao, Y. D., Middleton, R. E., & Sutton, K. G. (2006). Functional mapping of the transient receptor potential vanilloid 1 intracellular binding site. *Molecular Pharmacology, 70*, 1005–1012.

Joó, F., Szolcsányi, J., & Jancsó-Gábor, A. (1969). Mitochondrial alterations in the spinal ganglion cells of the rat accompanying the long-lasting sensory disturbance induced by capsaicin. *Life Sciences, 8*, 621–626.

Jordt, S. E., & Julius, D. (2002). Molecular basis for species-specific sensitivity to "hot" chili peppers. *Cell, 108*, 421–430.

Jung, J., Hwang, S. W., Kwak, J., Lee, S. Y., Kang, C. J., Kim, W. B., Kim, D., & Oh, U. (1999). Capsaicin binds to the intracellular domain of the capsaicin-activated ion channel. *The Journal of Neuroscience, 19*, 529–538.

Jung, J., Lee, S. Y., Hwang, S. W., Cho, H., Shin, J., Kang, Y. S., Kim, S., & Oh, U. (2002). Agonist recognition sites in the cytosolic tails of vanilloid receptor 1. *The Journal of Biological Chemistry, 277*, 44448–44454.

Kang, H. J., Soh, Y., Kim, M. S., Lee, E. J., Surh, Y. J., Kim, H. R., Kim, S. H., & Moon, A. (2003). Roles of JNK-1 and p38 in selective induction of apoptosis by capsaicin in ras-transformed human breast epithelial cells. *International Journal of Cancer, 103*, 475–482.

Karai, L., Brown, D. C., Mannes, A. J., Connelly, S. T., Brown, J., Gandal, M., Wellisch, O. M., Neubert, J. K., Olah, Z., & Iadarola, M. J. (2004). Deletion of vanilloid receptor 1-expressing primary afferent neurons for pain control. *The Journal of Clinical Investigation, 113*, 1344–1352.

Kissin, I. (2008). Vanilloid-induced conduction analgesia: Selective, dose-dependent, long-lasting, with a low level of potential neurotoxicity. *Anesthesia and Analgesia, 107*, 271–281.

Kitaguchi, T., & Swartz, K. J. (2005). An inhibitor of TRPV1 channels isolated from funnel Web spider venom. *Biochemistry, 44*, 15544–15549.

Knotkova, H., Pappagallo, M., & Szallasi, A. (2008). Capsaicin (TRPV1 Agonist) therapy for pain relief: Farewell or revival? *The Clinical Journal of Pain, 24*, 142–154.

Kostyuk, P., & Verkhratsky, A. (1994). Calcium stores in neurons and glia. *Neuroscience, 63*, 381–404.

Kroemer, G., & Reed, J. C. (2000). Mitochondrial control of cell death. *Nature Medicine, 6*, 513–519.

Kun, J., Helyes, Z., Perkecz, A., Bán, A., Polgár, B., Szolcsányi, J., & Pintér, E. (2012). Effect of surgical and chemical sensory denervation on non-neural expression of the Transient Receptor Potential Vanilloid 1 (TRPV1) receptors in the rat. *Journal of Molecular Neuroscience, 48*, 795–803.

Kuptniratsaikul, V., Thanakhumtorn, S., Chinswangwatanakul, P., Wattanamongkonsil, L., & Thamlikitkul, V. (2009). Efficacy and safety of Curcuma domestica extracts in patients with knee osteoarthritis. *Journal of Alternative and Complementary Medicine, 15*, 891–897.

Kuzhikandathil, E. V., Wang, H., Szabo, T., Morozova, N., Blumberg, P. M., & Oxford, G. S. (2001). Functional analysis of capsaicin receptor (vanilloid receptor subtype 1) multimerization and agonist responsiveness using a dominant negative mutation. *The Journal of Neuroscience, 21*, 8697–8706.

Lee, E., & Surh, Y. J. (1998). Induction of apoptosis in HL-60 cells by pungent vanilloids, [6]-gingerol and [6]-paradol. *Cancer Letters, 134*, 163–168.

Lee, Y. S., Nam, D. H., & Kim, J. A. (2000). Induction of apoptosis by capsaicin in A172 human glioblastoma cells. *Cancer Letters, 161*, 121–130.

Lee, Y. S., Kang, Y. S., Lee, J. S., Nicolova, S., & Kim, J. A. (2004). Involvement of NADPH oxidase-mediated generation of reactive oxygen species in the apototic cell death by capsaicin in HepG2 human hepatoma cells. *Free Radical Research, 38*, 405–412.

Lee, Y. M., Li, W. H., Kim, Y. K., Kim, K. H., & Chung, J. H. (2008). Heat-induced MMP-1 expression is mediated by TRPV1 through PKCalpha signaling in HaCaT cells. *Experimental Dermatology, 17*, 864–870.

Li, W. H., Lee, Y. M., Kim, J. Y., Kang, S., Kim, S., Kim, K. H., Park, C. H., & Chung, J. H. (2007). Transient receptor potential vanilloid-1 mediates heat-shock-induced matrix metalloproteinase-1 expression in human epidermal keratinocytes. *The Journal of Investigative Dermatology, 127*, 2328–2335.

Limtrakul, P., Lipigorngoson, S., Namwong, O., Apisariyakul, A., & Dunn, F. W. (1997). Inhibitory effect of dietary curcumin on skin carcinogenesis in mice. *Cancer Letters, 116*, 197–203.

Littarru, G. P., & Tiano, L. (2007). Bioenergetic and antioxidant properties of coenzyme Q10: Recent developments. *Molecular Biotechnology, 37*, 31–37.

Luo, X. J., Peng, J., & Li, Y. J. (2011). Recent advances in the study on capsaicinoids and capsinoids. *European Journal of Pharmacology, 650*, 1–7.

Lynn, B., & Shakhanbeh, J. (1988). Substance P content of the skin, neurogenic inflammation and numbers of C-fibres following capsaicin application to a cutaneous nerve in the rabbit. *Neuroscience, 24*, 769–775.

Macho, A., Blázquez, M. V., Navas, P., & Muñoz, E. (1998). Induction of apoptosis by vanilloid compounds does not require de novo gene transcription and activator protein 1 activity. *Cell Growth & Differentiation, 9*, 277–286.

Macho, A., Calzado, M. A., Muñoz-Blanco, J., Gómez-Díaz, C., Gajate, C., Mollinedo, F., Navas, P., & Muñoz, E. (1999). Selective induction of apoptosis by capsaicin in transformed cells: The role of reactive oxygen species and calcium. *Cell Death and Differentiation, 6*, 155–165.

Macho, A., Lucena, C., Calzado, M. A., Blanco, M., Donnay, I., Appendino, G., & Muñoz, E. (2000). Phorboid 20-homovanillates induce apoptosis through a VR1-independent mechanism. *Chemistry & Biology, 7*, 483–492.

Maggi, C. A. (1991). Capsaicin and primary afferent neurons: From basic science to human therapy? *Journal of the Autonomic Nervous System, 33*, 1–14.

Marsch, R., Foeller, E., Rammes, G., Bunck, M., Kössl, M., Holsboer, F., Zieglgänsberger, W., Landgraf, R., Lutz, B., & Wotjak, C. T. (2007). Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. *The Journal of Neuroscience, 27*, 832–839.

McConkey, D. J., & Orrenius, S. (1996). The role of calcium in the regulation of apoptosis. *Journal of Leukocyte Biology, 59*, 775–783.

Micale, V., Cristino, L., Tamburella, A., Petrosino, S., Leggio, G. M., Di Marzo, V., & Drago, F. (2010). Enhanced cognitive performance of dopamine D3 receptor "knock-out" mice in the step-through passive-avoidance test: assessing the role of the endocannabinoid/endovanilloid systems. *Pharmacological Research, 61*, 531–536.

Miller, C. (1995). The charybdotoxin family of K^+ channel-blocking peptides. *Neuron, 15*, 5–10.

Min, J. K., Han, K. Y., Kim, E. C., Kim, Y. M., Lee, S. W., Kim, O. H., Kim, K. W., Gho, Y. S., & Kwon, Y. G. (2004). Capsaicin inhibits in vitro and in vivo angiogenesis. *Cancer Research, 64*, 644–651.

Morré, D. J., Chueh, P. J., & Morré, D. M. (1995). Capsaicin inhibits preferentially the NADH oxidase and growth of transformed cells in culture. *Proceedings of the National Academy of Sciences USA, 92*, 1831–1835.

Morré, D. J., Sun, E., Geilen, C., Wu, L. Y., de Cabo, R., Krasagakis, K., Orfanos, C. E., & Morré, D. M. (1996). Capsaicin inhibits plasma membrane NADH oxidase and growth of human and mouse melanoma lines. *European Journal of Cancer, 32A*, 1995–2003.

Nagy, I., & Rang, H. (2000). Comparison of currents activated by noxious heat in rat and chicken primary sensory neurons. *Regulatory Peptides, 96*, 3–6.

Negri, L., Lattanzi, R., Giannini, E., Colucci, M., Margheriti, F., Melchiorri, P., Vellani, V., Tian, H., De Felice, M., & Porreca, F. (2006). Impaired nociception and inflammatory pain sensation in mice lacking the prokineticin receptor PKR1: Focus on interaction between PKR1 and the capsaicin receptor TRPV1 in pain behavior. *The Journal of Neuroscience, 26*, 6716–6727.

Neubert, J. K., Karai, L., Jun, J. H., Kim, H. S., Olah, Z., & Iadarola, M. J. (2003). Peripherally induced resiniferatoxin analgesia. *Pain, 104*, 219–228.

Neubert, J. K., Mannes, A. J., Karai, L. J., Jenkins, A. C., Zawatski, L., Abu-Asab, M., & Iadarola, M. J. (2008). Perineural resiniferatoxin selectively inhibits inflammatory hyperalgesia. *Molecular Pain, 4*, 03–13.

O'Connell, P. J., Pingle, S. C., & Ahern, G. P. (2005). Dendritic cells do not transduce inflammatory stimuli via the capsaicin receptor TRPV1. *FEBS Letters, 579*, 5135–5139.

Otten, U., Lorez, H. P., & Businger, F. (1983). Nerve growth factor antagonizes the neurotoxic action of capsaicin on primary sensory neurones. *Nature, 301*, 515–517.

Pákáski, M., Hugyecz, M., Sántha, P., Jancsó, G., Bjelik, A., Domokos, A., Janka, Z., & Kálmán, J. (2009). Capsaicin promotes the amyloidogenic route of brain amyloid precursor protein processing. *Neurochemistry International, 54*, 426–430.

Pan, H. L., Khan, G. M., Alloway, K. D., & Chen, S. R. (2003). Resiniferatoxin induces paradoxical changes in thermal and mechanical sensitivities in rats: Mechanism of action. *The Journal of Neuroscience, 23*, 2911–2919.

Park, K. K., & Surh, Y. J. (1997). Effects of capsaicin on chemically-induced two-stage mouse skin carcinogenesis. *Cancer Letters, 114*, 183–184.

Pecze, L., Szabó, K., Széll, M., Jósvay, K., Kaszás, K., Kúsz, E., Letoha, T., Prorok, J., Koncz, I., Tóth, A., Kemény, L., Vizler, C., & Oláh, Z. (2008). Human keratinocytes are vanilloid resistant. *PloS One, 3*, e3419.

Phillips, E., Reeve, A., Bevan, S., & McIntyre, P. (2004). Identification of species-specific determinants of the action of the antagonist capsazepine and the agonist PPAHV on TRPV1. *The Journal of Biological Chemistry, 279*, 17165–17172.

Pini, A., Baranowski, R., & Lynn, B. (1990). Long-term reduction in the number of C-Fibre Nociceptors following Capsaicin treatment of a cutaneous nerve in adult rats. *The European Journal of Neuroscience, 2*, 89–97.

Pórszász, R., Szolcsányi, J. (1991–1992). Circulatory and respiratory effects of capsaicin and resiniferatoxin on guinea pigs. *Acta Biochimica Biophysica Hungarica*, *26*, 131–138.

Pramanik, K. C., Boreddy, S. R., & Srivastava, S. K. (2011). Role of mitochondrial electron transport chain complexes in capsaicin mediated oxidative stress leading to apoptosis in pancreatic cancer cells. *PloS One, 6*, e20151.

Riera, C. E., Menozzi-Smarrito, C., Affolter, M., Michlig, S., Munari, C., Robert, F., Vogel, H., Simon, S. A., & le Coutre, J. (2009). Compounds from Sichuan and Melegueta peppers activate, covalently and non-covalently, TRPA1 and TRPV1 channels. *British Journal of Pharmacology, 157*, 1398–1409.

Ritter, S., & Dinh, T. T. (1988). Capsaicin-induced neuronal degeneration: Silver impregnation of cell bodies, axons, and terminals in the central nervous system of the adult rat. *The Journal of Comparative Neurology, 271*, 79–90.

Roberts, J. C., Davis, J. B., & Benham, C. D. (2004). [^{3}H]Resiniferatoxin autoradiography in the CNS of wild-type and TRPV1 null mice defines TRPV1 (VR-1) protein distribution. *Brain Research, 995*, 176–183.

Sardar, P., Kumar, A., Bhandari, A., & Goswami, C. (2012). Conservation of tubulin-binding sequences in TRPV1 throughout evolution. *PloS One, 7*, e31448.

Sasamura, T., Sasaki, M., Tohda, C., & Kuraishi, Y. (1998). Existence of capsaicin-sensitive glutamatergic terminals in rat hypothalamus. *Neuroreport, 9*, 2045–2048.

Sculptoreanu, A., de Groat, W. C., Buffington, C. A., & Birder, L. A. (2005). Abnormal excitability in capsaicin-responsive DRG neurons from cats with feline interstitial cystitis. *Experimental Neurology, 193*, 437–443.

Shimomura, Y., Kawada, T., & Suzuki, M. (1989). Capsaicin and its analogs inhibit the activity of NADH-coenzyme Q oxidoreductase of the mitochondrial respiratory chain. *Archives of Biochemistry and Biophysics, 270*, 573–577.

Siemens, J., Zhou, S., Piskorowski, R., Nikai, T., Lumpkin, E. A., Basbaum, A. I., King, D., & Julius, D. (2006). Spider toxins activate the capsaicin receptor to produce inflammatory pain. *Nature, 444*, 208–212.

Southall, M. D., Li, T., Gharibova, L. S., Pei, Y., Nicol, G. D., & Travers, J. B. (2003). Activation of epidermal vanilloid receptor-1 induces release of proinflammatory mediators in human keratinocytes. *The Journal of Pharmacology and Experimental Therapeutics, 304*, 217–222.

Starowicz, K., Cristino, L., & Di Marzo, V. (2008). TRPV1 receptors in the central nervous system: Potential for previously unforeseen therapeutic applications. *Current Pharmaceutical Design, 14*, 42–54.

Sugimoto, T., Xiao, C., & Ichikawa, H. (1998). Neonatal primary neuronal death induced by capsaicin and axotomy involves an apoptotic mechanism. *Brain Research, 807*, 147–154.

Surh, Y. J. (2002). More than spice: Capsaicin in hot chili peppers makes tumor cells commit suicide. *Journal of the National Cancer Institute, 94*, 1263–1265.

Surh, Y. J., Lee, R. C., Park, K. K., Mayne, S. T., Liem, A., & Miller, J. A. (1995). Chemoprotective effects of capsaicin and diallyl sulfide against mutagenesis or tumorigenesis by vinyl carbamate and N-nitrosodimethylamine. *Carcinogenesis, 16*, 2467–2471.

Sutton, K. G., Garrett, E. M., Rutter, A. R., Bonnert, T. P., Jarolimek, W., & Seabrook, G. R. (2005). Functional characterisation of the S512Y mutant vanilloid human TRPV1 receptor. *British Journal of Pharmacology, 146*, 702–711.

Svichar, N., Kostyuk, P., & Verkhratsky, A. (1997). Mitochondria buffer Ca^{2+} entry but not intracellular Ca^{2+} release in mouse DRG neurones. *Neuroreport, 8*, 3929–3932.

Swarnkar, S., Singh, S., Goswami, P., Mathur, R., Patro, I. K., & Nath, C. (2012). Astrocyte activation: A key step in rotenone induced cytotoxicity and DNA damage. *Neurochemical Research, 37*, 2178–2189.

Szallasi, A., & Blumberg, P. M. (1999). Vanilloid (Capsaicin) receptors and mechanisms. *Pharmacological Reviews, 51*, 159–212.

Szallasi, A., Goso, C., Blumberg, P. M., & Manzini, S. (1993). Competitive inhibition by capsazepine of [^{3}H]resiniferatoxin binding to central (spinal cord and dorsal root ganglia) and peripheral (urinary bladder and airways) vanilloid (capsaicin) receptors in the rat. *The Journal of Pharmacology and Experimental Therapeutics, 267*, 728–733.

Szallasi, A., Farkas-Szallasi, T., Tucker, J. B., Lundberg, J. M., Hökfelt, T., & Krause, J. E. (1999). Effects of systemic resiniferatoxin treatment on substance P mRNA in rat dorsal root ganglia and substance P receptor mRNA in the spinal dorsal horn. *Brain Research, 815*, 177–184.

Szöke, E., Seress, L., & Szolcsányi, J. (1998). Reevaluation of the effect of neonatal capsaicin treatment on the basis of morphometrical studies. *Neurobiology (Budapest, Hungary), 6*, 477–478.

Szöke, E., Seress, L., & Szolcsányi, J. (2002). Neonatal capsaicin treatment results in prolonged mitochondrial damage and delayed cell death of B cells in the rat trigeminal ganglia. *Neuroscience, 113*, 925–937.

Szolcsányi, J., Joó, F., & Jancsó-Gábor, A. (1971). Mitochondrial changes in preoptic neurons after capsaicin desensitization of the hypothalamic thermodetectors in rats. *Nature, 229*, 116–117.

Szolcsányi, J., Jancśo-Gábor, A., & Joo, F. (1975). Functional and fine structural characteristics of the sensory neuron blocking effect of capsaicin. *Naunyn-Schmiedeberg's Archives of Pharmacology, 287*, 157–169.

Tanaka, T., Kohno, H., Sakata, K., Yamada, Y., Hirose, Y., Sugie, S., & Mori, H. (2002). Modifying effects of dietary capsaicin and rotenone on 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. *Carcinogenesis, 23*, 1361–1367.

Tender, G. C., Walbridge, S., Olah, Z., Karai, L., Iadarola, M., Oldfield, E. H., & Lonser, R. R. (2005). Selective ablation of nociceptive neurons for elimination of hyperalgesia and neurogenic inflammation. *Journal of Neurosurgery, 102*, 522–525.

Terlau, H., & Olivera, B. M. (2004). Conus venoms: A rich source of novel ion channel-targeted peptides. *Physiological Reviews, 84*, 41–68.

Thoennissen, N. H., O'Kelly, J., Lu, D., Iwanski, G. B., La, D. T., Abbassi, S., Leiter, A., Karlan, B., Mehta, R., & Koeffler, H. P. (2010). Capsaicin causes cell-cycle arrest and apoptosis in ER-positive and -negative breast cancer cells by modulating the EGFR/HER-2 pathway. *Oncogene, 29*, 285–296.

Tóth, A., Boczán, J., Kedei, N., Lizanecz, E., Bagi, Z., Papp, Z., Edes, I., Csiba, L., & Blumberg, P. M. (2005). Expression and distribution of vanilloid receptor 1 (TRPV1) in the adult rat brain. *Brain Research. Molecular Brain Research, 135*, 162–168.

Tóth, B. I., Benko, S., Szöllosi, A. G., Kovács, L., Rajnavölgyi, E., & Bíró, T. (2009). Transient receptor potential vanilloid-1 signaling inhibits differentiation and activation of human dendritic cells. *FEBS Letters, 583*, 1619–1624.

Tsuchiya, H. (2001). Biphasic membrane effects of capsaicin, an active component in *Capsicum* species. *Journal of Ethnopharmacology, 75*, 295–299.

Valtschanoff, J. G., Rustioni, A., Guo, A., & Hwang, S. J. (2001). Vanilloid receptor VR1 is both presynaptic and postsynaptic in the superficial laminae of the rat dorsal horn. *The Journal of Comparative Neurology, 436*, 225–235.

Vellani, V., Colucci, M., Lattanzi, R., Giannini, E., Negri, L., Melchiorri, P., & McNaughton, P. A. (2006). Sensitization of transient receptor potential vanilloid 1 by the prokineticin receptor agonist Bv8. *The Journal of Neuroscience, 26*, 5109–5116.

Verkhratsky, A. J., & Petersen, O. H. (1998). Neuronal calcium stores. *Cell Calcium, 24*, 333–343.

Vlachova, V., Teisinger, J., Susankova, K., Lyfenko, A., Ettrich, R., & Vyklicky, L. (2003). Functional role of C-terminal cytoplasmic tail of rat vanilloid receptor 1. *The Journal of Neuroscience, 23*, 1340–1350.

Voets, T., Talavera, K., Owsianik, G., & Nilius, B. (2005). Sensing with TRP channels. *Nature Chemical Biology, 1*, 85–92.

Wardle, K. A., Furey, G., & Sanger, G. J. (1996). Pharmacological characterization of the vanilloid receptor in the rat isolated vas deferens. *The Journal of Pharmacy and Pharmacology, 48*, 285–291.

Welch, J. M., Simon, S. A., & Reinhart, P. H. (2000). The activation mechanism of rat vanilloid receptor 1 by capsaicin involves the pore domain and differs from the activation by either acid or heat. *Proceedings of the National Academy of Sciences USA, 97*, 13889–13894.

Winter, J. (1987). Characterization of capsaicin-sensitive neurones in adult rat dorsal root ganglion cultures. *Neuroscience Letters, 80*, 134–140.

Wolvetang, E. J., Larm, J. A., Moutsoulas, P., & Lawen, A. (1996). Apoptosis induced by inhibitors of the plasma membrane NADH-oxidase involves Bcl-2 and calcineurin. *Cell Growth & Differentiation, 7*, 1315–1325.

Wong, R., Steenbergen, C., & Murphy, E. (2012). Mitochondrial permeability transition pore and calcium handling. *Methods in Molecular Biology, 810*, 235–242.

Wood, J. N., Winter, J., James, I. F., Rang, H. P., Yeats, J., & Bevan, S. (1988). Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. *The Journal of Neuroscience, 8*, 3208–3320.

Xu, X. J., Farkas-Szallasi, T., Lundberg, J. M., Hökfelt, T., Wiesenfeld-Hallin, Z., & Szallasi, A. (1997). Effects of the capsaicin analogue resiniferatoxin on spinal nociceptive mechanisms in the rat: behavioral, electrophysiological and in situ hybridization studies. *Brain Research, 752*, 52–60.

Yagi, T. (1990). Inhibition by capsaicin of NADH-quinone oxidoreductases is correlated with the presence of energy-coupling site 1 in various organisms. *Archives of Biochemistry and Biophysics, 281*, 305–311.

Zhu, S., Darbon, H., Dyason, K., Verdonck, F., & Tytgat, J. (2003). Evolutionary origin of inhibitor cystine knot peptides. *The FASEB Journal, 17*, 1765–1767.

Part II

Dopamine Chemistry and Parkinson Disease

6-Hydroxydopamine as Preclinical Model of Parkinson's Disease

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Contents

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Abstract

Like many other diseases, Parkinson's disease (PD) requires preclinical models to understand the underlying molecular and cellular mechanisms that cause the disorder. Such models will also help to identify novel potential drug targets and to elucidate the effects of new drug candidates. The hydroxylated analogue of dopamine, 6-hydroxydopamine (6-OHDA), has been exploited as an experimental model to study PD. It also has been used extensively as a test system for novel symptomatic agents and for assessment of neuroprotective and neurorepair strategies. The 6-OHDA lesioned rat appears to be a good predictor for monitoring the efficacy of new dopaminergic drugs that enter phase II/III clinical trials. The aim of this chapter is the molecular and cellular mechanisms that are involved in the preclinical model of Parkinson's disease based on 6-OHDA. With special attention, we address the role of second messengers as the role of reactive oxygen species and the mitochondria as the headquarters of cell death. The role of molecular signaling pathways, for instance, the participation of Bcl-2 family members, will also be addressed.

List of Abbreviations

6-OHDA	6-hydroxydopamine
Bak	Bcl-2-antagonist/killer
Bax	X Bcl-2-associated protein
DA	Dopamine
GSH	Glutathione
MCC	Multiple conductance channel
MOMP	Permeabilization of the mitochondrial outer membrane
PD	Parkinson's disease
PTP	Permeability transition pore
ROS	Reactive oxygen species
$\Delta\Psi m$	Mitochondrial membrane potential

1 Introduction

6-Hydroxydopamine (6-OHDA), also known as oxidopamine, or 2,4,5-trihydroxyphenethylamine ($C_8H_{11}NO_3$) is a toxic oxidative metabolite of dopamine and is detected in the brains and urine of Parkinson's disease (PD) patients (Fig. 1). It has been applied broadly to generate experimental models of Parkinson's disease. 6-OHDA shares some structural similarities with dopamine and norepinephrine and exhibits high affinity for several catecholaminergic plasma membrane transporters, such as the dopamine and norepinephrine transporters. Consequently, 6-OHDA can enter both dopaminergic and noradrenergic neurons.

In this chapter we will focus on molecular and cellular mechanisms that are involved in neurodegenerative processes described in PD. We will discuss the preclinical model of Parkinson's disease (based on 6-OHDA), with special attention

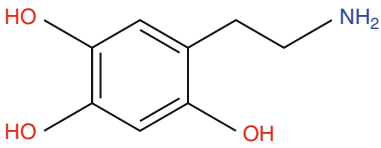

Fig. 1 Chemical structure of 6-OHDA

to the role of reactive oxygen species as second messengers and mitochondria as the headquarters of cell death. The role of molecular signaling pathways, for instance, the participation of Bcl-2 family members, will also be addressed.

2 In Vivo Experimental Animal Model

6-OHDA poorly crosses the blood–brain barrier, and, therefore, it does not accumulate within the brain parenchyma to meaningful neurotoxic concentrations following systemic injections. Thus, when 6-OHDA is administrated by systemic injection, it will not produce nigrostriatal lesions. Because of this, systemic injection is the preferred way of 6-OHDA administration to damage the peripheral nervous system and cause chemical sympathectomy. 6-OHDA has been found to deplete peripheral organs of noradrenaline (Porter et al. 1963; Laverty and Sharman 1965; Thoenen and Tranzer 1968). Former studies reported that 6-OHDA caused selective, acute degeneration of symptomatic nerve terminals in the peripheral nervous system (Malmfors and Sachs 1968; Thoenen and Tranzer 1968).

Already back in 1968, Thoenen et al. postulated that 6-OHDA can serve as a valuable tool for functional studies of the central nervous system (Thoenen and Tranzer 1968). The injection of 6-OHDA into the substantia nigra and the subsequent depletion of the nigro-neostriatal dopamine (DA) system were associated with marked motor asymmetry in the animals. This is in good agreement with data of Pottier et al. (1966). They found hypokinesia of the limbs ipsilateral to the depletion of striatal amines after the induction of lesions in monkeys. The same type of electrothermal lesion in rats also depleted natal DA (Hokfelt and Ungerstedt 1969). Moreover, it resulted in ipsilateral turning behavior, which is very similar to that found after nigral injection of 6-OHDA. Motor asymmetries were also reported upon removal of the corpus striatum and treatment with various drugs that interfere with catecholamine neurotransmission. Similar data to these were obtained after unilateral injection of DA into the nucleus caudatus putamen, which resulted in contralateral turning behavior (Ungerstedt 1968). From the results listed above, it seems probable that intracerebrally injected 6-OHDA is able to induce degeneration of catecholamine cell bodies, axons, and terminals in the vicinity of the site of injection. It thus offers the possibility of tracing these neuron systems in the brain, for instance, by studying the accumulation and disappearance of the transmitter after local injections of 6-OHDA into various cell groups, axon bundles, or terminal areas. Degeneration of the nigro-neostriatal DA neuron system, caused by 6-OHDA, produces marked motor disturbances, which are known to be associated with this system.

Several local sites of injection have been used to damage the central dopaminergic pathways, including intraventricular, intracisternal, and intracerebral tissues. For reviews on the animal model, see Bove et al. (2005), Cenci et al. (2002), and Duty and Jenner (2011).

3 In Vitro Experimental Cell Culture Models

There is accumulating evidence from in vitro and in vivo studies, implicating cell death in the etiology of the 6-OHDA model of PD. For instance, in neuronal (cerebellar granule cells, primary mesencephalic dopaminergic) and nonneuronal (mesencephalic-derived dopaminergic MN9D, NB41, SH-SY5Y, thymocytes, bovine chromaffin cells,) cell cultures, 6-OHDA induces characteristic biochemical, histochemical, and morphological changes that are characteristic for apoptosis (Blum et al. 2001). Furthermore, intracerebral injection of 6-OHDA causes apoptosis of dopaminergic neurons in the substantia nigra (Bove et al. 2005; Duty and Jenner 2011).

4 Cell Death Pathways Activated by 6-OHDA

4.1 Reactive Oxygen Species

Reactive oxygen species (ROS) are important for execution of physiological functions. However, excessive production of ROS is detrimental to the cell. Following an increase in ROS production, the cell's redox equilibrium is shifted to a more oxidized state, affecting both the structure and the function of different molecules. This may lead to specific toxic processes, which compromise the redox status of the cell and can cause cell death. Due to high levels of polyunsaturated fatty acids in their membranes and the relatively low activity of endogenous antioxidant enzymes, cells in the brain are particularly susceptible to oxidative damage. Increased oxidative stress together with a decline in endogenous antioxidants is an important determinant to develop PD. In our model, 6-OHDA initiates neurodegeneration through a combination of oxidative stress and mitochondrial respiratory dysfunction (Fig. 2).

Under physiological conditions, 6-OHDA is rapidly and nonenzymatically oxidized by molecular oxygen to form 1,4-para-quinone and its degradation products (Gee and Davison 1989), along with production of ROS such as hydrogen peroxide (H_2O_2), superoxide radical ($\bullet O_2^-$), and hydroxyl radical ($\bullet OH^-$). Quinones react with nucleotic groups of macromolecules, leading to inactive or destroyed quinoproteins. These do not seem to contribute significantly to the observed cytotoxic effects of 6-OHDA. H_2O_2 can enter the cells and reacts with trace metals to form highly reactive $\bullet OH^-$ (Koppenol 2001), which can oxidatively damage proteins, lipids, and DNA (Beckman and Ames 1997). We have shown that 6-OHDA concentrations that were nontoxic to cell cultures did not significantly

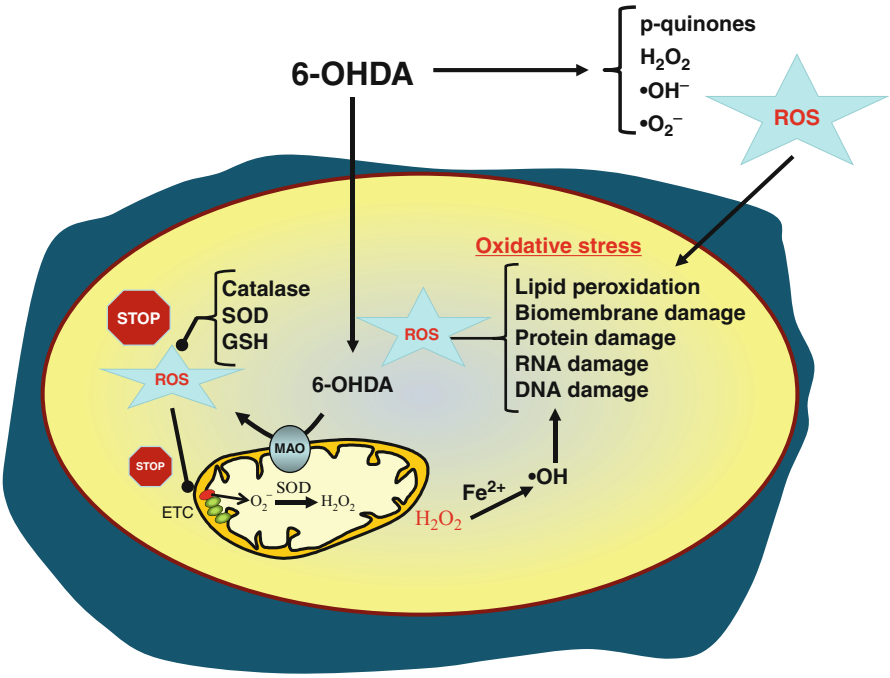

Fig. 2 6-OHDA initiates neurodegeneration through oxidative stress

increase H_2O_2 production (Galindo et al. 2003). Moreover, H_2O_2 addition to cultures produced a pattern of cell death similar to 6-OHDA.

In addition to the nonenzymatic self-auto-oxidation process, microinjection of 6-OHDA into the striatum may lead to the generation of H_2O_2 via a mitochondrial enzymatic oxidation process. Inhibition of complex I of the electron transport chain also stimulated mitochondrial production of superoxide radicals. These superoxide radicals were then catalyzed to H_2O_2 by superoxide dismutase, and, subsequently, •OH^- may arise from the breakdown of H_2O_2. This may be associated with the mitochondrial dysfunction seen in our experiments, because •OH^- rapidly attacks other biological molecules. The radicals produced in molecules such as lipids and proteins may also interact with mitochondrial enzymes to cause degradation.

4.2 Mitochondrial Dysfunction

Mitochondrial dysfunction is associated with many brain diseases including Parkinson's disease, Alzheimer's disease, and brain ischemia (Galindo et al. 2010; Jordan et al. 2011). However, the enzymatic basis for mitochondrial dysfunction remains largely unknown. Mitochondrial dysfunction in PD has been demonstrated as a reduction in complex I activity in the brains of patients (Mizuno et al. 1989; Schapira et al. 1989) and laboratory animals (Dabbeni-Sala et al. 2001).

Increased lipid peroxidation in the substantia nigra is typical of patients with PD (Dexter et al. 1989). This suggests that ROS are produced in excess, resulting in chronic local oxidative stress, which may cause progressive degeneration of the nigral dopaminergic neurons. Normally, ROS are probably produced in large quantities in the substantia nigra of humans, since there is an abundance of detoxifying enzymes, such as superoxide dismutase in dopaminergic neurons (Ceballos et al. 1990) and glutathione peroxidase in glial cells. In parkinsonian patients, the activity of these enzymes is not by definition abnormal (Marttila et al. 1988). However, decreased concentrations of glutathione (Perry et al. 1982) and increased activity of mitochondrial superoxide dismutase (Saggu et al. 1989) indicate that an excess of free radicals may be produced. Importantly, 6-OHDA readily reduces striatal levels of antioxidant enzymes (total glutathione (GSH) or superoxide dismutase) (Kunikowska and Jenner 2001; Perumal et al. 1992) to interact directly with complexes I and V of the mitochondrial respiratory chain. This leads to subsequent respiratory inhibition and further oxidative stress. Many of these effects are thought to mirror events that occur during PD in the brain, and this strongly supports the validity of the 6-OHDA model.

The oxidant H_2O_2 elicits the suppression of thiol-dependent electron transport, which appears to be related to the inhibition of complex I (nicotinamide adenine dinucleotide coenzyme Q reductase) in the respiratory chain (Mizuno et al. 1989). A 1-chloro-2,4-dinitrobenzene reductase in the mitochondrial matrix has been shown to be a thioredoxin reductase that reduces glutathione disulfide to glutathione (Lenartowicz and Wudarczyk 1995). 6-OHDA induced thiol oxidation in brain mitochondria, which was diminished by antioxidant enzymes. DA toxicity on neuronal cells is inhibited by thiol compounds and is potentiated by mitochondrial function inhibitors. Consequently, we and others have observed protection against 6-OHDA-induced toxicity in cell cultures by treatment with different antioxidant compounds (Galindo et al. 2003).

4.3 Mitochondria as Central Regulators of 6-OHDA Neurotoxicity

Mitochondria play critical roles in regulating cellular viability and show characteristic vulnerability to injury. Disruption of mitochondrial Ca^{2+} homeostasis may be involved in neuronal cell death. Mitochondria exposed to oxidative stress show enhanced Ca^{2+} uptake and increased formation of ROS. 6-OHDA slightly enhanced the Ca^{2+} uptake by mitochondria and apparently stimulated ruthenium red-induced Ca^{2+} release. The inhibitory effect of antioxidant enzymes indicates that ROS may be involved in the mitochondrial Ca^{2+} elevation induced by catecholamines.

Mitochondria can be considered as headquarters where the cell controls signaling pathways that under some circumstances can lead to cell death (Jordan et al. 2003a, 2011). Mitochondrial membrane permeabilization is a critical event during apoptosis and represents the point of no return of this lethal process (Galluzzi et al. 2009). Permeabilization of the mitochondrial outer membrane (MOMP) is a crucial

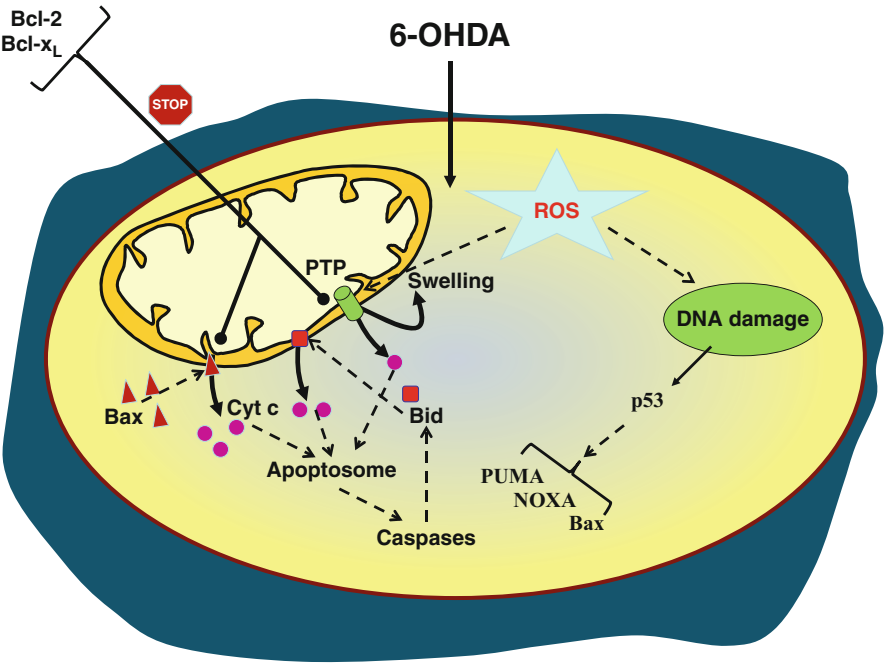

Fig. 3 Hypothetical mechanisms of 6-OHDA-induced MOMP

step both in apoptosis and necrosis. This phenomenon allows the release of mitochondrial death factors, which facilitates or triggers different signaling cascades. Ultimately this causes the execution of the cell involving release of cytochrome *c*. Treatment with 6-OHDA induced the release of cytochrome *c* from brain mitochondria (Gomez-Lazaro et al. 2008b).

MOMP is regulated either by the formation of the permeability transition pore (PTP) or by the insertion of Bcl-2 family members into the MOM (Fig. 3). There is some controversy about the role of the PTP in the 6-OHDA model. The PTP is a nonspecific large proteinaceous pore, spanning both mitochondrial membranes. It allows the passage of ions and substrates, in cost of loss of mitochondrial membrane potential. This leads to ATP depletion and energetic collapse and thus contributes to cell death (Qian et al. 1999). Onset of a PTP is such a severe perturbation of mitochondrial function that it basically assures cell death.

The PTP has been proposed to be another target of DA oxidation products in mitochondria. The oxidation of DA to dopamine quinone causes a significant increase in the swelling of brain and liver mitochondria. The effect of dopamine and 6-OHDA on mitochondrial membrane permeability has not been clearly elucidated. We have demonstrated that 6-OHDA did not induce PTP formation (Gomez-Lazaro et al. 2008b). First, 6-OHDA did not decrease the levels of mitochondrial calcein fluorescence, a hallmark of PTP opening. Second, 6-OHDA-induced

mitochondrial cytochrome c release was not inhibited in cell cultures that were pretreated with the PTP inhibitor cyclosporine A. Third, a mitochondria suspension isolated from brain did not readily undergo swelling upon exposure to 6-OHDA.

6-OHDA is also able to activate the multiple conductance channel (MCC), which is located in the inner membrane of mitochondria (Jordan et al. 2004). Normally, under physiological conditions, the MCC is closed, but it can be activated by different stimuli, such as Ca^{2+} at millimolar concentrations. Activation of the MCC leads to a multitude of changes in mitochondria, including mitochondrial membrane potential ($\Delta\Psi m$) dissipation.

6-OHDA induces profound mitochondrial fission in SH-SY5Y cell cultures, an event that precedes collapse of the $\Delta\Psi m$ and cytochrome *c* release (Gomez-Lazaro et al. 2008a). Mitochondria form a highly dynamic semi-tubular network, the morphology of which is regulated by frequent fission and fusion events as well as by movements along the cytoskeleton (Hoppins et al. 2007). Although the physiological significance of mitochondrial dynamics is not fully understood, growing evidence indicates that maintaining correct mitochondrial morphology through fission and fusion is critical for cell function (Chand et al. 2006; Chen and Chan 2006; Hoppins et al. 2007; Kiefel et al. 2006; Rouiller 1960). A reduction of the mitochondrial volume has been observed in pathological tissue (Arbustini et al. 1998; Nishino et al. 1998).

4.4 Bcl-2 Family Participation

Alternatively, MOMP may be initiated by insertion of the proto-oncogene Bcl-2 family into the MOM. The Bcl-2 family is composed of about 25 key regulators of apoptotic processes. These proteins are structural and functional homologs of the nematode protein CED-9 and are localized in the mitochondrial membrane. They contain up to four regions with a high homology to Bcl-2 (regions BH 1 to 4). Members containing only the BH3 region are proapoptotic proteins, and among them are Bax (X Bcl-2-associated protein), Bak (Bcl-2-antagonist/killer), BIM, and BID. How these proteins modulate changes in mitochondrial permeability is still an enigma. Inactive Bax resides in the cytosol or is anchored to the laxly face of the membranes of various organelles (Wolter et al. 1997). After a cell death signal, the Bax protein acquires a homo-oligomeric shape and is incorporated into the outer mitochondrial membrane. Postmortem studies indicated that the presence of Bax and its translocation to the outer mitochondrial membrane may contribute to the death of dopaminergic neurons in PD (Hartmann et al. 2001).

The protein BID is another Bcl-2 family member that migrates to mitochondria during processes of cell death. This protein is activated by caspase-8 and gives rise to a spoofed form t-bid, which is able to migrate to mitochondria, thereby altering their permeability. This migration is considered as the link between apoptotic caspase-8, triggered by signals of cell death, and downstream signaling cascades. On the other hand, some members of the Bcl-2 family have been proposed to modulate PTP formation. One example is Bcl-2, which inhibits the formation

of the PTP. Bcl-2 stabilizes the mitochondrial membrane, increases the buffering capacity of Ca^{2+}, and protects the $\Delta\Psi m$ to various stimuli. Yamada et al. have demonstrated that injection of a Bcl-2-producing vector into the substantia nigra 1 week prior to 6-OHDA injection increased neuronal survival in this tissue after the lesion (Yamada et al. 1999).

A potent induction of the BH3-only protein PUMA has been observed in response to 6-OHDA at both mRNA and protein levels (Gomez-Lazaro et al. 2008b). PUMA is thought to mediate the majority of the proapoptotic effects of p53 activity, which correlates with neuronal death in neurodegenerative diseases (Gomez-Lazaro et al. 2004; Gomez-Lazaro et al. 2005; Jordan et al. 2003b; Steffan et al. 2000; Tieu et al. 2001) including PD (Duan et al. 2002; Mandir et al. 2002).

Bcl-xL also belongs to the Bcl-2 family of proteins. Bcl-xL is able to protect cells from apoptosis induced by various stimuli, including serum deprivation, heat shock, malonate, and chemotherapeutic drugs (Fernandez-Gomez et al. 2005; Tsujimoto 1989). This suggests that the ability of Bcl-xL to prevent apoptosis is achieved by blocking a common cell death pathway. Our data indicate that Bcl-xL might prevent 6-OHDA effects by inhibiting MCC activation by the toxin (Jordan et al. 2004). TAT-Bcl-xL further reduces the number of cells that contain fragmented DNA or are caspase-3-reactive and increases the number of viable neurons in the striatum (Cao et al. 2002).

4.5 Caspases

Finally, the caspase family of proteases comprises fundamental components of many apoptotics signaling pathways. The percentage of active caspase-3-positive neurons among dopaminergic neurons was significantly higher in PD patients than in controls (Hartmann et al. 2000). Electron microscopy analysis of the human brain and in vitro data suggest that caspase-3 activation precedes and is not a consequence of apoptotic cell death in PD. Activation of caspase-3 was also demonstrated in 6-OHDA-induced apoptosis of SH-SY5Y cells (Gomez-Lazaro et al. 2008b). Pretreatment with caspase inhibitors prevented 6-OHDA-induced apoptosis (Hartmann et al. 2000; Takai et al. 1998). In human PD brain studies, a significantly higher percentage of dopaminergic substantia nigra neurons displayed activation of caspase-1, caspase-3, caspase-8, and caspase-9 (Hartmann et al. 2000; Mogi et al. 2000).

5 Flaws of the Model

However, the predictive validity of this model remains uncertain; strategies that reduced nigral dopaminergic cell loss in experimental animals so far have not been translated to the clinic. This can be due to the fact that the 6-OHDA model does not capture all features of PD. For example, it cannot exclude that PD pathological changes may occur in many brain areas outside of the basal ganglia locus coeruleus

and raphe nuclei, and this is not incorporated in the 6-OHDA model. Moreover, in the 6-OHDA model cell death occurs far more rapidly than in PD. Nevertheless, as long as these limitations are understood and accounted for when interpreting effects, the 6-OHDA model will remain at the forefront of preclinical drug discovery for PD.

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References

Arbustini, E., Diegoli, M., Fasani, R., Grasso, M., Morbini, P., Banchieri, N., Bellini, O., Dal Bello, B., Pilotto, A., Magrini, G., Campana, C., Fortina, P., Gavazzi, A., Narula, J., & Vigano, M. (1998). Mitochondrial DNA mutations and mitochondrial abnormalities in dilated cardiomyopathy. *American Journal of Pathology, 153*, 1501–1510.

Beckman, K. B., & Ames, B. N. (1997). Oxidative decay of DNA. *Journal of Biological Chemistry, 272*, 19633–19636.

Blum, D., Torch, S., Lambeng, N., Nissou, M., Benabid, A. L., Sadoul, R., & Verna, J. M. (2001). Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: Contribution to the apoptotic theory in Parkinson's disease. *Progress in Neurobiology, 65*, 135–172.

Bove, J., Prou, D., Perier, C., & Przedborski, S. (2005). Toxin-induced models of Parkinson's disease. *NeuroRx, 2*, 484–494.

Cao, G., Pei, W., Ge, H., Liang, Q., Luo, Y., Sharp, F. R., Lu, A., Ran, R., Graham, S. H., & Chen, J. (2002). In vivo delivery of a Bcl-xL fusion protein containing the TAT protein transduction domain protects against ischemic brain injury and neuronal apoptosis. *Journal of Neuroscience, 22*, 5423–5431.

Ceballos, I., Lafon, M., Javoy-Agid, F., Hirsch, E., Nicole, A., Sinet, P. M., & Agid, Y. (1990). Superoxide dismutase and Parkinson's disease. *The Lancet, 335*, 1035–1036.

Cenci, M. A., Whishaw, I. Q., & Schallert, T. (2002). Animal models of neurological deficits: How relevant is the rat? *Nature Reviews Neuroscience, 3*, 574–579.

Chand, D. K., Biradha, K., Kawano, M., Sakamoto, S., Yamaguchi, K., & Fujita, M. (2006). Dynamic self-assembly of an M3L6 molecular triangle and an M4L8 tetrahedron from naked Pd(II) ions and bis(3-pyridyl)-substituted arenes. *Chemistry, An Asian Journal, 1*, 82–90.

Chen, H., & Chan, D. C. (2006). Critical dependence of neurons on mitochondrial dynamics. *Current Opinion in Cell Biology, 18*, 453–459.

Dabbeni-Sala, F., Floreani, M., Franceschini, D., Skaper, S. D., & Giusti, P. (2001). Kainic acid induces selective mitochondrial oxidative phosphorylation enzyme dysfunction in cerebellar granule neurons: Protective effects of melatonin and GSH ethyl ester. *The FASEB Journal, 15*, 1786–1788.

Dexter, D. T., Carter, C. J., Wells, F. R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P., & Marsden, C. D. (1989). Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *Journal of Neurochemistry, 52*, 381–389.

Duan, W., Zhu, X., Ladenheim, B., Yu, Q. S., Guo, Z., Oyler, J., Cutler, R. G., Cadet, J. L., Greig, N. H., & Mattson, M. P. (2002). p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism. *Annals of Neurology, 52*, 597–606.

Duty, S., & Jenner, P. (2011). Animal models of Parkinson's disease: A source of novel treatments and clues to the cause of the disease. *British Journal of Pharmacology, 164*, 1357–1391.

Fernandez-Gomez, F. J., Galindo, M. F., Gomez-Lazaro, M., Yuste, V. J., Comella, J. X., Aguirre, N., & Jordan, J. (2005). Malonate induces cell death via mitochondrial potential collapse and delayed swelling through an ROS-dependent pathway. *British Journal of Pharmacology, 144*, 528–537.

Galindo, M. F., Jordan, J., Gonzalez-Garcia, C., & Cena, V. (2003). Chromaffin cell death induced by 6-hydroxydopamine is independent of mitochondrial swelling and caspase activation. *Journal of Neurochemistry, 84*, 1066–1073.

Galindo, M. F., Ikuta, I., Zhu, X., Casadesus, G., & Jordan, J. (2010). Mitochondrial biology in Alzheimer's disease pathogenesis. *Journal of Neurochemistry, 114*, 933–945.

Galluzzi, L., Blomgren, K., & Kroemer, G. (2009). Mitochondrial membrane permeabilization in neuronal injury. *Nature Reviews Neuroscience, 10*, 481–494.

Gee, P., & Davison, A. J. (1989). Intermediates in the aerobic autoxidation of 6-hydroxydopamine: Relative importance under different reaction conditions. *Free Radical Biology & Medicine, 6*, 271–284.

Gomez-Lazaro, M., Fernandez-Gomez, F. J., & Jordan, J. (2004). p53: Twenty five years understanding the mechanism of genome protection. *Journal of Physiology and Biochemistry, 60*, 287–307.

Gomez-Lazaro, M., Galindo, M. F., Fernandez-Gomez, F. J., Prehn, J. H., & Jordan, J. (2005). Activation of p53 and the pro-apoptotic p53 target gene PUMA during depolarization-induced apoptosis of chromaffin cells. *Experimental Neurology, 196*, 96–103.

Gomez-Lazaro, M., Bonekamp, N. A., Galindo, M. F., Jordan, J., & Schrader, M. (2008a). 6-Hydroxydopamine (6-OHDA) induces Drp1-dependent mitochondrial fragmentation in SH-SY5Y cells. *Free Radical Biology & Medicine, 44*, 1960–1969.

Gomez-Lazaro, M., Galindo, M. F., Concannon, C. G., Segura, M. F., Fernandez-Gomez, F. J., Llecha, N., Comella, J. X., Prehn, J. H., & Jordan, J. (2008b). 6-Hydroxydopamine activates the mitochondrial apoptosis pathway through p38 MAPK-mediated, p53-independent activation of Bax and PUMA. *Journal of Neurochemistry, 104*, 1599–1612.

Hartmann, A., Hunot, S., Michel, P. P., Muriel, M. P., Vyas, S., Faucheux, B. A., Mouatt-Prigent, A., Turmel, H., Srinivasan, A., Ruberg, M., Evan, G. I., Agid, Y., & Hirsch, E. C. (2000). Caspase-3: A vulnerability factor and final effector in apoptotic death of dopaminergic neurons in Parkinson's disease. *Proceedings of the National Academy of Sciences United States of America, 97*, 2875–2880.

Hartmann, A., Michel, P. P., Troadec, J. D., Mouatt-Prigent, A., Faucheux, B. A., Ruberg, M., Agid, Y., & Hirsch, E. C. (2001). Is Bax a mitochondrial mediator in apoptotic death of dopaminergic neurons in Parkinson's disease? *Journal of Neurochemistry, 76*, 1785–1793.

Hokfelt, T., & Ungerstedt, U. (1969). Electron and fluorescence microscopical studies on the nucleus caudatus putamen of the rat after unilateral lesions of ascending nigro-neostriatal dopamine neurons. *Acta Physiologica Scandinavica, 76*, 415–426.

Hoppins, S., Lackner, L., & Nunnari, J. (2007). The machines that divide and fuse mitochondria. *Annual Review of Biochemistry, 76*, 751–780.

Jordan, J., Cena, V., & Prehn, J. H. (2003a). Mitochondrial control of neuron death and its role in neurodegenerative disorders. *Journal of Physiology and Biochemistry, 59*, 129–141.

Jordan, J., Galindo, M. F., Gonzalez-Garcia, C., & Cena, V. (2003b). Role and regulation of p53 in depolarization-induced neuronal death. *Neuroscience, 122*, 707–715.

Jordan, J., Galindo, M. F., Tornero, D., Gonzalez-Garcia, C., & Cena, V. (2004). Bcl-x L blocks mitochondrial multiple conductance channel activation and inhibits 6-OHDA-induced death in SH-SY5Y cells. *Journal of Neurochemistry, 89*, 124–133.

Jordan, J., de Groot, P. W., & Galindo, M. F. (2011). Mitochondria: The headquarters in ischemia-induced neuronal death. *Central Nervous System Agents in Medicinal Chemistry, 11*(2), 98–106.

Kiefel, B. R., Gilson, P. R., & Beech, P. L. (2006). Cell biology of mitochondrial dynamics. *International Review of Cytology, 254*, 151–213.

Koppenol, W. H. (2001). The Haber-Weiss cycle – 70 years later. *Redox Report, 6*, 229–234.

Kunikowska, G., & Jenner, P. (2001). 6-Hydroxydopamine-lesioning of the nigrostriatal pathway in rats alters basal ganglia mRNA for copper, zinc- and manganese-superoxide dismutase, but not glutathione peroxidase. *Brain Research, 922*, 51–64.

Laverty, R., & Sharman, D. F. (1965). Modification by drugs of the metabolism of 3,4-dihydroxyphenylethylamine, noradrenaline and 5-hydroxytryptamine in the brain. *British Journal of Pharmacology and Chemotherapy, 24*, 759–772.

Lenartowicz, E., & Wudarczyk, J. (1995). Enzymatic reduction of 5,5′-dithiobis-(2-nitrobenzoic acid) by lysate of rat liver mitochondria. *The International Journal of Biochemistry & Cell Biology, 27*, 831–837.

Malmfors, T., & Sachs, C. (1968). Degeneration of adrenergic nerves produced by 6-hydroxydopamine. *European Journal of Pharmacology, 3*, 89–92.

Mandir, A. S., Simbulan-Rosenthal, C. M., Poitras, M. F., Lumpkin, J. R., Dawson, V. L., Smulson, M. E., & Dawson, T. M. (2002). A novel in vivo post-translational modification of p53 by PARP-1 in MPTP-induced parkinsonism. *Journal of Neurochemistry, 83*, 186–192.

Marttila, R. J., Lorentz, H., & Rinne, U. K. (1988). Oxygen toxicity protecting enzymes in Parkinson's disease. Increase of superoxide dismutase-like activity in the substantia nigra and basal nucleus. *Journal of the Neurological Sciences, 86*, 321–331.

Mizuno, Y., Ohta, S., Tanaka, M., Takamiya, S., Suzuki, K., Sato, T., Oya, H., Ozawa, T., & Kagawa, Y. (1989). Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease. *Biochemical and Biophysical Research Communications, 163*, 1450–1455.

Mogi, M., Togari, A., Kondo, T., Mizuno, Y., Komure, O., Kuno, S., Ichinose, H., & Nagatsu, T. (2000). Caspase activities and tumor necrosis factor receptor R1 (p55) level are elevated in the substantia nigra from parkinsonian brain. *Journal of Neural Transmission, 107*, 335–341.

Nishino, I., Kobayashi, O., Goto, Y., Kurihara, M., Kumagai, K., Fujita, T., Hashimoto, K., Horai, S., & Nonaka, I. (1998). A new congenital muscular dystrophy with mitochondrial structural abnormalities. *Muscle & Nerve, 21*, 40–47.

Perry, T. L., Godin, D. V., & Hansen, S. (1982). Parkinson's disease: A disorder due to nigral glutathione deficiency? *Neuroscience Letters, 33*, 305–310.

Perumal, A. S., Gopal, V. B., Tordzro, W. K., Cooper, T. B., & Cadet, J. L. (1992). Vitamin E attenuates the toxic effects of 6-hydroxydopamine on free radical scavenging systems in rat brain. *Brain Research Bulletin, 29*, 699–701.

Poirier, L. J., Sourkes, T. L., Bouvier, G., Boucher, R., & Carabin, S. (1966). Striatal amines, experimental tremor and the effect of harmaline in the monkey. *Brain, 89*, 37–52.

Porter, C. C., Totaro, J. A., & Stone, C. A. (1963). Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. *Journal of Pharmacology and Experimental Therapeutics, 140*, 308–316.

Qian, T., Herman, B., & Lemasters, J. J. (1999). The mitochondrial permeability transition mediates both necrotic and apoptotic death of hepatocytes exposed to Br-A23187. *Toxicology and Applied Pharmacology, 154*, 117–125.

Rouiller, C. (1960). Physiological and pathological changes in mitochondrial morphology. *International Review of Cytology, 9*, 227–292.

Saggu, H., Cooksey, J., Dexter, D., Wells, F. R., Lees, A., Jenner, P., & Marsden, C. D. (1989). A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. *Journal of Neurochemistry, 53*, 692–697.

Schapira, A. H., Cooper, J. M., Dexter, D., Jenner, P., Clark, J. B., & Marsden, C. D. (1989). Mitochondrial complex I deficiency in Parkinson's disease. *The Lancet, 1*, 1269.

Steffan, J. S., Kazantsev, A., Spasic-Boskovic, O., Greenwald, M., Zhu, Y. Z., Gohler, H., Wanker, E. E., Bates, G. P., Housman, D. E., & Thompson, L. M. (2000). The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proceedings of the National Academy of Sciences United States of America, 97*, 6763–6768.

Takai, N., Nakanishi, H., Tanabe, K., Nishioku, T., Sugiyama, T., Fujiwara, M., & Yamamoto, K. (1998). Involvement of caspase-like proteinases in apoptosis of neuronal PC12 cells and primary cultured microglia induced by 6-hydroxydopamine. *Journal of Neuroscience Research, 54*, 214–222.

Thoenen, H., & Tranzer, J. P. (1968). Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. *Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie, 261*, 271–288.

Tieu, K., Ashe, P. C., Zuo, D. M., & Yu, P. H. (2001). Inhibition of 6-hydroxydopamine-induced p53 expression and survival of neuroblastoma cells following interaction with astrocytes. *Neuroscience, 103*, 125–132.

Tsujimoto, Y. (1989). Stress-resistance conferred by high level of bcl-2 alpha protein in human B lymphoblastoid cell. *Oncogene, 4*, 1331–1336.

Ungerstedt, U. (1968). 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *European Journal of Pharmacology, 5*, 107–110.

Wolter, K. G., Hsu, Y. T., Smith, C. L., Nechushtan, A., Xi, X. G., & Youle, R. J. (1997). Movement of Bax from the cytosol to mitochondria during apoptosis. *The Journal of Cell Biology, 139*, 1281–1292.

Yamada, M., Oligino, T., Mata, M., Goss, J. R., Glorioso, J. C., & Fink, D. J. (1999). Herpes simplex virus vector-mediated expression of Bcl-2 prevents 6-hydroxydopamine-induced degeneration of neurons in the substantia nigra in vivo. *Proceedings of the National Academy of Sciences United States of America, 96*, 4078–4083.

Advances in Stem Cell Research for Parkinson Disease

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Contents

Abstract

There is significant interest in using stem cells as a cell source for treatment against Parkinson's and other diseases due to the limited self-repair ability of the central nervous system and the lack of a pharmacological treatment to replace lost

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neurons. Parkinson's disease is one of the main neurodegenerative disorders, and it is characterized by the degeneration of the nigrostriatal pathway caused by loss of ventral midbrain dopaminergic neurons in the substantia nigra pars compacta. Currently, there are no treatments to halt disease progress; the treatments merely ameliorate motor symptoms for a time, but the treatments are often followed by severe side effects. Cell-replacement therapy has been proposed as a treatment to replace damaged neurons and protect the remaining cells. Preliminary studies have utilized adult tissues, embryonic/fetal tissues, and embryonic stem cells as a source of dopaminergic cells. However, the ethical issues related to this type of procedure, the high quantity of required cells, the risk of tumor formation, and the immunological rejection associated with it have prevented this technique from being an effective treatment against Parkinson's disease. Researchers have been challenged to focus on new cell-replacement alternatives. In 2006, researchers found that only four transcription factors were necessary to reprogram an adult somatic cell to an induced pluripotent stem cell. This breakthrough revolutionized the scientific community, as this technique would allow scientists to obtain specific cells from the patient, avoiding rejection problems from non-autologous grafts and avoiding ethical problems. In addition, a large number of cells could be obtained. Although induced pluripotent stem (iPS) cells have great potential as a treatment against this disease, there are some obstacles for its clinical use such as the lack of standardized protocols that result in a dopaminergic cell phenotype that grafts without undifferentiated cells and without a risk of tumor formation. Currently, the iPS cell system is an ideal model of study for many other diseases besides Parkinson's, and the system is also good for drug screening. Recently, the direct reprogramming of somatic cells to induce neuronal (iN) cells without generating a pluripotent stage could be the solution to avoiding tumor formation risk. However, as a result of the low quantity of cells obtained, more investigations are necessary to convert this technique into an efficient cell-replacement therapy. If we overcome these obstacles, the use of iPS and iN cells could be, without a doubt, a promising therapy in the near future.

Keywords

Dopaminergic neurons • Induced neuronal cells • Induced pluripotent stem cells • Parkinson's disease • Pluripotency • Reprogramming • Stem cell • Therapy

List of Abbreviations

ASCs	Adult stem cells
ATRA	All-trans-retinoic acid
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
CB-SCs	Cord blood-derived multipotent stem cells
CNS	Central nervous system
DA	Dopamine
EGF	Epidermal growth factor

ESCs	Embryonic stem cells
FGF2	Fibroblast growth factor 2
FGF20	Fibroblast growth factor 20
FGF8	Fibroblast growth factor 8
GDNF	Glial cell line-derived neurotrophic factor
GSK-3	Glycogen synthase kinase 3
hESCs	Human embryonic stem cells
HGF	Hepatocyte growth factor
HSCs	Hematopoietic stem cells
HUVMSCs	Human umbilical vein mesenchymal stem cells
ICM	Inner cell mass
iN	Induced neuronal
iPSCs	Induced pluripotent stem cells
MSCs	Mesenchymal stem cells
NP	Neural progenitor
NPSCs	Neural progenitor stem cells
NSCs	Neural stem cells
NSPCs	Neural stem cell and neural progenitor cell
PD	Parkinson's disease
PGCs	Primordial germ cells
SCC	Stem cell coactivator complex
SDF1α	Stromal cell-derived factor-1α
SDIA	Stromal cell-derived inducing activity
sFRP1	Secreted frizzled-related protein 1
SHH	Sonic hedgehog
SNpc	Substantia nigra pars compacta
STEMCCA	Single excisable polycistronic lentiviral stem cell cassette
SVZ	Subventricular zone
VEGFD	Vascular endothelial growth factor D

1 Stem Cells

The human body consists of different cell types organized into tissues and organs. In the adult state, tissues are maintained because of the proliferative ability of stem cells (SCs). However, not all tissues have the same potential for renewal.

Different adult tissues differ in terms of the proportion of proliferative cells. For example, proliferative cells were discovered in the pancreas that have been described as being different from stem cells (Dor et al. 2004; Zhao et al. 2008). In the pancreas, the cells were able to renew constantly. Normally, the proliferative capacity of tissue is maintained by stem cells that have not only extensive renewal capacity, but also the ability to generate daughter cells capable of differentiating. These cells are called adult stem cells (ASCs) (Watt and Driskell 2010).

1.1 Characteristics

Stem cells are unspecialized cells in an undifferentiated state that have an extraordinary capacity to proliferate indefinitely without differentiating. However, stem cells are capable of differentiating into one or more specialized cell types. A stem cell divides to generate one daughter stem cell and one progenitor cell during self-renewal; later, the progenitor cell achieves a fully differentiated state.

The stem cells can also be categorized by their ability to divide and produce differentiated cells. These cells can be classified as totipotent, pluripotent, and multipotent cells. Totipotent cells are those that give rise to all cells of an organism including germ cells, as in a zygote. Pluripotent cells generate all cell types in an embryo except the cells of the embryonic membrane such as the placenta. The cells of the inner cell mass (ICM) are pluripotent cells that generate three embryonic germ layers. Unlike those cells, multipotent cells are adult stem cells that, despite the fact that they can give rise to different cell types, only generate a given cell lineage. Adult stem cells can differentiate into more than one mature cell. Examples of these adult stem cells are hematopoietic stem cells, mesenchymal stem cells, and neural stem cells (NSCs).

The stem cells present with different types of differentiation: (i) direct differentiation, where a specific cell type develops in a unidirectional pathway; (ii) transdifferentiation, where a direct conversion occurs from one cell type to another, different, cell type; and (iii) dedifferentiation, where a unipotent stem cell generates a multipotent one. Moreover, a stem cell can also fuse with a somatic cell resulting in another lineage (Nadig 2010; Lunn et al. 2011).

1.2 Classification

Stem cells can also be classified according to their origin: embryonic stem cells (ESCs) and adult stem cells (postnatal stem cells/somatic stem cells). ESCs can be obtained from blastocyst, epiblast, and primordial germ cells. These cells have been described as pluripotent because they are able to generate all cell types of the three primary germ layers (ectoderm, mesoderm, and endoderm). Initially, embryonic stem cells were isolated from preimplantation mouse embryos in the blastocyst state (Evans and Kaufman 1981; Martin 1981). In the year 1998, it was reported that stem cells could also be derived from human blastocysts (Thomson et al. 1998). Pluripotent ESCs can be obtained from the post-implantation epiblasts of the mouse embryo (Brons et al. 2007; Tesar et al. 2007). In this case, ESCs were isolated from 12-day-old embryos called epiblasts. Likewise, pluripotent ESCs can be obtained from primordial germ cells, which are progenitors of adult gametes (Kerr et al. 2006). The use of embryonic tissue in research and clinical fields generates cultural disagreement, as cells are generally obtained from "stored" in vitro fertilization embryos. Ethical and cultural matters make the clinical practice with stem cell-based therapies difficult (Watt and Driskell 2010).

In contrast, ASCs are found in most adult tissues. Unlike ESCs, ASCs are not pluripotent cells but rather multipotent cells. ASCs have the ability to differentiate into more than one cell type but not all cell types, as a pluripotent cell does. Examples of such cells are hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). Hematopoietic stem cells can be obtained from cord or peripheral blood. These stem cells have been widely used in the treatment of several diseases such as leukemia, immunodeficiency, metabolic disorders, and tumors (Reimann et al. 2009; Perl et al. 2010). Mesenchymal stem cells originate in the stromal component of the bone marrow, although it is also possible to derive them from peripheral and umbilical cord blood (Perl et al. 2010). Mesenchymal stem cells are found in the bone marrow and hepatic, dermal, orofacial, and adult dental pulp tissues, among others (Gronthos et al. 2000; Nadig 2009; Nesti et al. 2011). Consequently, the use of MSCs as a treatment has been investigated for several diseases (Mundra et al. 2013; Morigi and Benigni 2012; Vawda and Fehlings 2012). Likewise, the role of MSCs in the contribution to tumor growth and progression has also been studied (Yang et al. 2013).

Currently, several human stem cell lines have been characterized that exhibit similar expression patterns for several pluripotency and lineage markers. However, these lines also present differences mainly due to the techniques used for their derivation and maintenance. Consequently, epigenetic factors and intrinsic genetic variations are important factors inherent to human samples, which could contribute to these variations and thus affect gene expression patterns (Adewumi et al. 2007).

1.3 Generation of Pluripotency

In the past, it was believed that only ESCs are pluripotent. However, further investigations have shown that not only are ESCs pluripotent, but adult cells can, in some circumstances, also produce progeny that differentiate across the three primary germ layers (ectoderm, mesoderm, and endoderm), which makes them pluripotent. A mechanism of reprogramming an adult cell to a pluripotent state is the transference of an adult nucleus into the cytoplasm of an oocyte (Gurdon et al. 1958; Gurdon and Melton 2008). An example of this technique is the creation of Dolly the sheep (Wilmut et al. 1997). Likewise, another mechanism to generate pluripotent cells is the fusion of adult cells with a pluripotent cell (Miller and Ruddle 1976). As described above, a pluripotent state can be generated through somatic nuclear transfer. Adult cells can also be reprogrammed to a pluripotent state by the transfection of only four transcription factors: Oct4, Sox2, Klf4, and Myc (Takahashi and Yamanaka 2006).

1.4 Markers of Pluripotency

During early embryonic development, cells change their gene expression profile. Some pluripotent state-related genes are expressed, whereas those related to

differentiation should be transcriptionally silenced. Many studies have identified the diverse pluripotency factors that are expressed in both embryos and stem cells.

Several methods have allowed for the identification of pluripotency factors. Studies performed in embryos, cell lines, and ESCs have relied on candidate gene analysis. Phenotypes resulting from specific knockdown, RNAi screens (Chambers et al. 2003; Rodda et al. 2005; Ivanova et al. 2006), gene expression profiles (Voutila et al. 2012; Zheng and Hu 2012), epigenetic profiles (Tomkins et al. 2012; Seisenberger et al. 2012; Al-Khtib et al. 2012), and protein expression profiles (Loh et al. 2006; Walker et al. 2007) have extended our knowledge of the genes and molecules associated with pluripotency.

These studies have allowed for the description of the role of the transcription factors Oct4, Sox2, Klf4, c-Myc, and Nanog for the establishment and/or maintenance of the pluripotent state.

However, a substantial amount remains to be learned regarding the changes in gene expression that occur during embryo development. Such changes are responsible for the pluripotent stem cell state and later to cell differentiation. In addition, it is crucial to understand the role of exogenous and endogenous factors for stem cell maintenance and how, through signaling pathways, gene expression levels are impacted.

These studies have helped determine a list of pluripotency-related genes that might be possible candidates for cellular reprogramming. However, the studies have not clarified the differential expression of genes leading to the pluripotent stem cell state nor how these genes determine the maintenance of the pluripotent state. It is important to emphasize that signaling pathways controlling pluripotency are extensively complex.

2 Stem Cells and Parkinson's Disease

2.1 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder that affects approximately 1 % of the population over 65 years of age worldwide (Twelves et al. 2003). However, the prevalence of this disease increases with the age. Studies performed in the USA have shown that PD incidence varies among Whites, Blacks, and Asians, and PD is substantially more common in Whites (Wright Willis et al. 2010). PD was described for the first time by Dr. James Parkinson in the year 1817. Although the disease etiology remains unknown, several factors are believed to be involved in PD development. This disease involves widespread dysfunction and cell degeneration that is characterized mainly by the gradual degeneration of ventral midbrain dopaminergic neurons in the substantia nigra pars compacta (SNpc), causing nigrostriatal pathway dysfunction. This degeneration leads to concomitant depletion dopamine (DA) in the striatum corpus, mostly in the caudate and putamen areas (Olanow 2004). This nigrostriatal pathway is related to controlling movement and gait. It should be noted that many patients suffering from

Parkinson's disease experience not only motor symptoms but also non-motor ones (Storch et al. 2013).

Parkinson's disease is mainly diagnosed by the motor clinical manifestations presented by patients. Unfortunately, the first motor symptoms are normally observed when more than 60 % of the dopaminergic neurons have died (Bernheimer et al. 1973). Currently, strong evidence shows the presence of non-motor symptoms such as olfactory dysfunction, sleep abnormalities, cardiac sympathetic denervation, depression, and pain long before the presence of motor symptoms (O'Sullivan et al. 2008; Obeso et al. 2010; Storch et al. 2013). These findings are particularly encouraging because they could aid in disease diagnosis at an early stage and thus result in therapies to halt its progress.

Currently, there are no effective treatments to cure this disease, and pharmacological therapies with dopaminergic drugs or functional neurosurgery merely improves the motor symptoms. The pharmacological treatments include dopamine precursors or dopamine agonists. It has been noted that treatment with L-dopa, the precursor of dopamine, may alleviate symptoms for only a few years. Moreover, long-term treatment with L-dopa is not satisfactory because the remaining dopaminergic neurons continue degenerating (Marsden 1994). In addition, chronic drug use is related to a variety of adverse effects such as L-dopa-induced dyskinesias (Politis et al. 2003; Fisone and Bezard 2011). Likewise, deep brain stimulation directed to the subthalamic nucleus or globus pallidus also may relieve symptoms in some patients (Odekerken et al. 2013). Current treatment options are oriented to reverse motors features of this disease such as tremor, rigidity, and bradykinesia/akinesia; however, these treatments do not halt PD progress. As the main cause of symptoms is ongoing degeneration, new treatments should, in the future, focus on halting dopaminergic neuron degeneration using neuroprotective agents or cell therapies to replace the degenerated neurons. Therefore, replacement therapy with stem cells or fetal tissue might be the best approach (Pawitan 2011), although many issues remain to be resolved before this technique can be used therapeutically. Transplanted cell survival is a major obstacle for the development of cell therapies for PD.

2.2 Stem Cell Therapy for Parkinson's Disease

Currently, PD patients are treated with various drugs including L-dopa, dopaminergic agonists, and inhibitors of dopamine-degrading enzymes. Likewise, deep brain stimulation has been utilized in numerous patients. While these treatments are effective early on to ameliorate the motor symptoms, the treatments do not halt the disease course. In recent decades, research efforts have focused on replacing the dopaminergic neurons that are lost in PD patients. To this end, cellular therapies that replace degenerated cells are the main interest focus today. These therapies involve the use of tissue transplants such as fetal, embryonic, or adult tissues as a source of dopaminergic cells or neural progenitor cells (Lunn et al. 2011). Over time, investigations included not only transplants from the same species but also

used autotransplantation and xenotransplantation between different species such as rats, mice, monkeys, pigs, and humans (Huffaker et al. 1989; Bankiewicz et al. 1990; Freed et al. 1990; Galpern et al. 1996; Hara et al. 1997; Molenaar et al. 1997; Barinaga 2000; Yurek and Fletcher-Turner 2004; Puschban et al. 2005; Arias-Carrión and Yuan 2009). Furthermore, investigations have performed the direct transplantation of SC-derived dopaminergic cells such as those derived from ESCs, induced pluripotent stem cells (iPSCs), fetal brain NSCs, and bone marrow-derived SC (Kim 2011). As recently described, other cell types, different from stem cells, may also be suitable for cell therapy. These cells include the induced neuronal (iN) cells, which are derived from somatic cells (Caiazzo et al. 2011; Politis and Lindvall 2012; Lévêque et al. 2012). Moreover, innovative therapies have emerged involving transplantation with genetically modified neuronal or nonneuronal cells. In some cases, the cells were genetically manipulated by introducing genes such as tyrosine hydroxylase, Bcl2, Nurr1, or neurotrophic factors (Holm et al. 2001; Torres et al. 2005; Shim et al. 2007; Ramos-Moreno et al. 2012).

One of the first transplantation studies was performed using a brain tissue graft containing dopamine-secreting cells in the rat striatum. The results were encouraging because the motor abnormalities present in these rats were significantly reduced after the fetal brain tissue graft (Perlow et al. 1979). Subsequent studies proceeded with searching for new sources of donor dopaminergic cells; some studies were very promising but others had poor results. The adrenal medulla, fetal or embryonic mesencephalic tissue, and the carotid body have been used as a source of dopamine-secreting cells (Strömberg et al. 1986; Espejo et al. 1998). Transplantation of human adrenal chromaffin cells or sympathetic neurons from the cervical ganglion was not effective (Yong et al. 1989). Adrenal medulla transplants also showed inconsistent results. Despite the fact that co-transplantation of peripheral nerves and the adrenal medulla in patients with Parkinson's disease can promote long-term improvement, other studies performed in patients showed that the adrenal medullary transplants do not survive long-term (López-Lozano et al. 2000; Kompoliti et al. 2007). On the other hand, the transplantation of mesencephalic dopaminergic neurons or the intrastriatal transplantation of carotid body tissues show more promise as future therapies for this disease (Mendez et al. 2005; Mínguez-Castellanos et al. 2007; Pardal and López-Barneo 2012).

The carotid body consists of adult tissue that contains dopamine-secreting cells and expresses high levels of glial cell line-derived neurotrophic factor GDNF (Toledo-Aral et al. 2003; Porzionato et al. 2008). Studies have shown that intrastriatal autotransplantation of carotid tissues induced functional recovery in parkinsonian rats and monkeys and in some patients with advanced PD (Espejo et al. 1998; Luquin et al. 1999; Arjona et al. 2003). More recently, a population of neural progenitor stem cells (NPSCs) was discovered in this organ (Pardal et al. 2007). Hence, not only is the transplantation of dopaminergic cells necessary for cell therapy, but also the presence of neurotrophic factors as a neuroprotection mechanism. These studies suggest the use of neurotrophic factors as a new experimental approach to combat this disease (Minguez-Castellanos and Escamilla-Sevilla 2005). There is concern because the grafted cells not only

produce dopamine, but also deliver neurotrophic factors that protect nigrostriatal neurons. Thus, the trophic factors would be capable of promoting restoration of the nigrostriatal pathway. In this regard, patient-specific adult stem cells derived from the carotid body could be an interesting candidate for PD treatment that avoids human embryonic/fetal tissue or embryonic stem cell use. The cells derived from adult tissues could also be expanded and differentiated into DA precursor cells in vitro (Pardal and López-Barneo 2012). More recently, studies of co-grafts of a cell suspension of rat dopaminergic neuroblasts with carotid body cells increased survival of dopaminergic neurons in rats with total unilateral dopaminergic denervation (Rodriguez-Pallares et al. 2012).

In spite of the fact that cell therapies with carotid tissues appeared to be effective in animal models and patients, the amount of tissue necessary for obtaining significant clinical benefit is one of the main factors limiting their use clinically. Although we are able to develop optimized protocols that could improve the safety and efficacy of these procedures, a major constraint is the availability of tissue for these grafts.

In this respect, progress in stem cell research appears to be the future for cell therapy against Parkinson's disease. Due to the self-renewal capacity and multilineage plasticity of stem cells, stem cells can generate numerous cell types needed for cell therapy. As mentioned previously, several types of stem cells have been isolated from many sources such as embryonic tissues, peripheral blood, umbilical cord blood, bone marrow, and the olfactory bulb. Stem cells have also been obtained from somatic cells; these stem cells comprise the ESCs, iPSCs, NSCs, and MSCs.

2.2.1 Embryonic/Fetal Tissue Cell Therapy for Parkinson's Disease

In the year 1988, Brundin and collaborators performed the first transplantation of human fetal mesencephalic neurons. The results were promising, showing that patients can effectively restore dopaminergic neurotransmission (Brundin et al. 1988). Because the utilization of human fetal tissue as source of dopamine cells is not easy, xenografting of the pig ventral mesencephalon may be an appropriate alternative to the use of human fetal tissue as a PD therapy. These studies showed how implants could survive and mediate functional recovery in animal model of PD and also in some patients, although immunological rejection is the main limitation in humans (Huffaker et al. 1989; Bankiewicz et al. 1990; Galpern et al. 1996; Molenaar et al. 1997; Schumacher et al. 2000; Larsson and Widner 2000).

One of the most commonly used therapies has been the intrastriatal transplantation of embryonic or fetal mesencephalic neurons, which have produced the best clinical results (Madrazo et al. 1990; Freed et al. 1990, 1992, 2001; Sirinathsinghji et al. 1990; Lindvall et al. 1994; Wenning et al. 1997; Piccini et al. 1999). Although in some cases the implanted neuron can survive many years, this phenomenon was not observed in all patients (Piccini et al. 1999). Thus, one of the main difficulties observed with these grafts is the low survival of implanted neurons (Piccini et al. 1999; Emgård et al. 1999; Kaminski Schierle et al. 1999). For this reason, further therapies were oriented to the application of neurotrophic factors as

a neuroprotective mechanism of neuronal degeneration (Kupsch et al. 1995; Ballagi et al. 1994; Collier et al. 1999). On the other hand, to improve transplant efficiency, new alternatives emerged including double grafts, either sequentially transplanted or co-grafted (Mendez et al. 1996; Sortwell et al. 1998; Hagell et al. 1999; Kaddis et al. 2000). Simultaneous intrastriatal and intranigral neuronal transplants in rat lesions contribute to striatal reinnervation and reestablishment of the nigrostriatal circuitry (Mendez et al. 1996). Moreover, sequential transplantation of human embryonic mesencephalic tissue in PD patients showed restoring of fluorodopa uptake without affecting graft survival (Hagell et al. 1999). Interestingly, co-grafts of DA neurons with Schwann cells, muscle cells with mesencephalic tissue, or mesencephalic tissue with striatal cell suspensions increase the survival and outgrowth of the DA neurons present in mesencephalic tissue. These effects were related to the neurotrophic factors delivered by the Schwann, skeletal muscle, and striatal cells (Sortwell et al. 1998; Collier et al. 1999; Kaddis et al. 2000). An interesting study performed in 250 patients with advanced PD showed how transplanted embryonic neuronal grafts survive and induce significant therapeutic effects (Björklund and Lindvall 1999).

All investigations performed in animals and humans have contributed to establishing the requirements of the transplanted cells to be used as a cell therapy for Parkinson's disease. Therefore, some important points to consider regarding establishing transplanted cells as possible cell therapy are the following: the presence of functional and morphological characteristics similar to dopaminergic neurons; the existence of the capacity to reestablish nigrostriatal circuitry; the presence of greater cell survival in tissue; the absence of adverse effects such as transplant-associated dyskinesias, transplant rejection, or tumor formation; and the reversion of motor symptoms in PD patients and animal models. On the other hand, difficulties in obtaining fetal tissue have added to the adverse effects caused by transplantation (the graft-induced dyskinesias in some patients), and the low survival of grafted cells makes this technique unsuitable for practice in numerous patients and cannot be recommended as a PD therapy (Freed et al. 2001; Olanow et al. 2003). In this regard, other neuronal cell sources such as embryonic stem cells could be an alternative cell therapy for PD.

2.2.2 Embryonic Stem Cell Therapy for Parkinson's Disease

Embryonic stem cells can be derived from different embryonic developmental states such as blastocyst, epiblast, and also primordial germ cells (Evans and Kaufman 1981; Martin 1981; Thomson et al. 1998; Kerr et al. 2006; Brons et al. 2007). These ESCs possess a self-renewal ability and retain pluripotency over prolonged periods of expansion in culture without differentiation (Dushnik-Levinson and Benvenisty 1995; Amit et al. 2000). Unlike other cell types, ESCs have a higher plasticity and the capacity to differentiate into any cell type including dopamine neurons (Park et al. 2004; Zeng et al. 2004; Yan et al. 2005; Datta et al. 2013). One of the main limitations of using ESCs for transplantation in PD patients is the pluripotent state of these cells because under these conditions, the cells could form tumors once grafted, hindering their clinical application. To avoid adverse

effects, an early study showed that an enriched population of midbrain NSCs could be derived from ESCs. Thus, the dopamine neurons obtained from neural SCs are suitable for transplantation (Kim et al. 2002). A recent report showed that dopamine neurons derived from human ESCs efficiently survive after transplantation in animal PD models without forming tumors (Kriks et al. 2011).

The decreased survival of grafted cells after transplantation is one of the main obstacles for this surgery. One procedure involves the injection of adenoviral vectors into the dopamine-depleted striatum of hemiparkinsonian rats to deliver the differentiation factor sonic hedgehog (SHH) or the glial cell line-derived neurotrophic factor (GDNF) to dopamine-rich grafts. Later, embryonic ventral mesencephalic cells were implanted in the striatum. Both trophic and differentiation factors enhanced the yield of dopamine neurons in the ventral mesencephalic grafts (Torres et al. 2005).

2.2.3 MSCs as Cell Therapy for Parkinson's Disease

Mesenchymal stem cells are multipotent stem cells with the capacity for differentiation into various cell types including neurons (MacKenzie & Flake 2002; Choi et al. 2012). These cells can be obtained from human umbilical cord, bone marrow, or adipose tissue (Fu et al. 2002; Kestendjieva et al. 2008; Li et al. 2010; Włodarski et al. 2012). Autotransplantation is possible for cell therapy because the cells can be obtained from the same patient. One interesting immunosuppressive property of these cells is the prevention of an inflammatory response, thus avoiding transplanted cell rejection (Guo et al. 2006; Mathew et al. 2003). Other interesting properties of these cells are their neuroprotective capacity mediated by the delivery of neurotrophic factors (Wilkins et al. 2009; Whone et al. 2012). Several studies have shown that the presence of neurotrophic factors protects the transplanted neurons and favors NSC proliferation and differentiation (Park et al. 2008). These features make MSCs good candidates for cell-replacement therapy in PD. Bone marrow-derived MSCs can be induced to secrete neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF). When these neurotrophic factor-secreting cells were transplanted into the lesioned brain of a Parkinson's disease rat model, regeneration of the damaged dopaminergic nerve terminal in the striatum was observed (Sadan et al. 2009). On the other hand, Li and colleagues differentiated human umbilical vein mesenchymal stem cells (HUVMSCs) into dopaminergic-like cells. Utilizing a rat model of PD, the authors observed that the transplanted cells together with NGF administration considerably increased grafted cell survival (Li et al. 2010).

2.2.4 Neural Stem/Progenitor Cells

The transplantation of fetal or embryonic tissue has significant limitations for use as a cell-replacement therapy such as ethical issues due to the use of human fetal/embryonic tissue, deleterious immune reactions by the central nervous system after intracerebral transplantation, and the high quantity of cells necessary for an efficient transplantation. In addition, this treatment could have side effects such as dyskinesia and a risk of tumor formation. Consequently, researchers have focused

their efforts on isolating and differentiating in vitro neural stem cells before performing the transplantation. Therefore, NSCs would be obtained first and, later, dopaminergic cells. Thus, fetal brain neural SC-derived dopaminergic cells were observed to have little risk of tumor formation and immune rejection in contrast to stem cells. In addition, neural stem/progenitor cells (NSPCs) are an alternative cell source for neural transplantation because they display important neuroprotective and immunomodulator features that favor survival of the remaining or transplanted neurons (Bonnamain et al. 2012).

Stem cell differentiation generates progenitors of a specific lineage cell such as NSCs. Neural stem cells are multipotent cells with the capacity to proliferate and give rise to cell types with restricted neural lineages (Bai et al. 2007). NSCs differentiate into neural progenitor cells (NPCs) and finally into dopaminergic neurons (Riaz et al. 2002). Although NSCs have a more restricted self-renewal potential, they proliferate in culture forming neurospheres and may be differentiated either before or after transplantation. Both neural stem cell and neural progenitor cells (NSPCs) are present in neurospheres. It has been discussed whether the term NSCs, neural precursor cells, or NPCs are the same cells. However, it is not clear which exact cell types are present within the neurospheres. Recently, it was demonstrated that neurospheres can be maintained in a culture with different cell populations (Narayanan et al. 2012). Currently, it is believed that the cell identities present in neurospheres might differ between species (Steffenhagen et al. 2011).

The NSPCs can be derived from embryonic nerve tissue, adult nerve tissue such as the subventricular zone or carotid body, or from differentiated embryonic/fetal stem cells (Okabe et al. 1996; Pardal et al. 2007; Ara et al. 2010). Autologous transplantation of NSCs derived from adult tissue is an attractive cell therapy against Parkinson's disease (Arias-Carrión and Yuan 2009). The intrastriatal transplantation of NSCs isolated from the developing human CNS in adult rats with unilateral dopaminergic lesions partially ameliorated lesion-induced behavioral deficits in some animals (Svendsen et al. 1997). Pre-differentiated neural progenitor cells are more effective in enhancing the therapeutic potency for PD (Wang et al. 2004). Others studies have shown that transplanted NSCs survive into the substantia nigra pars compacta or striatum in PD rat models (Zhu et al. 2009).

NSCs were isolated from the adult pig subventricular zone, maintaining their capacities for proliferation, self-renewal, and multipotency. Because the pig is immunologically closest to humans, pigs can be an efficient source for cell therapy (Liard et al. 2009). Interestingly, the use of xenografts of porcine neural precursor cell in a rat model of Parkinson's disease showed long-term survival of grafts in the host brain (Harrower et al. 2006).

2.2.5 Other Stem Cell Sources

From bone marrow, it is possible to obtain various types of stem cells such as MSCs and hematopoietic stem cells. One study showed that bone marrow-derived stem cells transfected with tyrosine hydroxylase and a GFP-containing plasmid can be later differentiated to neurons. Injection of these cells into the brain of a Parkinson's disease model rat showed favorable results (Zou et al. 2010). On the other hand,

stem cells can be isolated either from the dermis or hair follicles. Both cell types, dermis-derived neural crest-related and epidermal neural crest stem cells, can be differentiated into cells with distinct cell lineages including dopaminergic neuron-like cells. Transplanting these cells into Parkinson's disease model rats showed encouraging results (Kubo et al. 2009). In addition, implantation of epidermal neural crest stem cells into a brain injury rat model leads to cell differentiation into immature astrocytes, which secrete neurotrophic factors (Jackson et al. 2010).

2.3 Other Cell Sources for Parkinson's Disease Therapy

Although the tremendous potential of stem cells as an effective cell therapy in Parkinson's disease is well established, cells other than stem cells have also been investigated as alternative sources for cell therapy. These cells may be appropriate for use as supportive or replacement cells for neurons. Among these are amniotic epithelial cells, retinal pigment epithelium (RPE) cells, and fetal dopamine neurons (Sheng et al. 1993; Mendez et al. 2005; Yang et al. 2009; Kakishita et al. 2000). The ability of some cells to secrete neurotrophic factors, differentiate into dopamine-secreting cells in vitro, survive in the striatum in vivo, and increase dopaminergic marker expression levels in Parkinson's disease model rats makes them an excellent candidate for cell therapy against Parkinson's disease (Mendez et al. 2005; Stover and Watts 2008; Yang et al. 2009). The clinical results obtained from the transplant of fetal dopamine neurons in patients with Parkinson's disease were very interesting. When these neurons were transplanted into the striatum or both the striatum and substantia nigra, they presented as functional dopaminergic neurons at the transplantation sites. Likewise, postmortem analyses showed the survival of the transplanted dopaminergic neurons (Mendez et al. 2005).

Despite the progress achieved in this field, numerous complications have prevented the practical application of stem cell-based therapy: ethical problems of fetal and embryonic tissue use, low safety due to teratoma formation risk (Kuroda et al. 2013), and rejection issues and little efficacy caused by very low cell survival. Before the clinical application of cell therapy for patients with Parkinson's disease, safety and efficacy studies, standardized protocols that utilize animal compound-free defined media for culture in vitro, and differentiation procedures that allow us to isolate primarily dopaminergic neurons are required.

2.4 Differentiation of Stem Cells into NSCs and Dopaminergic Neurons

It is essential to increase our knowledge on nervous system development and to understand how numerous factors participate in the intrinsic and extrinsic signaling pathways inducing cell differentiation. As a result of these advances, we can design more efficient strategies for cell differentiation in vitro in the future.

The differentiation of stem cells involves the formation of neural precursor cells or neural progenitor cells, which can be expanded in vitro. Later, these cells are differentiated further into dopaminergic neurons (Cho et al. 2008). During the differentiation process, neuroepithelial rosette structures are formed (Zhang & Zhang 2010). It was found that not only are stem cells potentially tumorigenic, but neuroepithelial rosettes are as well. An efficient protocol for neuronal differentiation that eliminates ESCs and neuroepithelial rosettes is necessary to avoid risk of tumors or teratoma formation before transplantation. Although some studies performed in rats have shown that multipassage neural precursor cells do not form tumors, low survival and loss of the dopaminergic neuronal phenotype make them unsuitable for replacement therapies (Ko et al. 2009; Jo et al. 2009; Pawitan 2011).

In general, numerous protocols have been described to differentiate stem cells into dopaminergic neurons or dopaminergic-like cells. These protocols have presented with different degrees of efficiency (Hwang et al. 2010). Some protocols utilized a co-culture with PA6 or MS5 mouse stromal cells, and others used chemically defined media or the specific activation of an intrinsic pathway (Momčilović et al. 2012; Watmuff et al. 2012). Currently, there is notable heterogeneity in the yield of tyrosine hydroxylase (TH)-positive cells (Hwang et al. 2010). Although iPSCs and ESCs can be differentiated using the same transcriptional network with same set of morphogens, the differentiation occurs with reduced efficiency and increased variability (Hu et al. 2010). Derivation of only one cell population and increased efficiency during this process are important factors to consider.

Stromal feeder cell-based differentiation protocols have been described by several authors (Perrier et al. 2004; Sonntag et al. 2007). Stromal cells are a source of stromal cell-derived inducing activity (SDIA). Recent work has identified stromal candidate factors inducing the neuronal differentiation of hiPSCs such as hepatocyte growth factor (HGF), stromal cell-derived factor-1α (SDF1α), secreted frizzled-related protein 1 (sFRP1), and vascular endothelial growth factor D (VEGFD) (Schwartz et al. 2012).

Recent evidence has shown that Lmx1a overexpression generates enriched populations of human A9-subtype ventral midbrain DA neurons from hESC or hiPSCs, which should be suitable for cell-replacement strategies (Sánchez-Danés et al. 2012a). Similarly, other studies have shown how overexpression of the nuclear receptor Nurr1 (a transcriptional factor specific to midbrain dopamine (DA) neuronal development) in NPCs isolated from the subventricular zone (SVZ) could induce a DA neuron phenotype (Shim et al. 2007).

As a consequence of the progress in this area, it has been determined that a diverse combination of growth factors and molecules contributes to the expansion and/or differentiation of either stem cells or neural progenitor cells. Among those described include basic fibroblast growth factor (bFGF), fibroblast growth factor 2 (FGF2), fibroblast growth factor 8 (FGF8), fibroblast growth factor 20 (FGF20), docosahexaenoic acid, all-trans-retinoic acid (ATRA), sonic hedgehog (SHH), transcription factor Nato3, a 3 % oxygen level, and ascorbic acid (Volpicelli et al. 2004; Correia et al. 2007; Cooper et al. 2010; Morizane et al. 2010; Chang et al. 2012; Stacpoole et al. 2011; Li et al. 2012; Nissim-Eliraz et al. 2012; Wang et al. 2013).

Similarly, several studies have shown how the bone morphogenic protein antagonist noggin, sonic hedgehog (SHH), FGF8, and Wnt5a promote the differentiation, survival, and functional integration of stem cell-derived DA neurons in vivo (Jensen et al. 2008; Parish et al. 2008; Chiba et al. 2008). Likewise, the BMP, TGF-β, and Wnt signaling pathways are involved in midbrain dopamine differentiation. The BMP and TGF-β pathway members participate how upstream regulators of the Wnt signaling pathway during midbrain dopamine differentiation of human pluripotent stem cells (Cai et al. 2013). Likewise, another study showed that BDNF contributes to NSC proliferation and differentiation through the activation of the Wnt/β-catenin signaling pathway (Chen et al. 2013). Wnts, a family of secreted proteins, are involved in stem cell differentiation (Prakash and Wurst 2007). It was reported that loss of Wnt1 generates the loss of Lmx1a and Ngn2 expression, affecting the formation of DA neurons in the midbrain floor plate (Andersson et al. 2013). In addition, it has been observed that the Wnt signaling pathway leads to the inhibition of glycogen synthase kinase 3 (GSK-3). GSK-3 inhibition in the presence of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) increases hiPSC-NP proliferation. However, an absence of these growth factors generates a switch to neuronal differentiation and a dopaminergic fate (Esfandiari et al. 2012).

The effect of retinoic acid in the neuronal differentiation has been described. It was shown that retinoic acid induced neuronal differentiation of NTERA2 cells through the modulation of Sox2 and Sox3 gene expression. The authors showed downregulation of Sox2 and upregulation of the Sox3 gene (Stevanovic 2003).

Utilizing different stem cell types, it is possible to give rise to dopamine neurons. Recently, Li and colleagues were able to derive dopamine neurons from human cord blood-derived multipotent stem cells (CB-SCs) before treatment with all-trans-retinoic acid (ATRA) (Li et al. 2012). Similarly, the isolation and differentiation of MSCs from the umbilical cord matrix tissue alleviate Parkinsonian symptoms in a rodent model in vivo (Shetty et al. 2013).

On the other hand, iPSCs may also become an important source of dopaminergic neurons. Several differentiation protocols performed in embryonic stem cells were applicable to iPSCs. Thus, it was observed that docosahexaenoic acid stimulated the differentiation of murine iPSCs into dopaminergic neurons (Chang et al. 2012). The neurons obtained from reprogrammed fibroblasts improved symptoms in a rat model of Parkinson's disease (Wernig et al. 2008). Likewise, differentiated Parkinson patient-derived iPSCs reduce motor asymmetry in parkinsonian rats (Hargus et al. 2010).

3 Induced Pluripotent Stem Cells (iPSCs)

Although most of our tissues contain adult stem cells that differentiate into any cell type when required, including different tissues (Abdanipour et al. 2011; Darabi et al. 2013), the ability for renewing damaged cells is limited. Over time, the amount of adult stem cells decreases and, thus, the ability to regenerate damaged tissues also decreases. A greater understanding of the molecular mechanisms

and signaling pathways involved in the maintenance and division of these adult stem cells could help design strategies to maintain the tissue regenerative capacity. Certainly, this strategy could be the solution to many diseases involving cell loss. Unfortunately, knowledge in this field remains scarce (Beltrami et al. 2007).

Currently, global research efforts have focused on designing strategies to develop stem cells from different sources. In previous work, different technologies to develop patient-specific stem cells, such as somatic cell nuclear transfer or cell fusion, have been described. In these methodologies, either the somatic nuclear content is transferred to an oocyte or ES cells are fused with somatic cells (Wilmut et al. 1997; Tada et al. 2001; Cowan et al. 2005). Unfortunately, both methods involve technical and ethical considerations that hinder the technology. Likewise, the isolation of adult or embryonic stem cells is not easy to realize, mainly due to technical difficulties and cultural issues. Currently, iPSC technology is a new tool for increasing our understanding of the molecular mechanisms involved in the development of several disorders. Thus, there is great interest in researching the possible use of iPSCs, not just as model of PD but also in future clinical applications (Sun et al. 2010; Stadtfeld and Hochedlinger 2010; Ebben et al. 2011; Han et al. 2011). Currently, these technologies have generated several PD patient-derived iPSC lines (Sánchez-Danés et al. 2012b; Byers et al. 2012). These iPSC lines will certainly help our understanding of the molecular mechanisms of PD and help us to design patient-specific stem cell treatments.

3.1 History

In the year 2006, a remarkable finding was reported by Takahashi and Yamanaka in the Yamanaka group in Japan. They showed, for the first time, that an adult somatic cell could be reprogrammed into the pluripotent state by reproducing the ESC signaling pathways (Takahashi and Yamanaka 2006). To define the transcription factors necessary for inducing the reprogramming of somatic cells, the Yamanaka group initially selected a total of 24 separate genes. Previous experience suggested that these genes could play a role in ESC identity development and maintenance (Schöler et al. 1989; Avilion et al. 2003; Rizzino 2009). Thus, they determined a final "cocktail" of transcription factors required to induce pluripotent cell development. A number of these genes are involved in rapid cell proliferation, and some are considered oncogenes. After the analysis of different transcription factor combinations, the authors found that only four factors are essential to reprogram an adult somatic cell to the undifferentiated state, a so-called induced pluripotent stem cell (iPSC). Thus, the authors developed iPSCs by inducing overexpression through the retroviral transduction of Oct4, Sox2, Klf4, and c-Myc in mouse embryonic and adult fibroblasts and subsequent selection for Fbx15 expression (Takahashi and Yamanaka 2006). Shortly after, these studies were successfully confirmed by Wernig and colleagues (Wernig et al. 2007). Subsequently, new selection markers were reviewed for identifying iPSC colonies. The authors showed that selection for

Nanog expression generates a germline-competent iPSCs with characteristics similar to ESCs. Under these conditions, they found that the four transgenes (Oct3/4, Sox2, c-Myc, and Klf4) are silenced in Nanog iPSCs (Okita et al. 2007).

Due to the rapid progress in iPSC research, the Yamanaka and Thomson groups reported in 2007 the generation of iPSCs from human cells. These findings are particularly interesting because the contribution of pluripotent cells to disease modeling, drug development, or regenerative medicine was surprising. The Yamanaka group generated iPSCs from adult human dermal fibroblasts using the same four factors: Oct3/4, Sox2, Klf4, and c-Myc (Takahashi et al. 2007). Tumor development is linked with the reactivation of the c-Myc transgene, which is present in the reprogramming cocktail. The presence of this gene could increase tumorigenicity in both the chimeras and progeny mice, which is a serious hurdle for the therapeutic application of iPSCs technology. However, the Thomson group reported that four factors (Oct4, Sox2, Nanog, and Lin28) are sufficient for reprogramming human somatic cells to pluripotent stem cells. Interestingly, Klf4 and c-Myc were not included in the reprogramming cocktail, and the technical limitations that generate viral integration were also eliminated (Yu et al. 2007). Furthermore, new investigations were able to generate human iPSCs from adult dermal fibroblasts without c-Myc (Yu et al. 2007; Nakagawa et al. 2008).

In accordance with the previous descriptions from the Yamanaka, Jaenisch, or Thomson groups, the reprogramming process involved simultaneous overexpression of four transcription factors. In early studies, the authors utilized Oct4, Sox2, Klf4, and c-Myc as pluripotency factors (Takahashi and Yamanaka 2006; Takahashi et al. 2007; Wernig et al. 2007). Thereafter, the Thompson group achieved reprogramming using different pluripotent factors such as Nanog and Lin28 (Yu et al. 2007). Later, reprogramming was also obtained using a fewer number of transcription factors related to stem cell pluripotency (Patel and Yang 2010; Stadtfeld and Hochedlinger 2010; Robinton and Daley 2012). Moreover, some researchers achieved reprogramming including only one or two factors (Grinnell et al. 2007; Kim et al. 2008, 2009a, b, c).

On the other hand, iPSCs have been generated from cells of numerous origins and different species. First, iPSCs were obtained from mice and humans; later, they were generated from other species: rat, pig, rhesus monkey, marmoset, canine, sheep, and snow leopard (Stadtfeld and Hochedlinger 2010; Verma et al. 2012; Sumer et al. 2011; Piedrahita et al. 2011). Certainly, the reprogramming technology offers powerful new opportunities for research not only in the biomedical area. Similarly, iPSCs have been derived from different somatic cell types such as embryonic fibroblasts, adult fibroblasts, hepatocytes, epithelial cells, keratinocytes, melanocytes, mature B cells, mature T cells, pancreatic B cells, and the hepatic endoderm (Nakagawa et al. 2008; Shi et al. 2008; Silva et al. 2008; Stadtfeld et al. 2008a; Guo et al. 2009). Undifferentiated cells have also been useful for reprogramming to iPSCs. These cells include neural stem cells, myeloid progenitor cells, hematopoietic stem cells, adipose-derived stem cells, and cord blood stem cells (Stadtfeld and Hochedlinger 2010). Because the iPS cells can be differentiated into any type of cell with three germ layers, they can be

a cell source relevant for replacement therapy for several disorders. Low transformation efficiency has been a main problem in obtaining these cells.

These findings were startling and altered our knowledge of the epigenetic landscape. Although there has been extensive research to understand how these factors induce changes in gene expression profiles and chromatin modifications through global epigenetic changes, the molecular mechanisms involved in these dedifferentiation processes are incompletely understood (Bernstein et al. 2006).

3.1.1 Oct3/4

The octamer-binding transcription factor POU5F1 (also known as Oct3, Oct4, Oct3/4) is encoded by the POU5F1 gene and expressed during early mouse embryogenesis (Schöler et al. 1989; Palmieri et al. 1994). Oct3/4 has been described as a crucial regulator maintaining pluripotency, which in conjunction with other transcription factors such as Nanog and Sox2, forms a transcription factor molecular network that regulates ESC pluripotency (Boyer et al. 2005; Niwa 2007). Previous studies have shown that Oct3/4 at later stages is expressed in adult stem cells and is considered a pluripotent cell marker (Tai et al. 2005). However, Oct3/4 expression is not limited to stem cells, as differentiated cells also express it (Zangrossi et al. 2007). The differential expression of the isoforms Oct3/4 as Oct3/4A and Oct3/4B may correlate with different processes such as proliferation and differentiation. In fact, a recent study showed that the isoform Oct3/4A is expressed in the nucleus, whereas Oct3/4B is expressed in the cytoplasm in the developing tooth germ (Nakagawa et al. 2012).

Oct3/4 is an essential reprogramming factor, allowing mature somatic cells to return to a pluripotent state as observed in stem cells (Takahashi and Yamanaka 2006; Yu et al. 2007; Nakagawa et al. 2008). Interestingly, their extraordinary ability to reprogram somatic cells into pluripotent cells can be achieved either alone or in collaboration with other factors (Kim et al. 2008, 2009a, b; Li et al. 2011). Currently, a recent study has demonstrated that transcriptional activation of specific genes by Oct3/4 without the gene repression required for differentiation is sufficient for promoting pluripotency (Hammachi et al. 2012). These results contradict those previously reported by researchers who described that the repression of genes required for differentiation is an essential role of Oct3/4 (Boyer et al. 2006; Bilodeau et al. 2009). Oct3/4 does not appear to be required for somatic stem cell self-renewal (Berg and Goodell 2007; Lengner et al. 2007). Moreover, it was observed that Oct3/4 prevents the differentiation and expansion of progenitor cells when overexpressed in adult epithelia (Hochedlinger et al. 2005). Oct3/4 cooperates with a wide set of cofactors. Together with Sox2, Oct3/4 can form a protein-DNA ternary complex that is able to promote the transcriptional activation of fibroblast growth factor 4 (FGF4) (Yuan et al. 1995). Moreover, an interaction between Oct4 and Sox2 might promote expression of itself (Okumura-Nakanishi et al. 2005) and other factors such as Nanog (Kuroda et al. 2005). More recently, Oct3/4 together with Sox2 interacts with a multi-subunit stem cell coactivator complex (SCC) that affects Nanog and Oct4 gene expression as well as genomic regions occupied by Oct4 and Sox2 (Fong et al. 2011).

3.1.2 Sox2

The Sox genes consist of a family of transcriptional regulators involved in the control of nervous system development. Among these is the protein Sox2, an HMG box transcription factor, which is predominantly expressed in undifferentiated cells such as ESCs and is required for the maintaining pluripotency and/or self-renewal (Rizzino 2009).

Studies have shown that Sox2 can form a ternary complex with Oct3 on the FGF4 enhancer DNA sequences promoting FGF4 gene and Nanog gene transcriptional activation. FGF4 is one protein expressed in early embryonic development (Yuan et al. 1995; Kuroda et al. 2005). Likewise, a Sox2 regulatory region, which is also under the control of this Oct3/4-Sox2 complex, was identified (Tomioka et al. 2002). It is believed that the Oct3/4 and Sox2 expression levels are maintained by the Oct4/Sox2 complex in pluripotent cells by a reciprocal transcriptional regulation mechanism (Chew et al. 2005). It has been proposed that the major role of Sox2 is to regulate multiple transcription factors that affect Oct3/4 expression, thus maintaining Oct3/4 expression in pluripotent cells (Masui et al. 2007). Importantly, the transcription factor Sox2 and Oct3/4 have been implicated in early lineage decisions. While both factors cooperate to suppress trophoblast fates in ESCs, they also establish the three lineages present at implantation (Avilion et al. 2003; Li et al. 2007). Sox2 is highly expressed in dividing neural progenitor cells throughout the central nervous system (CNS) (Ellis et al. 2004; Ferri et al. 2004; Komitova and Eriksson 2004). Thus, Sox2 is expressed in embryonic early neural precursors of the ventricular zone and in the ependymal of adult cells. Sox2 is also expressed in lower proportions in differentiated neurons. An important role for Sox2 in neuron maintenance has been described (Ferri et al. 2004). Indeed, Sox2 expression is characteristic of NSCs in the adult brain; however, not all NSCs have the capability to differentiate into all neural cell types in vivo (Brazel et al. 2005). An interesting difference in Sox2 gene expression levels between human and mouse has been described. Mouse primordial germ cells (PGCs) express Oct3/4, Nanog, and Sox2, whereas human PGCs express Oct3/4 and Nanog but not Sox2 (Perrett et al. 2008; De Jong et al. 2008). Sox2 is considered a neural stem cell marker for ICM stem cells. Sox2 has been used as a key transcription factor for reprogramming somatic cells into induced pluripotent cells, but it was not considered essential (Kim et al. 2008, 2009a). Reprogramming neural progenitor cells into iPSCs can be achieved by the combination of factors necessary for reprogramming. Depending on the cellular context, exogenous Sox2 expression is not necessary if cells already express high levels of Sox2 (Eminli et al. 2008). Likewise, Sox2 is not absolutely necessary for neural stem cell self-renewal or multipotency (Miyagi et al. 2008). On the other hand, Sox2 has also been shown to be implicated in tumorigenesis in various organs (Lengerke et al. 2011). These observations indicate that there is a relationship between pluripotency and tumor development (Bernhardt et al. 2012).

3.1.3 Nanog

The transcription factor Nanog plays a key role during lineage specification and in the maintenance of pluripotency in both ICM and ESCs. Nanog in conjunction with other pluripotent factors such as Oct3/4 and Sox2 controls expression of target genes that

possess important functions in pluripotency development. Thus, it was found that Nanog-deficient ESCs lost their ability to self-renew (Cavaleri and Schöler 2003; Mitsui et al. 2003). High levels of Nanog can allow for ESC self-renewal and prevent differentiation in the absence of cytokine LIF or feeder layers (Pan and Thomson 2007). These key transcription factors form a complex regulatory network affecting the expression levels of different target genes related to pluripotency or differentiation (Wang et al. 2006). It was observed that Oct3/4 and Sox2 binding to the Nanog promoter is essential for Nanog transcription in mouse and human embryonic stem cells (Rodda et al. 2005). Moreover, it was observed that Nanog regulates downstream genes necessary for both pluripotency and the differentiation of stem cells through two distinct transactivation domains (Pan and Pei 2003). Other studies described that Nanog dimerization is necessary for interaction with several proteins that promote stem cell pluripotency (Wang et al. 2008). In fact, Nanog is a transcriptional activator for Rex-1, which is a marker for undifferentiated ESCs (Shi et al. 2006). When embryonic stem cells are differentiated into primitive endoderm, Nanog is repressed. Thus, Nanog downregulation plays a crucial role in cell fate during the early embryonic development (Hamazaki et al. 2004). Several studies have shown that Nanog expression is not exclusive to pluripotent cells because it is also present in tumor cells and plays a novel role in tumor development (Jeter et al. 2009). While mouse Nanog expression is high in undifferentiated ESCs in the ICM of the blastocyst, germ cell tumor, and teratoma-derived cell lines, expression is low in several adult tissues (Hart et al. 2004; Hoei-Hansen et al. 2005). Thus, Nanog levels may act as a control mechanism to decide between cell self-renewal and differentiation. Moreover, the expression of pluripotent genes such as Nanog in both seminomas and breast carcinomas suggests a direct role in the progression of different types of carcinomas and could serve as an important marker of tumorigenesis (Ezeh et al. 2005). It was observed that many characteristics present in stem cells are also present in cancer cells. This suggests that any changes in the complex regulation mechanism existing in pluripotent cells could lead to tumor development. Furthermore, it was found that Nanog priming before full reprogramming may generate germ cell tumors (Grad et al. 2011). It is fundamental to discriminate both cell types correctly. On the other hand, regulation of the pluripotent state involves the participation of various factors. Thus, many endogenous noncoding RNA molecules, polycomb complexes, and DNA methylation may play a role in regulating the pluripotent state (Kashyap et al. 2009). Tay and colleagues described how different microRNAs targeting Nanog, Oct4, and Sox2 coding regions participate in embryonic stem cell differentiation (Tay et al. 2008). Likewise, the identification of conserved lncRNA-encoding genes such as lncRNAs, AK028326, and AK141205 was described as direct targets of Oct4 and Nanog (Sheik Mohamed et al. 2010).

3.2 Delivery Systems into Donor Cell

Certainly, the main problem in generating iPS cells is an extremely inefficient transformation process. Thus, iPSCs reprogramming is influenced by numerous

variables that must be considered before choosing a reprogramming method. In fact, reproducibility and the quality of the resulting iPSCs are also affected by the delivery system.

Currently, there are several strategies to generate iPS cells, as rapid progress has been made in this field. These methods utilize several mechanisms for factor delivery as non-integrating vectors, excisable vectors, retrovirus, adenovirus, microRNA, and even soluble proteins, and each has different reprogramming efficiencies (Okita et al. 2008; Stadtfeld et al. 2008b; Kaji et al. 2009; Soldner et al. 2009; Woltjen et al. 2009; Zhou and Freed 2009). Several systems have been described to deliver transgenes into donor cells including integrative and non-integrative delivery systems. Integrative methods have been described such as viral delivery vectors, lentiviral delivery vectors, transfection of linear DNA, and the piggyBac transposon (González et al. 2011; Robinton and Daley 2012; Bellin et al. 2012). Wide variations in the efficiency of reprogramming were observed if reprogramming was performed in cells at different differentiation states. Less differentiated cells as progenitor cells can be reprogrammed more easily. Moreover, less differentiated cells may require fewer transcription factors to achieve reprogramming (Stadtfeld and Hochedlinger 2010).

The firsts iPSCs were obtained by retroviral transduction using pMX-based retroviral vectors (Takahashi and Yamanaka 2006; Takahashi et al. 2007; Okita et al. 2007; Nakagawa et al. 2008). In this case, the retroviruses expressing each gene separately showed an iPSC generation efficiency of less than 0.02 % when either mouse or human fibroblasts were reprogrammed (Stadtfeld and Hochedlinger 2010). Likewise, polycistronic vectors were designed resulting in the simultaneous, almost stoichiometric, expression of transcription factors encoded by the polycistron.

Later, other groups utilized effective lentiviral vectors as delivery systems of reprogramming factors (Yu et al. 2007). In contrast to other retroviruses, lentiviral vectors have a higher infection efficiency, allowing for the infection of both dividing and nondividing cells. Moreover, the development of new lentiviral vectors such as Tet-inducible reprogramming lentiviruses or the STEMCCA lentiviral vector permits the successful ectopic expression of reprogramming factors (Major et al. 2011; Sommer et al. 2012). However, there are several limitations to the safe use of lentiviral vectors for reprogramming. Viral integration into the genome can be random, thus affecting expression of tumor suppressor genes or oncogenes. Therefore, the possibility that viral transgenes can ultimately be spontaneously reactivated increases the risk of tumor formation (Okita et al. 2007).

To avoid viral integration, new alternatives have emerged for factor delivery through a non-integrative method. Adenoviral reprogramming of mouse fibroblasts or liver cells to iPSCs has been described (Stadtfeld et al. 2008b; Okita et al. 2008). Likewise, human iPS cells were derived using a non-integrating episomal vector. Because of the removal of the episoma, the human iPSCs were free of vector and transgene sequences (Yu et al. 2009; Chou et al. 2011). Human iPSCs were also derived using non-viral minicircle DNA vectors (Okita et al. 2010). The major

advantage of these technologies is the lack of genomic integration. One of the main issues in obtaining iPSCs is not just the lower reprogramming efficiency, but also choosing a method that does not change the genomic DNA. Other interesting reprogramming methods involve the use of proteins (Zhou et al. 2009). Although the technique has been described as having a very low reprogramming efficiency, multiple hiPSC lines have been derived through protein-based reprogramming. These cells rescued motor deficits in a PD model (Rhee et al. 2011).

One of the methods that have been proposed to significantly increase reprogramming efficiency is the use of RNA and certain molecules (Warren et al. 2010; Wang et al. 2012).

4 Direct Reprogramming into Neurons

One of the problems with the use of induced pluripotent stem cells is that they can generate tumors. Recently, different groups of researchers have shown that only a few transcription factors were sufficient to generate the direct conversion of fibroblasts to functional neurons without generating a pluripotent stem cell stage (Kim et al. 2011). These cells were less likely to form tumors, avoiding much of the carcinogenic risks of pluripotent cells; they are called induced neuronal (iN) cells. Vierbuchen and colleagues analyzed a pool of 19 neural-lineage-specific transcription factors as possible candidates for direct reprogramming. They showed that the combination of three factors, Ascl1, Brn2, and Myt1l, is able to transform mouse embryonic and postnatal fibroblasts into functional neurons in vitro. The iN cells generated expressed neuronal markers such as GABA (Vierbuchen et al. 2010). They were also capable of converting fetal and postnatal human fibroblasts into iN cells in the presence of the basic helix-loop-helix transcription factor NeuroD1 (Pang et al. 2011). Functional neurons were also obtained from adult human fibroblasts (Pfisterer et al. 2011). Similarly, Caiazzo and collaborates identified three other transcription factors, Mash1, Nurr1, and Lmx1a, that also can be utilized to induce the reprogramming of mouse and human fibroblasts into dopaminergic neurons (Caiazzo et al. 2011). On the other hand, another group of investigators found that the combination of five transcriptional factors, Mash1, Ngn2, Sox2, Nurr1, and Pitx3, were capable of reprogramming human fibroblasts into DA neuron-like cells (Liu et al. 2012).

One of the major disadvantages of this technology in cell-replacement therapy is the low number of cells generated. A new procedure that directly reprograms mouse fibroblasts into neural progenitor cells could avoid this problem. Unlike iN cells, these cells can be expanded in vitro (Kim et al. 2011).

Furthermore, before being used for cell transplantation, the cells must survive at the transplantation site in addition to being effective in the animal model. However, this tool is an attractive option for other cell types for generating patient- and disease-specific neurons to be used in a cell disease models.

5 Conclusion

In recent decades, research efforts have focused on replacing dopaminergic neurons that are lost in patients with Parkinson's disease. To this end, cellular therapies that replace degenerated cells have been the major focus and may be promising therapeutic options in patients with Parkinson's disease in the future. Preliminary studies have utilized adult tissue, embryonic/fetal tissue, and embryonic stem cells as a source of dopaminergic cells. However, safety issues such as the risk of tumor formation and immunological rejection have prevented this type of technique from being an effective treatment against Parkinson's disease. Advances in iPSC research have allowed scientists to obtain specific cells from the same patient, avoiding rejection problems. However, obstacles remain for its clinical use such as the lack of standardized protocols that result in a dopaminergic cell phenotype that grafts without undifferentiated cells and without a risk of tumor formation. Direct reprogramming of somatic cells into induced neuronal (iN) cells without generating a pluripotent stage could be a solution that avoids tumor risk. However, as a result of the low quantity of cells obtained, more investigations are necessary to turn this technique into an efficient cell-replacement therapy. If we overcome these obstacles, iPS and iN cells may be, without a doubt, a very promising therapy in the near future.

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References

Abdanipour, A., Tiraihi, T., & Delshad, A. (2011). Trans-differentiation of the adipose tissue-derived stem cells into neuron-like cells expressing neurotrophins by selegiline. *Iranian Biomedical Journal, 15*, 113–121.

Adewumi, O., Aflatoonian, B., Ahrlund-Richter, L., Amit, M., Andrews, P. W., Beighton, G., Bello, P. A., Benvenisty, N., Berry, L. S., Bevan, S., et al. (2007). Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nature Biotechnology, 25*, 803–816.

Amit, M., Carpenter, M. K., Inokuma, M. S., Chiu, C. P., Harris, C. P., Waknitz, M. A., Itskovitz-Eldor, J., & Thomson, J. A. (2000). Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Developmental Biology, 227*, 271–278.

Andersson, E. R., Saltó, C., Villaescusa, J. C., Cajanek, L., Yang, S., Bryjova, L., Nagy, I. I., Vainio, S. J., Ramirez, C., Bryja, V., & Arenas, E. (2013). Wnt5a cooperates with canonical Wnts to generate midbrain dopaminergic neurons in vivo and in stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 110*, E602–E610.

Ara, J., Fekete, S., Zhu, A., & Frank, M. (2010). Characterization of neural stem/progenitor cells expressing VEGF and its receptors in the subventricular zone of newborn piglet brain. *Neurochemical Research, 35*, 1455–1470.

Arias-Carrión, O., & Yuan, T. F. (2009). Autologous neural stem cell transplantation: A new treatment option for Parkinson's disease? *Medical Hypotheses, 73*, 757–759.

Arjona, V., Mínguez-Castellanos, A., Montoro, R. J., Ortega, A., Escamilla, F., Toledo-Aral, J. J., Pardal, R., Méndez-Ferrer, S., Martín, J. M., Pérez, M., Katati, M. J., Valencia, E., García, T., & López-Barneo, J. (2003). Autotransplantation of human carotid body cell aggregates for treatment of Parkinson's disease. *Neurosurgery, 53*, 321–328.

Avilion, A. A., Nicolis, S. K., Pevny, L. H., Perez, L., Vivian, N., & Lovell-Badge, R. (2003). Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes & Development, 17*, 126–140.

Bai, L., Caplan, A., Lennon, D., & Miller, R. H. (2007). Human mesenchymal stem cells signals regulate neural stem cell fate. *Neurochemical Research, 32*, 353–362.

Ballagi, A. E., Odin, P., Othberg-Cederström, A., Smits, A., Duan, W. M., Lindvall, O., & Funa, K. (1994). Platelet-derived growth factor receptor expression after neural grafting in a rat model of Parkinson's disease. *Cell Transplantation, 3*, 453–460.

Bankiewicz, K. S., Plunkett, R. J., Jacobowitz, D. M., Porrino, L., di Porzio, U., London, W. T., Kopin, I. J., & Oldfield, E. H. (1990). The effect of fetal mesencephalon implants on primate MPTP-induced parkinsonism. Histochemical and behavioral studies. *Journal of Neurosurgery, 72*, 231–244.

Barinaga, M. (2000). Fetal neuron grafts pave the way for stem cell therapies. *Science, 287*, 1421–1422.

Bellin, M., Marchetto, M. C., Gage, F. H., & Mummery, C. L. (2012). Induced pluripotent stem cells: The new patient? *Nature Reviews Molecular Cell Biology, 13*, 713–726.

Beltrami, A. P., Cesselli, D., Bergamin, N., Marcon, P., Rigo, S., Puppato, E., D'Aurizio, F., Verardo, R., Piazza, S., Pignatelli, A., Poz, A., Baccarani, U., Damiani, D., Fanin, R., Mariuzzi, L., Finato, N., Masolini, P., Burelli, S., Belluzzi, O., Schneider, C., & Beltrami, C. A. (2007). Multipotent cells can be generated in vitro from several adult human organs (heart, liver, and bone marrow). *Blood, 110*, 3438–3446.

Berg, J. S., & Goodell, M. A. (2007). An argument against a role for Oct4 in somatic stem cells. *Cell Stem Cell, 1*, 359–360.

Bernhardt, M., Galach, M., Novak, D., & Utikal, J. (2012). Mediators of induced pluripotency and their role in cancer cells – current scientific knowledge and future perspectives. *Biotechnology Journal, 7*, 810–821.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *Journal of Neurological Sciences, 20*, 415–455.

Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., Jaenisch, R., Wagschal, A., Feil, R., Schreiber, S. L., & Lander, E. S. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell, 125*, 315–326.

Bilodeau, S., Kagey, M. H., Frampton, G. M., Rahl, P. B., & Young, R. A. (2009). SetDB1 contributes to repression of genes encoding developmental regulators and maintenance of ES cell state. *Genes & Development, 23*, 2484–2489.

Björklund, A., & Lindvall, O. (1999). Transplanted nerve cells survive and are functional for many years. *Lakartidningen, 96*, 3407–3412.

Bonnamain, V., Neveu, I., & Naveilhan, P. (2012). Neural stem/progenitor cells as a promising candidate for regenerative therapy of the central nervous system. *Frontiers in Cellular Neuroscience, 6*, 17.

Boyer, L. A., Lee, T. I., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., Guenther, M. G., Kumar, R. M., Murray, H. L., Jenner, R. G., Gifford, D. K., Melton, D. A., Jaenisch, R., & Young, R. A. (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell, 122*, 947–956.

Boyer, L. A., Plath, K., Zeitlinger, J., Brambrink, T., Medeiros, L. A., Lee, T. I., Levine, S. S., Wernig, M., Tajonar, A., Ray, M. K., Bell, G. W., Otte, A. P., Vidal, M., Gifford, D. K., Young, R. A., & Jaenisch, R. (2006). Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature, 441*, 349–353.

Brazel, C. Y., Limke, T. L., Osborne, J. K., Miura, T., Cai, J., Pevny, L., & Rao, M. S. (2005). Sox2 expression defines a heterogeneous population of neurosphere-forming cells in the adult murine brain. *Aging Cell, 4*, 197–207.

Brons, I. G., Smithers, L. E., Trotter, M. W., Rugg-Gunn, P., Sun, B., Chuva de Sousa Lopes, S. M., Howlett, S. K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R. A., & Vallier, L. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature, 448*, 191–195.

Brundin, P., Strecker, R. E., Gage, F. H., Lindvall, O., & Björklund, A. (1988). Intracerebral transplantation of dopamine neurons: Understanding the functional role of the mesolimbocortical dopamine system and developing a therapy for Parkinson's disease. *Annals of the New York Academy of Sciences, 537*, 148–160.

Byers, B., Lee, H. L., & Reijo Pera, R. (2012). Modeling Parkinson's disease using induced pluripotent stem cells. *Current Neurology and Neuroscience Reports, 12*, 237–242.

Cai, J., Schleidt, S., Pelta-Heller, J., Hutchings, D., Cannarsa, G., & Iacovitti, L. (2013). BMP and TGF-β pathway mediators are critical upstream regulators of Wnt signaling during midbrain dopamine differentiation in human pluripotent stem cells. *Developmental Biology, 1606*, 30–34.

Caiazzo, M., Dell'Anno, M. T., Dvoretskova, E., Lazarevic, D., Taverna, S., Leo, D., Sotnikova, T. D., Menegon, A., Roncaglia, P., Colciago, G., Russo, G., Carninci, P., Pezzoli, G., Gainetdinov, R. R., Gustincich, S., Dityatev, A., & Broccoli, V. (2011). Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature, 476*, 224–227.

Cavaleri, F., & Schöler, H. R. (2003). Nanog: A new recruit to the embryonic stem cell orchestra. *Cell, 113*, 551–552.

Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S., Tweedie, S., & Smith, A. (2003). Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell, 113*, 643–655.

Chang, Y. L., Chen, S. J., Kao, C. L., Hung, S. C., Ding, D. C., Yu, C. C., Chen, Y. J., Ku, H. H., Lin, C. P., Lee, K. H., Chen, Y. C., Wang, J. J., Hsu, C. C., Chen, L. K., Li, H. Y., & Chiou, S. H. (2012). Docosahexaenoic acid promotes dopaminergic differentiation in induced pluripotent stem cells and inhibits teratoma formation in rats with Parkinson-like pathology. *Cell Transplantation, 21*, 313–332.

Chen, B. Y., Wang, X., Wang, Z. Y., Wang, Y. Z., Chen, L. W., & Luo, Z. J. (2013). Brain-derived neurotrophic factor stimulates proliferation and differentiation of neural stem cells, possibly by triggering the Wnt/β-catenin signaling pathway. *Journal of Neuroscience Research, 91*, 30–41.

Chew, J. L., Loh, Y. H., Zhang, W., Chen, X., Tam, W. L., Yeap, L. S., Li, P., Ang, Y. S., Lim, B., Robson, P., & Ng, H. H. (2005). Reciprocal transcriptional regulation of Pou5f1 and Sox2 via the Oct4/Sox2 complex in embryonic stem cells. *Molecular and cellular biology, 25*, 6031–6046.

Chiba, S., Lee, Y. M., Zhou, W., & Freed, C. R. (2008). Noggin enhances dopamine neuron production from human embryonic stem cells and improves behavioral outcome after transplantation into Parkinsonian rats. *Stem Cells, 26*, 2810–2820.

Cho, M. S., Lee, Y. E., Kim, J. Y., Chung, S., Cho, Y. H., Kim, D. S., Kang, S. M., Lee, H., Kim, M. H., Kim, J. H., Leem, J. W., Oh, S. K., Choi, Y. M., Hwang, D. Y., Chang, J. W., & Kim, D. W. (2008). Highly efficient and large scale generation of functional dopamine neurons from human embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 3392–3397.

Choi, Y. K., Cho, H., Seo, Y. K., Yoon, H. H., & Park, J. K. (2012). Stimulation of sub-sonic vibration promotes the differentiation of adipose tissue-derived mesenchymal stem cells into neural cells. *Life Sciences, 91*, 329–337.

Chou, B. K., Mali, P., Huang, X., Ye, Z., Dowey, S. N., Resar, L. M., Zou, C., Zhang, Y. A., Tong, J., & Cheng, L. (2011). Efficient human iPS cell derivation by a non-integrating plasmid from blood cells with unique epigenetic and gene expression signatures. *Cell Research, 21*, 518–529.

Collier, T. J., Sortwell, C. E., & Daley, B. F. (1999). Diminished viability, growth, and behavioral efficacy of fetal dopamine neuron grafts in aging rats with long-term dopamine depletion: An argument for neurotrophic supplementation. *Journal of Neuroscience, 19*, 5563–5573.

Cooper, O., Hargus, G., Deleidi, M., Blak, A., Osborn, T., Marlow, E., Lee, K., Levy, A., Perez-Torres, E., Yow, A., & Isacson, O. (2010). Differentiation of human ES and Parkinson's disease iPS cells into ventral midbrain dopaminergic neurons requires a high activity form of

SHH, FGF8a and specific regionalization by retinoic acid. *Molecular and Cellular Neuroscience, 45*, 258–266.

Correia, A. S., Anisimov, S. V., Roybon, L., Li, J. Y., & Brundin, P. (2007). Fibroblast growth factor-20 increases the yield of midbrain dopaminergic neurons derived from human embryonic stem cells. *Frontiers in Neuroanatomy, 1*, 4.

Cowan, C. A., Atienza, J., Melton, D. A., & Eggan, K. (2005). Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science, 309*, 1369–1373.

Darabi, S., Tiraihi, T., Delshad, A., & Sadeghizadeh, M. (2013). A new multistep induction protocol for the transdifferentiation of bone marrow stromal stem cells into GABAergic neuron-like cells. *Iranian Biomedical Journal, 17*, 8–14.

Datta, I., Ganapathy, K., Tattikota, S. M., & Bhonde, R. (2013). Directed differentiation of human embryonic stem cell-line HUES9 to dopaminergic neurons in a serum-free defined culture niche. *Cell Biology International, 37*, 54–64.

de Jong, J., Stoop, H., Gillis, A. J., van Gurp, R. J., van de Geijn, G. J., Boer, M. D., Hersmus, R., Saunders, P. T., Anderson, R. A., Oosterhuis, J. W., & Looijenga, L. H. (2008). Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications. *The Journal of Pathology, 215*, 21–30.

Dor, Y., Brown, J., Martinez, O. I., & Melton, D. A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature, 429*, 41–46.

Dushnik-Levinson, M., & Benvenisty, N. (1995). Embryogenesis in vitro: Study of differentiation of embryonic stem cells. *Biology of the Neonate, 67*, 77–83.

Ebben, J. D., Zorniak, M., Clark, P. A., & Kuo, J. S. (2011). Introduction to induced pluripotent stem cells: Advancing the potential for personalized medicine. *World Neurosurgery, 76*, 270–275.

Ellis, P., Fagan, B. M., Magness, S. T., Hutton, S., Taranova, O., Hayashi, S., McMahon, A., Rao, M., & Pevny, L. (2004). SOX2, a persistent marker for multipotential neural stem cells derived from embryonic stem cells, the embryo or the adult. *Developmental Neuroscience, 26*, 148–165.

Emgård, M., Karlsson, J., Hansson, O., & Brundin, P. (1999). Patterns of cell death and dopaminergic neuron survival in intrastriatal nigral grafts. *Experimental Neurology, 160*, 279–288.

Eminli, S., Utikal, J., Arnold, K., Jaenisch, R., & Hochedlinger, K. (2008). Reprogramming of neural progenitor cells into induced pluripotent stem cells in the absence of exogenous Sox2 expression. *Stem Cells, 26*, 2467–2474.

Esfandiari, F., Fathi, A., Gourabi, H., Kiani, S., Nemati, S., & Baharvand, H. (2012). Glycogen synthase kinase-3 inhibition promotes proliferation and neuronal differentiation of human-induced pluripotent stem cell-derived neural progenitors. *Stem Cells and Development, 21*, 3233–3243.

Espejo, E. F., Montoro, R. J., Armengol, J. A., & López-Barneo, J. (1998). Cellular and functional recovery of Parkinsonian rats after intrastriatal transplantation of carotid body cell aggregates. *Neuron, 20*, 197–206.

Evans, M., & Kaufman, M. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature, 292*, 154–156.

Ezeh, U. I., Turek, P. J., Reijo, R. A., & Clark, A. T. (2005). Human embryonic stem cell genes OCT4, NANOG, STELLAR, and GDF3 are expressed in both seminoma and breast carcinoma. *Cancer, 104*, 2255–2265.

Ferri, A. L., Cavallaro, M., Braida, D., Di Cristofano, A., Canta, A., Vezzani, A., Ottolenghi, S., Pandolfi, P. P., Sala, M., DeBiasi, S., & Nicolis, S. K. (2004). Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. *Development, 131*, 3805–3819.

Fisone, G., & Bezard, E. (2011). Molecular mechanisms of l-DOPA-induced dyskinesia. *International Review of Neurobiology, 98*, 95–122.

Fong, Y. W., Inouye, C., Yamaguchi, T., Cattoglio, C., Grubisic, I., & Tjian, R. (2011). A DNA repair complex functions as an Oct4/Sox2 coactivator in embryonic stem cells. *Cell, 147*, 120–131.

Freed, C. R., Breeze, R. E., Rosenberg, N. L., Schneck, S. A., Wells, T. H., Barrett, J. N., Grafton, S. T., Huang, S. C., Eidelberg, D., & Rottenberg, D. A. (1990). Transplantation of human fetal dopamine cells for Parkinson's disease. Results at 1 year. *Archives of Neurology, 47*, 505–512.

Freed, C. R., Breeze, R. E., Rosenberg, N. L., Schneck, S. A., Kriek, E., Qi, J. X., Lone, T., Zhang, Y. B., Snyder, J. A., & Wells, T. H. (1992). Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. *The New England Journal of Medicine, 327*, 1549–1555.

Freed, C. R., Greene, P. E., Breeze, R. E., Tsai, W. Y., DuMouchel, W., Kao, R., Dillon, S., Winfield, H., Culver, S., Trojanowski, J. Q., Eidelberg, D., & Fahn, S. (2001). Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *The New England Journal of Medicine, 344*, 710–719.

Fu, W., Lu, Y., & Piao, Y. (2002). Culture and pluripotentiality of human marrow mesenchymal stem cells. *Zhonghua Xue Ye Xue Za Zhi, 23*, 202–204.

Galpern, W. R., Burns, L. H., Deacon, T. W., Dinsmore, J., & Isacson, O. (1996). Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: Functional recovery and graft morphology. *Experimental Neurology, 140*, 1–13.

González, F., Boué, S., & Izpisúa Belmonte, J. C. (2011). Methods for making induced pluripotent stem cells: Reprogramming à la carte. *Nature Reviews Genetics, 12*, 231–242.

Grad, I., Hibaoui, Y., Jaconi, M., Chicha, L., Bergström-Tengzelius, R., Sailani, M. R., Pelte, M. F., Dahoun, S., Mitsiadis, T. A., Töhönen, V., Bouillaguet, S., Antonarakis, S. E., Kere, J., Zucchelli, M., Hovatta, O., & Feki, A. (2011). NANOG priming before full reprogramming may generate germ cell tumours. *European Cells & Materials, 22*, 258–274.

Grinnell, K. L., Yang, B., Eckert, R. L., & Bickenbach, J. R. (2007). De-differentiation of mouse interfollicular keratinocytes by the embryonic transcription factor Oct-4. *The Journal of Investigative Dermatology, 127*, 372–380.

Gronthos, S., Mankani, M., Brahim, J., Robey, P. G., & Shi, S. (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 13625–13630.

Guo, Z., Li, H., Li, X., Yu, X., Wang, H., Tang, P., & Mao, N. (2006). In vitro characteristics and in vivo immunosuppressive activity of compact bone-derived murine mesenchymal progenitor cells. *Stem Cells, 24*, 992–1000.

Guo, G., Yang, J., Nichols, J., Hall, J. S., Eyres, I., Mansfield, W., & Smith, A. (2009). Klf4 reverts developmentally programmed restriction of ground state pluripotency. *Development, 136*, 1063–1069.

Gurdon, J. B., & Melton, D. A. (2008). Nuclear reprogramming in cells. Review. *Science, 322*, 1811–1815.

Gurdon, J. B., Elsdale, T. R., & Fischberg, M. (1958). Sexually mature individuals of Xenopus laevis from the transplantation of single somatic nuclei. *Nature, 182*, 64–65.

Hagell, P., Schrag, A., Piccini, P., Jahanshahi, M., Brown, R., Rehncrona, S., Widner, H., Brundin, P., Rothwell, J. C., Odin, P., Wenning, G. K., Morrish, P., Gustavii, B., Björklund, A., Brooks, D. J., Marsden, C. D., Quinn, N. P., & Lindvall, O. (1999). Sequential bilateral transplantation in Parkinson's disease: Effects of the second graft. *Brain, 122*, 1121–1132.

Hamazaki, T., Oka, M., Yamanaka, S., & Terada, N. (2004). Aggregation of embryonic stem cells induces Nanog repression and primitive endoderm differentiation. *Journal of Cell Science, 117*, 5681–5686.

Hammachi, F., Morrison, G. M., Sharov, A. A., Livigni, A., Narayan, S., Papapetrou, E. P., O'Malley, J., Kaji, K., Ko, M. S., Ptashne, M., & Brickman, J. M. (2012). Transcriptional activation by Oct4 is sufficient for the maintenance and induction of pluripotency. *Cell Reports, 1*, 99–109.

Han, S. S., Williams, L. A., & Eggan, K. C. (2011). Constructing and deconstructing stem cell models of neurological disease. *Neuron, 70*, 626–644.

Hara, K., Uchida, K., Fukunaga, A., Toya, S., & Kawase, T. (1997). Implantation of xenogeneic transgenic neural plate tissues into parkinsonian rat brain. *Cell Transplantation, 6*, 515–519.

Hargus, G., Cooper, O., Deleidi, M., Levy, A., Lee, K., Marlow, E., Yow, A., Soldner, F., Hockemeyer, D., Hallett, P. J., Osborn, T., Jaenisch, R., & Isacson, O. (2010). Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 15921–15926.

Harrower, T. P., Tyers, P., Hooks, Y., & Barker, R. A. (2006). Long-term survival and integration of porcine expanded neural precursor cell grafts in a rat model of Parkinson's disease. *Experimental Neurology, 197*, 56–69.

Hart, A. H., Hartley, L., Ibrahim, M., & Robb, L. (2004). Identification, cloning and expression analysis of the pluripotency promoting Nanog genes in mouse and human. *Developmental Dynamics, 230*, 187–198.

Hochedlinger, K., Yamada, Y., Beard, C., & Jaenisch, R. (2005). Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. *Cell, 121*, 465–477.

Hoei-Hansen, C. E., Almstrup, K., Nielsen, J. E., Brask Sonne, S., Graem, N., Skakkebaek, N. E., Leffers, H., & Rajpert-De Meyts, E. (2005). Stem cell pluripotency factor NANOG is expressed in human fetal gonocytes, testicular carcinoma in situ and germ cell tumours. *Histopathology, 47*, 48–56.

Holm, K. H., Cicchetti, F., Bjorklund, L., Boonman, Z., Tandon, P., Costantini, L. C., Deacon, T. W., Huang, X., Chen, D. F., & Isacson, O. (2001). Enhanced axonal growth from fetal human bcl-2 transgenic mouse dopamine neurons transplanted to the adult rat striatum. *Neuroscience, 104*, 397–405.

Hu, B. Y., Weick, J. P., Yu, J., Ma, L. X., Zhang, X. Q., Thomson, J. A., & Zhang, S. C. (2010). Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 4335–4340.

Huffaker, T. K., Boss, B. D., Morgan, A. S., Neff, N. T., Strecker, R. E., Spence, M. S., & Miao, R. (1989). Xenografting of fetal pig ventral mesencephalon corrects motor asymmetry in the rat model of Parkinson's disease. *Experimental Brain Research, 77*, 329–336.

Hwang, D. Y., Kim, D. S., & Kim, D. W. (2010). Human ES and iPS cells as cell sources for the treatment of Parkinson's disease: Current state and problems. *Journal of Cellular Biochemistry, 109*, 292–301.

Ivanova, N., Dobrin, R., Lu, R., Kotenko, I., Levorse, J., DeCoste, C., Schafer, X., Lun, Y., & Lemischka, I. R. (2006). Dissecting self-renewal in stem cells with RNA interference. *Nature, 442*, 533–538.

Jackson, J. S., Golding, J. P., Chapon, C., Jones, W. A., & Bhakoo, K. K. (2010). Homing of stem cells to sites of inflammatory brain injury after intracerebral and intravenous administration: A longitudinal imaging study. *Stem Cell Research & Therapy, 1*, 17.

Jensen, P., Pedersen, E. G., Zimmer, J., Widmer, H. R., & Meyer, M. (2008). Functional effect of FGF2- and FGF8-expanded ventral mesencephalic precursor cells in a rat model of Parkinson's disease. *Brain Research, 1218*, 13–20.

Jeter, C. R., Badeaux, M., Choy, G., Chandra, D., Patrawala, L., Liu, C., Calhoun-Davis, T., Zaehres, H., Daley, G. Q., & Tang, D. G. (2009). Functional evidence that the self-renewal gene NANOG regulates human tumor development. *Stem Cells, 27*, 993–1005.

Jo, A. Y., Kim, M. Y., Lee, H. S., Rhee, Y. H., Lee, J. E., Baek, K. H., Park, C. H., Koh, H. C., Shin, I., Lee, Y. S., & Lee, S. H. (2009). Generation of dopamine neurons with improved cell survival and phenotype maintenance using a degradation-resistant nurr1 mutant. *Stem Cells, 27*, 2238–2246.

Kaddis, F. G., Clarkson, E. D., Bell, K. P., Choi, P. K., & Freed, C. R. (2000). Co-grafts of muscle cells and mesencephalic tissue into hemiparkinsonian rats: Behavioral and histochemical effects. *Brain Research Bulletin, 51*, 203–211.

Kaji, K., Norrby, K., Paca, A., Mileikovsky, M., Mohseni, P., & Woltjen, K. (2009). Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature, 458*, 771–775.

Kakishita, K., Elwan, M. A., Nakao, N., Itakura, T., & Sakuragawa, N. (2000). Human amniotic epithelial cells produce dopamine and survive after implantation into the striatum of a rat model of Parkinson's disease: A potential source of donor for transplantation therapy. *Experimental Neurology, 165*, 27–34.

Kaminski Schierle, G. S., Hansson, O., & Brundin, P. (1999). Flunarizine improves the survival of grafted dopaminergic neurons. *Neuroscience, 94*, 17–20.

Kashyap, V., Rezende, N. C., Scotland, K. B., Shaffer, S. M., Persson, J. L., Gudas, L. J., & Mongan, N. P. (2009). Regulation of stem cell pluripotency and differentiation involves a mutual regulatory circuit of the NANOG, OCT4, and SOX2 pluripotency transcription factors with polycomb repressive complexes and stem cell microRNAs. *Stem Cells and Development, 18*, 1093–1108.

Kerr, C. L., Gearhart, J. D., Elliott, A. M., & Donovan, P. J. (2006). Embryonic germ cells: When germ cells become stem cells. *Seminars in Reproductive Medicine, 24*, 304–313.

Kestendjieva, S., Kyurkchiev, D., Tsvetkova, G., Mehandjiev, T., Dimitrov, A., Nikolov, A., & Kyurkchiev, S. (2008). Characterization of mesenchymal stem cells isolated from the human umbilical cord. *Cell Biology International, 32*, 724–732.

Kim, H. J. (2011). Stem cell potential in Parkinson's disease and molecular factors for the generation of dopamine neurons. *Biochimica et Biophysica Acta, 1812*, 1–11.

Kim, J. H., Auerbach, J. M., Rodríguez-Gómez, J. A., Velasco, I., Gavin, D., Lumelsky, N., Lee, S. H., Nguyen, J., Sánchez-Pernaute, R., Bankiewicz, K., & McKay, R. (2002). Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature, 418*, 50–56.

Kim, J. B., Zaehres, H., Wu, G., Gentile, L., Ko, K., Sebastiano, V., Araúzo-Bravo, M. J., Ruau, D., Han, D. W., Zenke, M., & Schöler, H. R. (2008). Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature, 454*, 646–650.

Kim, J. B., Greber, B., Araúzo-Bravo, M. J., Meyer, J., Park, K. I., Zaehres, H., & Schöler, H. R. (2009a). Direct reprogramming of human neural stem cells by OCT4. *Nature, 461*, 649–653.

Kim, J. B., Sebastiano, V., Wu, G., Araúzo-Bravo, M. J., Sasse, P., Gentile, L., Ko, K., Ruau, D., Ehrich, M., van den Boom, D., Meyer, J., Hübner, K., Bernemann, C., Ortmeier, C., Zenke, M., Fleischmann, B. K., Zaehres, H., & Schöler, H. R. (2009b). Oct4-induced pluripotency in adult neural stem cells. *Cell, 136*, 411–419.

Kim, J. B., Zaehres, H., Araúzo-Bravo, M. J., & Schöler, H. R. (2009c). Generation of induced pluripotent stem cells from neural stem cells. *Nature Protocols, 4*, 1464–1470.

Ko, J. Y., Lee, H. S., Park, C. H., Koh, H. C., Lee, Y. S., & Lee, S. H. (2009). Conditions for tumor-free and dopamine neuron-enriched grafts after transplanting human ES cell-derived neural precursor cells. *Molecular Therapy, 17*, 1761–1770.

Komitova, M., & Eriksson, P. S. (2004). Sox-2 is expressed by neural progenitors and astroglia in the adult rat brain. *Neuroscience Letters, 369*, 24–27.

Kompoliti, K., Chu, Y., Shannon, K. M., & Kordower, J. H. (2007). Neuropathological study 16 years after autologous adrenal medullary transplantation in a Parkinson's disease patient. *Movement Disorders, 22*, 1630–1633.

Kriks, S., Shim, J. W., Piao, J., Ganat, Y. M., Wakeman, D. R., Xie, Z., Carrillo-Reid, L., Auyeung, G., Antonacci, C., Buch, A., Yang, L., Beal, M. F., Surmeier, D. J., Kordower, J. H., Tabar, V., & Studer, L. (2011). Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature, 480*, 547–551.

Kubo, A., Yoshida, T., Kobayashi, N., Yokoyama, T., Mimura, T., Nishiguchi, T., Higashida, T., Yamamoto, I., & Kanno, H. (2009). Efficient generation of dopamine neuron-like cells from skin-derived precursors with a synthetic peptide derived from von Hippel- Lindau protein. *Stem Cells and Development, 18*, 1523–1532.

Kupsch, A., Oertel, W. H., Earl, C. D., & Sautter, J. (1995). Neuronal transplantation and neurotrophic factors in the treatment of Parkinson's disease–update February 1995. *Journal of Neural Transmission. Supplementum, 46*, 193–207.

Kuroda, T., Tada, M., Kubota, H., Kimura, H., Hatano, S. Y., Suemori, H., Nakatsuji, N., & Tada, T. (2005). Octamer and Sox elements are required for transcriptional cis regulation of Nanog gene expression. *Molecular and Cellular Biology, 25*, 2475–2485.

Kuroda, T., Yasuda, S., & Sato, Y. (2013). Tumorigenicity studies for human pluripotent stem cell-derived products. *Biological and Pharmaceutical Bulletin, 36*, 189–192.

Larsson, L. C., & Widner, H. (2000). Neural tissue xenografting. *Scandinavian Journal of Immunology, 52*, 249–256.

Lengerke, C., Fehm, T., Kurth, R., Neubauer, H., Scheble, V., Müller, F., Schneider, F., Petersen, K., Wallwiener, D., Kanz, L., Fend, F., Perner, S., Bareiss, P. M., & Staebler, A. (2011). Expression of the embryonic stem cell marker SOX2 in early-stage breast carcinoma. *BMC Cancer, 11*, 42.

Lengner, C. J., Camargo, F. D., Hochedlinger, K., Welstead, G. G., Zaidi, S., Gokhale, S., Scholer, H. R., Tomilin, A., & Jaenisch, R. (2007). Oct4 expression is not required for mouse somatic stem cell self-renewal. *Cell Stem Cell, 1*, 403–415.

Lévêque, X., Nerrière-Daguin, V., Neveu, I., & Naveilhan, P. (2012). Pig neural cells derived from foetal mesencephalon as cell source for intracerebral xenotransplantation. *Methods in Molecular Biology, 885*, 233–243.

Li, J., Pan, G., Cui, K., Liu, Y., Xu, S., & Pei, D. A. (2007). A dominant-negative form of mouse SOX2 induces trophectoderm differentiation and progressive polyploidy in mouse embryonic stem cells. *Journal of Biological Chemistry, 282*, 19481–19492.

Li, M., Zhang, S. Z., Guo, Y. W., Cai, Y. Q., Yan, Z. J., Zou, Z., Jiang, X. D., Ke, Y. Q., He, X. Y., Jin, Z. L., Lu, G. H., & Su, D. Q. (2010). Human umbilical vein-derived dopaminergic-like cell transplantation with nerve growth factor ameliorates motor dysfunction in a rat model of Parkinson's disease. *Neurochemical Research, 35*, 1522–1529.

Li, Y., Zhang, Q., Yin, X., Yang, W., Du, Y., Hou, P., Ge, J., Liu, C., Zhang, W., Zhang, X., Wu, Y., Li, H., Liu, K., Wu, C., Song, Z., Zhao, Y., Shi, Y., & Deng, H. (2011). Generation of iPSCs from mouse fibroblasts with a single gene, Oct4, and small molecules. *Cell Research, 21*, 196–204.

Li, X., Li, H., Bi, J., Chen, Y., Jain, S., & Zhao, Y. (2012). Human cord blood-derived multipotent stem cells (CB-SCs) treated with all-trans-retinoic acid (ATRA) give rise to dopamine neurons. *Biochemical and Biophysical Research Communications, 419*, 110–116.

Liard, O., Segura, S., Pascual, A., Gaudreau, P., Fusai, T., & Moyse, E. (2009). In vitro isolation of neural precursor cells from the adult pig subventricular zone. *Journal of Neuroscience Methods, 182*, 172–179.

Lindvall, O., Sawle, G., Widner, H., Rothwell, J. C., Björklund, A., Brooks, D., Brundin, P., Frackowiak, R., Marsden, C. D., & Odin, P. (1994). Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. *Annals of Neurology, 35*, 172–180.

Liu, X., Li, F., Stubblefield, E. A., Blanchard, B., Richards, T. L., Larson, G. A., He, Y., Huang, Q., Tan, A. C., Zhang, D., Benke, T. A., Sladek, J. R., Zahniser, N. R., & Li, C. Y. (2012). Direct reprogramming of human fibroblasts into dopaminergic neuron-like cells. *Cell Research, 22*, 321–332.

Loh, Y. H., Wu, Q., Chew, J. L., Vega, V. B., Zhang, W., Chen, X., Bourque, G., George, J., Leong, B., Liu, J., Wong, K. Y., Sung, K. W., Lee, C. W., Zhao, X. D., Chiu, K. P., Lipovich, L., Kuznetsov, V. A., Robson, P., Stanton, L. W., Wei, C. L., Ruan, Y., Lim, B., & Ng, H. H. (2006). The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nature Genetics, 38*, 431–440.

López-Lozano, J. J., Mata, M., & Bravo, G. (2000). Neural transplants en Parkinson disease: Clinical results of 10 years of experience. Group of Neural Transplants of the CPH. *Revista de Neurologia, 30*, 1077–1083.

Lunn, J. S., Sakowski, S. A., Hur, J., & Feldman, E. L. (2011). Stem cell technology for neurodegenerative diseases. *Annals of Neurology, 70*, 353–361.

Luquin, M. R., Montoro, R. J., Guillén, J., Saldise, L., Insausti, R., Del Río, J., & López-Barneo, J. (1999). Recovery of chronic parkinsonian monkeys by autotransplants of carotid body cell aggregates into putamen. *Neuron, 22*, 743–750.

MacKenzie, T. C., & Flake, A. W. (2002). Human mesenchymal stem cells: Insights from a surrogate in vivo assay system. *Cells, Tissues, Organs, 171*, 90–95.

Madrazo, I., Franco-Bourland, R., Ostrosky-Solis, F., Aguilera, M., Cuevas, C., Zamorano, C., Morelos, A., Magallon, E., & Guizar-Sahagun, G. (1990). Fetal homotransplants (ventral mesencephalon and adrenal tissue) to the striatum of parkinsonian subjects. *Archives of Neurology, 47*, 1281–1285.

Major, T., Menon, J., Auyeung, G., Soldner, F., Hockemeyer, D., Jaenisch, R., & Tabar, V. (2011). Transgene excision has no impact on in vivo integration of human iPS derived neural precursors. *PLoS One, 6*, e24687.

Marsden, C. D. (1994). Problems with long-term levodopa therapy for Parkinson's disease. *Clinical Neuropharmacology, 17*(Suppl 2), 32–44.

Martin, G. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 78*, 7634–7638.

Masui, S., Nakatake, Y., Toyooka, Y., Shimosato, D., Yagi, R., Takahashi, K., Okochi, H., Okuda, A., Matoba, R., Sharov, A. A., Ko, M. S., & Niwa, H. (2007). Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nature Cell Biology, 9*, 625–635.

Mathew, J. M., Garcia-Morales, R. O., Carreno, M., Jin, Y., Fuller, L., Blomberg, B., Cirocco, R., Burke, G. W., Ciancio, G., Ricordi, C., Esquenazi, V., Tzakis, A. G., & Miller, J. (2003). Immune responses and their regulation by donor bone marrow cells in clinical organ transplantation. *Transplant Immunology, 11*, 307–321.

Mendez, I., Sadi, D., & Hong, M. (1996). Reconstruction of the nigrostriatal pathway by simultaneous intrastriatal and intranigral dopaminergic transplants. *Journal of Neuroscience, 16*, 7216–7227.

Mendez, I., Sanchez-Pernaute, R., Cooper, O., Viñuela, A., Ferrari, D., Björklund, L., Dagher, A., & Isacson, O. (2005). Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with Parkinson's disease. *Brain, 128*, 1498–1510.

Miller, R. A., & Ruddle, F. H. (1976). Pluripotent teratocarcinoma-thymus somatic cell hybrids. *Cell, 9*, 45–55.

Minguez-Castellanos, A., & Escamilla-Sevilla, F. (2005). Pallidal vs subthalamic deep brain stimulation for Parkinson disease: Winner and loser or a sharing of honors? *Archives of Neurology, 62*, 1642–1643.

Mínguez-Castellanos, A., Escamilla-Sevilla, F., Hotton, G. R., Toledo-Aral, J. J., Ortega-Moreno, A., Méndez-Ferrer, S., Martín-Linares, J. M., Katati, M. J., Mir, P., Villadiego, J., Meersmans, M., Pérez-García, M., Brooks, D. J., Arjona, V., & López-Barneo, J. (2007). Carotid body autotransplantation in Parkinson disease: A clinical and positron emission tomography study. *Journal of Neurology, Neurosurgery, and Psychiatry, 7*, 825–831.

Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., Maruyama, M., Maeda, M., & Yamanaka, S. (2003). The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell, 113*, 631–642.

Miyagi, S., Masui, S., Niwa, H., Saito, T., Shimazaki, T., Okano, H., Nishimoto, M., Muramatsu, M., Iwama, A., & Okuda, A. (2008). Consequence of the loss of Sox2 in the developing brain of the mouse. *FEBS Letters, 582*, 2811–2815.

Molenaar, G. J., Hogenesch, R. I., Sprengers, M. E., & Staal, M. J. (1997). Ontogenesis of embryonic porcine ventral mesencephalon in the perspective of its potential use as a xenograft in Parkinson's disease. *The Journal of Comparative Neurology, 382*, 19–28.

Momčilović, O., Montoya-Sack, J., & Zeng, X. (2012). Dopaminergic differentiation using pluripotent stem cells. *Journal of Cellular Biochemistry, 113*, 3610–3619.

Morigi, M., & Benigni, A. (2012). Mesenchymal stem cells and kidney repair. *Nephrol Dial Transplant, 28*, 788–793.

Morizane, A., Darsalia, V., Guloglu, M. O., Hjalt, T., Carta, M., Li, J. Y., & Brundin, P. A. (2010). Simple method for large-scale generation of dopamine neurons from human embryonic stem cells. *Journal of Neuroscience Research, 88*, 3467–3478.

Mundra, V., Gerling, I. C., & Mahato, R. I. (2013). Mesenchymal stem cell-based therapy. *Molecular Pharmaceutics, 10*, 77–89.

Nadig, R. R. J. (2009). Stem cell therapy – Hype or hope? A review. *Journal of Conservative Dentistry: JCD, 12*, 131–138.

Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., Aoi, T., Okita, K., Mochiduki, Y., Takizawa, N., & Yamanaka, S. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nature Biotechnology, 26*, 101–106.

Nakagawa, E., Zhang, L., Kim, E. J., Shin, J. O., Cho, S. W., Ohshima, H., & Jung, H. S. (2012). The novel function of Oct3/4 in mouse tooth development. *Histochemistry and Cell Biology, 137*, 367–376.

Narayanan, G., Poonepalli, A., Chen, J., Sankaran, S., Hariharan, S., Yu, Y. H., Robson, P., Yang, H., & Ahmed, S. (2012). Single-cell mRNA profiling identifies progenitor subclasses in neurospheres. *Stem Cells and Development, 21*, 3351–3362.

Nesti, C., Pardini, C., Barachini, S., D'Alessandro, D., Siciliano, G., Murri, L., Petrini, M., & Vaglini, F. (2011). Human dental pulp stem cells protect mouse dopaminergic neurons against MPP+ or rotenone. *Brain Research, 1367*, 94–102.

Nissim-Eliraz, E., Zisman, S., Schatz, O., & Ben-Arie, N. (2012). Nato3 integrates with the Shh-Foxa2 transcriptional network regulating the differentiation of midbrain dopaminergic neurons. *Journal of Molecular Neuroscience, 21*. doi: 10.1007/s12031-012-9939-6.

Niwa, H. (2007). How is pluripotency determined and maintained? *Development, 134*, 635–646.

O'Sullivan, S. S., Williams, D. R., Gallagher, D. A., Massey, L. A., Silveira-Moriyama, L., & Lees, A. J. (2008). Nonmotor symptoms as presenting complaints in Parkinson's disease: A clinicopathological study. *Movement Disorders, 23*, 101–106.

Obeso, J. A., Rodriguez-Oroz, M. C., Goetz, C. G., Marin, C., Kordower, J. H., Rodriguez, M., Hirsch, E. C., Farrer, M., Schapira, A. H., & Halliday, G. (2010). Missing pieces in the Parkinson's disease puzzle. *Nature Medicine, 16*, 653–661.

Odekerken, V. J., van Laar, T., Staal, M. J., Mosch, A., Hoffmann, C. F., Nijssen, P. C., Beute, G. N., van Vugt, J. P., Lenders, M. W., Contarino, M. F., Mink, M. S., Bour, L. J., van den Munckhof, P., Schmand, B. A., de Haan, R. J., Schuurman, P. R., & de Bie, R. M. (2013). Subthalamic nucleus versus globus pallidus bilateral deep brain stimulation for advanced Parkinson's disease (NSTAPS study): A randomised controlled trial. *Lancet Neurology, 12*, 37–44.

Okabe, S., Forsberg-Nilsson, K., Spiro, A. C., Segal, M., & McKay, R. D. (1996). Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro. *Mechanisms of Development, 59*, 89–102.

Okita, K., Ichisaka, T., & Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. *Nature, 448*, 313–317.

Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T., & Yamanaka, S. (2008). Generation of mouse induced pluripotent stem cells without viral vectors. *Science, 322*, 949–953.

Okita, K., Hong, H., Takahashi, K., & Yamanaka, S. (2010). Generation of mouse-induced pluripotent stem cells with plasmid vectors. *Nature Protocols, 5*, 418–428.

Okumura-Nakanishi, S., Saito, M., Niwa, H., & Ishikawa, F. (2005). Oct-3/4 and Sox2 regulate Oct-3/4 gene in embryonic stem cells. *Journal of Biological Chemistry, 280*, 5307–5317.

Olanow, C. W. (2004). The scientific basis for the current treatment of Parkinson's disease. *Annual Review of Medicine, 55*, 41–60.

Olanow, C. W., Goetz, C. G., Kordower, J. H., Stoessl, A. J., Sossi, V., Brin, M. F., Shannon, K. M., Nauert, G. M., Perl, D. P., Godbold, J., & Freeman, T. B. (2003). A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Annals of Neurology, 54*, 403–414.

Palmieri, S. L., Peter, W., Hess, H., & Schöler, H. R. (1994). Oct-4 transcription factor is differentially expressed in the mouse embryo during establishment of the first two extraembryonic cell lineages involved in implantation. *Developmental Biology, 166*, 259–267.

Pan, G. J., & Pei, D. Q. (2003). Identification of two distinct transactivation domains in the pluripotency sustaining factor nanog. *Cell Research, 13*, 499–502.

Pan, G., & Thomson, J. A. (2007). Nanog and transcriptional networks in embryonic stem cell pluripotency. *Cell Research, 17*, 42–49.

Pang, Z. P., Yang, N., Vierbuchen, T., Ostermeier, A., Fuentes, D. R., Yang, T. Q., Citri, A., Sebastiano, V., Marro, S., Südhof, T. C., & Wernig, M. (2011). Induction of human neuronal cells by defined transcription factors. *Nature, 476*, 220–223.

Pardal, R., & López-Barneo, J. (2012). Neural stem cells and transplantation studies in Parkinson's disease. *Advances in Experimental Medicine and Biology, 741*, 206–216.

Pardal, R., Ortega-Sáenz, P., Durán, R., & López-Barneo, J. (2007). Glia-like stem cells sustain physiologic neurogenesis in the adult mammalian carotid body. *Cell, 131*, 364–377.

Parish, C. L., Castelo-Branco, G., Rawal, N., Tonnesen, J., Sorensen, A. T., Salto, C., Kokaia, M., Lindvall, O., & Arenas, E. (2008). Wnt5a-treated midbrain neural stem cells improve dopamine cell replacement therapy in parkinsonian mice. *The Journal of Clinical Investigation, 118*, 149–160.

Park, S., Lee, K. S., Lee, Y. J., Shin, H. A., Cho, H. Y., Wang, K. C., Kim, Y. S., Lee, H. T., Chung, K. S., Kim, E. Y., & Lim, J. (2004). Generation of dopaminergic neurons in vitro from human embryonic stem cells treated with neurotrophic factors. *Neuroscience Letters, 359*, 99–103.

Park, H. J., Lee, P. H., Bang, O. Y., Lee, G., & Ahn, Y. H. (2008). Mesenchymal stem cells therapy exerts neuroprotection in a progressive animal model of Parkinson's disease. *Journal of Neurochemistry, 107*, 141–151.

Patel, M., & Yang, S. (2010). Advances in reprogramming somatic cells to induced pluripotent stem cells. *Stem Cell Reviews, 6*, 367–380.

Pawitan, J. A. (2011). Prospect of cell therapy for Parkinson's disease. *Anatomy & Cell Biology, 44*, 256–264.

Perl, L., Weissler, A., Mekori, Y. A., & Mor, A. (2010). Cellular therapy in 2010: Focus on autoimmune and cardiac diseases. *The Israel Medical Association Journal, 12*, 110–115.

Perlow, M. J., Freed, W. J., Hoffer, B. J., Seiger, A., Olson, L., & Wyatt, R. J. (1979). Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science, 1204*, 643–647.

Perrett, R. M., Turnpenny, L., Eckert, J. J., O'Shea, M., Sonne, S. B., Cameron, I. T., Wilson, D. I., Rajpert-De Meyts, E., & Hanley, N. A. (2008). The early human germ cell lineage does not express SOX2 during in vivo development or upon in vitro culture. *Biology of Reproduction, 78*, 852–858.

Perrier, A. L., Tabar, V., Barberi, T., Rubio, M. E., Bruses, J., Topf, N., Harrison, N. L., & Studer, L. (2004). Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 12543–12548.

Pfisterer, U., Wood, J., Nihlberg, K., Hallgren, O., Bjermer, L., Westergren-Thorsson, G., Lindvall, O., & Parmar, M. (2011). Efficient induction of functional neurons from adult human fibroblasts. *Cell Cycle, 10*, 3311–3316.

Piccini, P., Brooks, D. J., Björklund, A., Gunn, R. N., Grasby, P. M., Rimoldi, O., Brundin, P., Hagell, P., Rehncrona, S., Widner, H., & Lindvall, O. (1999). Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. *Nature Neuroscience, 2*, 1137–1140.

Politis, M., & Lindvall, O. (2012). Clinical application of stem cell therapy in Parkinson's disease. *BMC Medicine, 10*, 1.

Porzionato, A., Macchi, V., Parenti, A., & De Caro, R. (2008). Trophic factors in the carotid body. *International Review of Cell and Molecular Biology, 269*, 1–58.

Prakash, N., & Wurst, W. (2007). A Wnt signal regulates stem cell fate and differentiation in vivo. *Neurodegenerative Diseases, 4*, 333–338.

Puschban, Z., Stefanova, N., Petersén, A., Winkler, C., Brundin, P., Poewe, W., & Wenning, G. K. (2005). Evidence for dopaminergic re-innervation by embryonic allografts in an optimized rat model of the Parkinsonian variant of multiple system atrophy. *Brain Research Bulletin, 68*, 54–58.

Ramos-Moreno, T., Castillo, C. G., & Martínez-Serrano, A. (2012). Long term behavioral effects of functional dopaminergic neurons generated from human neural stem cells in the rat 6-OH-DA Parkinson's disease model. Effects of the forced expression of BCL-X(L). *Behavioural Brain Research, 232*, 225–232.

Reimann, V., Creutzig, U., & Kögler, G. (2009). Stem cells derived from cord blood in transplantation and regenerative medicine. *Deutsches Ärzteblatt International, 106*, 831–836.

Rhee, Y. H., Ko, J. Y., Chang, M. Y., Yi, S. H., Kim, D., Kim, C. H., Shim, J. W., Jo, A. Y., Kim, B. W., Lee, H., Lee, S. H., Suh, W., Park, C. H., Koh, H. C., Lee, Y. S., Lanza, R., Kim, K. S., & Lee, S. H. (2011). Protein-based human iPS cells efficiently generate functional dopamine neurons and can treat a rat model of Parkinson disease. *The Journal of Clinical Investigation, 121*, 2326–2335.

Riaz, S. S., Jauniaux, E., Stern, G. M., & Bradford, H. F. (2002). The controlled conversion of human neural progenitor cells derived from foetal ventral mesencephalon into dopaminergic neurons in vitro. *Brain Research. Developmental Brain Research, 136*, 27–34.

Rizzino, A. (2009). Sox2 and Oct-3/4: A versatile pair of master regulators that orchestrate the self-renewal and pluripotency of embryonic stem cells. *Wiley Interdisciplinary Reviews. Systems Biology and Medicine, 1*, 228–236.

Robinton, D. A., & Daley, G. Q. (2012). The promise of induced pluripotent stem cells in research and therapy. *Nature, 481*, 295–305.

Rodda, D. J., Chew, J. L., Lim, L. H., Loh, Y. H., Wang, B., Ng, H. H., & Robson, P. (2005). Transcriptional regulation of nanog by OCT4 and SOX2. *Journal of Biological Chemistry, 280*, 24731–24737.

Rodriguez-Pallares, J., Joglar, B., Muñoz-Manchado, A. B., Villadiego, J., Toledo-Aral, J. J., & Labandeira-Garcia, J. L. (2012). Cografting of carotid body cells improves the long-term survival, fiber outgrowth and functional effects of grafted dopaminergic neurons. *Regenerative Medicine, 7*, 309–322.

Sadan, O., Bahat-Stromza, M., Barhum, Y., Levy, Y. S., Pisnevsky, A., Peretz, H., Ilan, A. B., Bulvik, S., Shemesh, N., Krepel, D., Cohen, Y., Melamed, E., & Offen, D. (2009). Protective effects of neurotrophic factor-secreting cells in a 6-OHDA rat model of Parkinson disease. *Stem Cells and Development, 18*, 1179–1190.

Sánchez-Danés, A., Consiglio, A., Richaud, Y., Rodríguez-Pizà, I., Dehay, B., Edel, M., Bové, J., Memo, M., Vila, M., Raya, A., & Izpisua-Belmonte, J. C. (2012a). Efficient generation of A9 midbrain dopaminergic neurons by lentiviral delivery of LMX1A in human embryonic stem cells and induced pluripotent stem cells. *Human Gene Therapy, 23*, 56–69.

Sánchez-Danés, A., Richaud-Patin, Y., Carballo-Carbajal, I., Jiménez-Delgado, S., Caig, C., Mora, S., Di Guglielmo, C., Ezquerra, M., Patel, B., Giralt, A., Canals, J. M., Memo, M., Alberch, J., López-Barneo, J., Vila, M., Cuervo, A. M., Tolosa, E., Consiglio, A., & Raya, A. (2012b). Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease. *EMBO Molecular Medicine, 4*, 380–395.

Schöler, H. R., Hatzopoulos, A. K., Balling, R., Suzuki, N., & Gruss, P. (1989). A family of octamer-specific proteins present during mouse embryogenesis: Evidence for germline-specific expression of an Oct factor. *EMBO Journal, 8*, 2543–2550.

Schumacher, J. M., Ellias, S. A., Palmer, E. P., Kott, H. S., Dinsmore, J., Dempsey, P. K., Fischman, A. J., Thomas, C., Feldman, R. G., Kassissieh, S., Raineri, R., Manhart, C., Penney, D., Fink, J. S., & Isacson, O. (2000). Transplantation of embryonic porcine mesencephalic tissue in patients with PD. *Neurology, 54*, 1042–1050.

Schwartz, C. M., Tavakoli, T., Jamias, C., Park, S. S., Maudsley, S., Martin, B., Phillips, T. M., Yao, P. J., Itoh, K., Ma, W., Rao, M. S., Arenas, E., & Mattson, M. P. (2012). Stromal factors SDF1α, sFRP1, and VEGFD induce dopaminergic neuron differentiation of human pluripotent stem cells. *Journal of Neuroscience Research, 90*, 1367–1381.

Sheik Mohamed, J., Gaughwin, P. M., Lim, B., Robson, P., & Lipovich, L. (2010). Conserved long noncoding RNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells. *RNA, 16*, 324–337.

Sheng, J. G., McShane, L. M., Plunkett, R. J., Cummins, A. C., Oldfield, E. H., Kopin, I. J., & Palmatier, M. A. (1993). Dopaminergic neuronal sprouting and behavioral recovery in hemiparkinsonian rats after implantation of amnion cells. *Experimental Neurology, 123*, 192–203.

Shetty, P., Thakur, A. M., & Viswanathan, C. (2013). Dopaminergic cells, derived from a high efficiency differentiation protocol from umbilical cord derived mesenchymal stem cells, alleviate symptoms in a Parkinson's disease rodent model. *Cell Biology International, 37*, 167–180.

Shi, W., Wang, H., Pan, G., Geng, Y., Guo, Y., & Pei, D. (2006). Regulation of the pluripotency marker Rex-1 by Nanog and Sox2. *Journal of Biological Chemistry, 281*, 23319–23325.

Shi, Y., Desponts, C., Do, J. T., Hahm, H. S., Schöler, H. R., & Ding, S. (2008). Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell, 3*, 568–574.

Shim, J. W., Park, C. H., Bae, Y. C., Bae, J. Y., Chung, S., Chang, M. Y., Koh, H. C., Lee, H. S., Hwang, S. J., Lee, K. H., Lee, Y. S., Choi, C. Y., & Lee, S. H. (2007). Generation of functional dopamine neurons from neural precursor cells isolated from the subventricular zone and white matter of the adult rat brain using Nurr1 overexpression. *Stem Cells, 25*, 1252–1262.

Silva, J., Barrandon, O., Nichols, J., Kawaguchi, J., Theunissen, T. W., & Smith, A. (2008). Promotion of reprogramming to ground state pluripotency by signal inhibition. *PLoS Biology, 6*, e253.

Sirinathsinghji, D. J., Dunnett, S. B., Northrop, A. J., & Morris, B. J. (1990). Experimental hemiparkinsonism in the rat following chronic unilateral infusion of MPP+ into the nigrostriatal dopamine pathway–III. Reversal by embryonic nigral dopamine grafts. *Neuroscience, 37*, 757–766.

Soldner, F., Hockemeyer, D., Beard, C., Gao, Q., Bell, G. W., Cook, E. G., Hargus, G., Blak, A., Cooper, O., Mitalipova, M., Isacson, O., & Jaenisch, R. (2009). Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell, 136*, 964–977.

Sommer, A. G., Rozelle, S. S., Sullivan, S., Mills, J. A., Park, S. M., Smith, B. W., Iyer, A. M., French, D. L., Kotton, D. N., Gadue, P., Murphy, G. J., & Mostoslavsky, G. (2012). Generation of human induced pluripotent stem cells from peripheral blood using the STEMCCA lentiviral vector. Journal of Visualized Experiments*, 68*. doi: 10.3791/4327.

Sonntag, K. C., Pruszak, J., Yoshizaki, T., van Arensbergen, J., Sanchez-Pernaute, R., & Isacson, O. (2007). Enhanced yield of neuroepithelial precursors and midbrain-like dopaminergic neurons from human embryonic stem cells using the bone morphogenic protein antagonist noggin. *Stem Cells, 25*, 411–418.

Sortwell, C. E., Collier, T. J., & Sladek, J. R., Jr. (1998). Co-grafted embryonic striatum increases the survival of grafted embryonic dopamine neurons. *The Journal of Comparative Neurology, 399*, 530–540.

Stacpoole, S. R., Bilican, B., Webber, D. J., Luzhynskaya, A., He, X. L., Compston, A., Karadottir, R., Franklin, R. J., & Chandran, S. (2011). Efficient derivation of NPCs, spinal motor neurons and midbrain dopaminergic neurons from hESCs at 3% oxygen. *Nature Protocols, 6*, 1229–1240.

Stadtfeld, M., & Hochedlinger, K. (2010). Induced pluripotency: History, mechanisms, and applications. *Genes & Development, 24*, 2239–22363.

Stadtfeld, M., Brennand, K., & Hochedlinger, K. (2008a). Reprogramming of pancreatic beta cells into induced pluripotent stem cells. *Current Biology, 18*, 890–894.

Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G., & Hochedlinger, K. (2008b). Induced pluripotent stem cells generated without viral integration. *Science, 322*, 945–949.

Steffenhagen, C., Kraus, S., Dechant, F. X., Kandasamy, M., Lehner, B., Poehler, A. M., Furtner, T., Siebzehnrubl, F. A., Couillard-Despres, S., Strauss, O., Aigner, L., & Rivera, F. J. (2011). Identity, fate and potential of cells grown as neurospheres: Species matters. *Stem Cell Reviews, 7*, 815–835.

Stevanovic, M. (2003). Modulation of SOX2 and SOX3 gene expression during differentiation of human neuronal precursor cell line NTERA2. *Molecular Biology Reports, 30*, 127–132.

Storch, A., Schneider, C. B., Wolz, M., Stürwald, Y., Nebe, A., Odin, P., Mahler, A., Fuchs, G., Jost, W. H., Chaudhuri, K. R., Koch, R., Reichmann, H., & Ebersbach, G. (2013). Nonmotor fluctuations in Parkinson disease: Severity and correlation with motor complications. *Neurology, 80*, 800–809.

Stover, N. P., & Watts, R. L. (2008). Spheramine for treatment of Parkinson's disease. *Neurotherapeutics, 5*, 252–259.

Strömberg, I., Bygdeman, M., Goldstein, M., Seiger, A., & Olson, L. (1986). Human fetal substantia nigra grafted to the dopamine-denervated striatum of immunosuppressed rats: Evidence for functional reinnervation. *Neuroscience Letters, 71*, 271–276.

Sumer, H., Liu, J., Malaver-Ortega, L. F., Lim, M. L., Khodadadi, K., & Verma, P. J. (2011). NANOG is a key factor for induction of pluripotency in bovine adult fibroblasts. *Journal of Animal Science, 89*, 2708–2716.

Sun, N., Longaker, M. T., & Wu, J. C. (2010). Human iPS cell-based therapy: Considerations before clinical applications. *Cell Cycle, 9*, 880–885.

Svendsen, C. N., Caldwell, M. A., Shen, J., ter Borg, M. G., Rosser, A. E., Tyers, P., Karmiol, S., & Dunnett, S. B. (1997). Long-term survival of human central nervous system progenitor cells transplanted into a rat model of Parkinson's disease. *Experimental Neurology, 148*, 135–146.

Tada, M., Takahama, Y., Abe, K., Nakatsuji, N., Tada, T. (2001). Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. *Curr Biol, 11*, 1553–1558.

Tai, M. H., Chang, C. C., Kiupel, M., Webster, J. D., Olson, L. K., & Trosko, J. E. (2005). Oct4 Expression in adult human stem cells: Evidence in support of the stem cell theory of carcinogenesis. *Carcinogenesis, 26*, 495–502.

Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell, 126*, 663–676.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell, 131*, 861–872.

Tay, Y., Zhang, J., Thomson, A. M., Lim, B., & Rigoutsos, I. (2008). MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature, 455*, 1124–1128.

Tesar, P. J., Chenoweth, J. G., Brook, F. A., Davies, T. J., Evans, E. P., Mack, D. L., Gardner, R. L., & Mckay, R. D. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature, 448*, 196–199.

Thomson, J., Itskovitz-Eldor, J., Shapiro, S., Waknitz, M., Swiergiel, J., Marshall, V., & Jones, J. (1998). Embryonic stem cell lines derived from human blastocysts. *Science, 282*, 1145–1147.

Toledo-Aral, J. J., Méndez-Ferrer, S., Pardal, R., Echevarría, M., & López-Barneo, J. (2003). Trophic restoration of the nigrostriatal dopaminergic pathway in long-term carotid body-grafted parkinsonian rats. *Journal of Neuroscience, 23*, 141–148.

Tomioka, M., Nishimoto, M., Miyagi, S., Katayanagi, T., Fukui, N., Niwa, H., Muramatsu, M., & Okuda, A. (2002). Identification of Sox-2 regulatory region which is under the control of Oct-3/4-Sox-2 complex. *Nucleic Acids Research, 30*, 3202–3213.

Torres, E. M., Monville, C., Lowenstein, P. R., Castro, M. G., & Dunnett, S. B. (2005). Delivery of sonic hedgehog or glial derived neurotrophic factor to dopamine-rich grafts in a rat model of Parkinson's disease using adenoviral vectors increased yield of dopamine cells is dependent on embryonic donor age. *Brain Research Bulletin, 68*, 31–41.

Twelves, D., Perkins, K. S., & Counsell, C. (2003). Systematic review of incidence studies of Parkinson's disease. *Movement Disorders, 18*, 19–31.

Vawda, R., & Fehlings, M. G. (2012). Mesenchymal cells in the treatment of spinal cord injury: Current & future perspectives. *Current Stem Cell Research & Therapy, 8*, 25–38.

Verma, R., Holland, M. K., Temple-Smith, P., & Verma, P. J. (2012). Inducing pluripotency in somatic cells from the snow leopard (Panthera uncia), an endangered felid. *Theriogenology, 77*, 220–228.

Vierbuchen, T., Ostermeier, A., Pang, Z. P., Kokubu, Y., Südhof, T. C., & Wernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. *Nature, 463*, 1035–1041.

Volpicelli, F., Consales, C., Caiazzo, M., Colucci-D'Amato, L., Perrone-Capano, C., & di Porzio, U. (2004). Enhancement of dopaminergic differentiation in proliferating midbrain neuroblasts by sonic hedgehog and ascorbic acid. *Neural Plasticity, 11*, 45–57.

Walker, E., Ohishi, M., Davey, R. E., Zhang, W., Cassar, P. A., Tanaka, T. S., Der, S. D., Morris, Q., Hughes, T. R., Zandstra, P. W., & Stanford, W. L. (2007). Prediction and testing of novel transcriptional networks regulating embryonic stem cell self-renewal and commitment. *Cell Stem Cell, 1*, 71–86.

Wang, X., Lu, Y., Zhang, H., Wang, K., He, Q., Wang, Y., Liu, X., Li, L., & Wang, X. (2004). Distinct efficacy of pre-differentiated versus intact fetal mesencephalon-derived human neural progenitor cells in alleviating rat model of Parkinson's disease. *International Journal of Developmental Neuroscience, 22*, 175–183.

Wang, J., Rao, S., Chu, J., Shen, X., Levasseur, D. N., Theunissen, T. W., & Orkin, S. H. (2006). A protein interaction network for pluripotency of embryonic stem cells. *Nature, 444*, 364–368.

Wang, J., Levasseur, D. N., & Orkin, S. H. (2008). Requirement of Nanog dimerization for stem cell self-renewal and pluripotency. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 6326–6331.

Wang, J., He, Q., Han, C., Gu, H., Jin, L., Li, Q., Mei, Y., & Wu, M. (2012). p53-facilitated miR-199a-3p regulates somatic cell reprogramming. *Stem Cells, 30*, 1405–1413.

Wang, Y., Yang, J., Li, H., Wang, X., Zhu, L., Fan, M., & Wang, X. (2013). Hypoxia promotes dopaminergic differentiation of mesenchymal stem cells and shows benefits for transplantation in a rat model of Parkinson's disease. *PLoS One, 8*, e54296.

Warren, L., Manos, P. D., Ahfeldt, T., Loh, Y. H., Li, H., Lau, F., Ebina, W., Mandal, P. K., Smith, Z. D., Meissner, A., Daley, G. Q., Brack, A. S., Collins, J. J., Cowan, C., Schlaeger, T. M., & Rossi, D. J. (2010). Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell, 7*, 618–630.

Watmuff, B., Pouton, C. W., & Haynes, J. M. (2012). In vitro maturation of dopaminergic neurons derived from mouse embryonic stem cells: Implications for transplantation. *PLoS One, 7*, e31999.

Watt, F. M., & Driskell, R. R. (2010). The therapeutic potential of stem cells. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 365*, 155–163.

Wenning, G. K., Odin, P., Morrish, P., Rehncrona, S., Widner, H., Brundin, P., Rothwell, J. C., Brown, R., Gustavii, B., Hagell, P., Jahanshahi, M., Sawle, G., Björklund, A., Brooks, D. J., Marsden, C. D., Quinn, N. P., & Lindvall, O. (1997). Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. *Annals of Neurology, 42*, 95–107.

Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., Bernstein, B. E., & Jaenisch, R. (2007). In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature, 448*, 318–324.

Wernig, M., Zhao, J. P., Pruszak, J., Hedlund, E., Fu, D., Soldner, F., Broccoli, V., Constantine-Paton, M., Isacson, O., & Jaenisch, R. (2008). Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 5856–5861.

Whone, A. L., Kemp, K., Sun, M., Wilkins, A., & Scolding, N. J. (2012). Human bone marrow mesenchymal stem cells protect catecholaminergic and serotonergic neuronal perikarya and transporter function from oxidative stress by the secretion of glial-derived neurotrophic factor. *Brain Research, 1431*, 86–96.

Wilkins, A., Kemp, K., Ginty, M., Hares, K., Mallam, E., & Scolding, N. (2009). Human bone marrow-derived mesenchymal stem cells secrete brain-derived neurotrophic factor which promotes neuronal survival in vitro. *Stem Cell Research, 3*, 63–70.

Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J., & Campbell, K. H. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature, 385*, 810–813.

Włodarski, K. H., Włodarski, P., Galus, R., & Mazur, S. (2012). Adipose mesenchymal stem cells. Their characteristics and potential application in tissue repair. *Polish Orthopedics & Traumatology, 77*, 97–99.

Woltjen, K., Michael, I. P., Mohseni, P., Desai, R., Mileikovsky, M., Hämäläinen, R., Cowling, R., Wang, W., Liu, P., Gertsenstein, M., Kaji, K., Sung, H. K., & Nagy, A. (2009). PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature, 458*, 766–770.

Wright Willis, A., Evanoff, B. A., Lian, M., Criswell, S. R., & Racette, B. A. (2010). Geographic and ethnic variation in Parkinson disease: A population-based study of US medicare beneficiaries. *Neuroepidemiology, 34*, 143–151.

Yan, Y., Yang, D., Zarnowska, E. D., Du, Z., Werbel, B., Valliere, C., Pearce, R. A., Thomson, J. A., & Zhang, S. C. (2005). Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. *Stem Cells, 23*, 781–790.

Yang, X. X., Xue, S. R., Dong, W. L., & Kong, Y. (2009). Therapeutic effect of human amniotic epithelial cell transplantation into the lateral ventricle of hemiparkinsonian rats. *Chinese Medical Journal, 122*, 2449–2454.

Yang, X., Hou, J., Han, Z., Wang, Y., Hao, C., Wei, L., & Shi, Y. (2013). One cell, multiple roles: Contribution of mesenchymal stem cells to tumor development in tumor microenvironment. *Cell & Bioscience, 3*, 5, Epub ahead of print.

Yong, V. W., Guttman, M., Kim, S. U., Calne, D. B., Turnbull, I., Watabe, K., & Tomlinson, R. W. (1989). Transplantation of human sympathetic neurons and adrenal chromaffin cells into parkinsonian monkeys: No reversal of clinical symptoms. *Journal of Neurological Sciences, 94*, 51–67.

Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R., Slukvin, I. I., & Thomson, J. A. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science, 318*, 1917–1920.

Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, I. I., & Thomson, J. A. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. *Science, 324*, 797–801.

Yuan, H., Corbi, N., Basilico, C., & Dailey, L. (1995). Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. *Genes & Development, 9*, 2635–2645.

Yurek, D. M., & Fletcher-Turner, A. (2004). Comparison of embryonic stem cell-derived dopamine neuron grafts and fetal ventral mesencephalic tissue grafts: Morphology and function. *Cell Transplantation, 3*, 295–306.

Zangrossi, S., Marabese, M., Broggini, M., Giordano, R., D'Erasmo, M., Montelatici, E., Intini, D., Neri, A., Pesce, M., Rebulla, P., & Lazzari, L. (2007). Oct-4 expression in adult human differentiated cells challenges its role as a pure stem cell marker. *Stem Cells, 25*, 1675–1680.

Zeng, X., Cai, J., Chen, J., Luo, Y., You, Z. B., Fotter, E., Wang, Y., Harvey, B., Miura, T., Backman, C., Chen, G. J., Rao, M. S., & Freed, W. J. (2004). Dopaminergic differentiation of human embryonic stem cells. *Stem Cells, 22*, 925–940.

Zhang, X. Q., & Zhang, S. C. (2010). Differentiation of neural precursors and dopaminergic neurons from human embryonic stem cells. *Methods in Molecular Biology, 584*, 355–366.

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell, 132*, 645–660.

Zhou, W., & Freed, C. R. (2009). Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. *Stem Cells, 27*, 2667–2674.

Zhou, H., Wu, S., Joo, J. Y., Zhu, S., Han, D. W., Lin, T., Trauger, S., Bien, G., Yao, S., Zhu, Y., Siuzdak, G., Schöler, H. R., Duan, L., & Ding, S. (2009). Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell, 4*, 381–384.

Zhu, Q., Ma, J., Yu, L., & Yuan, C. (2009). Grafted neural stem cells migrate to substantia nigra and improve behavior in Parkinsonian rats. *Neuroscience Letters, 462*, 213–218.

Zou, Z., Jiang, X., Zhang, W., Zhou, Y., Ke, Y., Zhang, S., & Xu, R. (2010). Efficacy of Tyrosine Hydroxylase gene modified neural stem cells derived from bone marrow on Parkinson's disease a rat model study. *Brain Research, 1346*, 279–286.

Alpha Synuclein in Parkinson's Disease

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Contents

Abstract

The perception of Parkinson's disease (PD) as a disease centered on dopaminergic striatonigral neurodegeneration has changed fundamentally since 1997 when the first mutation in the SNCA gene (PARK1) encoding α-synuclein was discovered (Polymeropoulos et al. 1997). This discovery formed the basis for a new description of brain pathology characterized by the presence of α-synuclein

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aggregates in brain cell inclusions that are the hallmarks of PD and other synucleinopathies: dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). This field has been thoroughly covered by many reviews during the last decade (Gai et al. 1998; Spillantini and Goedert 2000; Huang et al. 2004; Ubhi et al. 2011). This review will briefly highlight the historical breakthroughs but focus on α-synuclein modifications, human neuropathology, biomarker potential, current animal models and the new concepts emerging after the significance of extracellular α-synuclein has gained support.

Keywords
Animal models • Biomarkers • Dementia with Lewy bodies • Lewy bodies • Multiple systems atrophy • Parkinson disease • Phosphorylation • Truncation • α-synuclein

1 Introduction

The perception of Parkinson's disease (PD) as a disease centered on dopaminergic striatonigral neurodegeneration has changed fundamentally since 1997 when the first mutation in the SNCA gene (PARK1) encoding α-synuclein was discovered (Polymeropoulos et al. 1997). This discovery formed the basis for a new description of brain pathology characterized by the presence of α-synuclein aggregates in brain cell inclusions that are the hallmarks of PD and other synucleinopathies: dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). This field has been thoroughly covered by many reviews during the last decade (Gai et al. 1998; Spillantini and Goedert 2000; Huang et al. 2004; Ubhi et al. 2011). This review will briefly highlight the historical breakthroughs but focus on recent developments in concepts and hypotheses.

2 Genetics

Missense mutations in the SNCA gene substituting single amino acids (A30P, E46K, and A53T) cause early-onset autosomal dominant PD and DLB (Polymeropoulos et al. 1997; Kruger et al. 1998; Zarranz et al. 2004). Additionally, genomic multiplications of the SNCA gene have been found in patients with early-onset PD and DLB (Singleton et al. 2003) demonstrating that simple overexpression and perhaps developmentally dysregulated expression of the normal protein is sufficient to initiate disease development. The discovery of these rare cases was essential because they causally tie α-synuclein to the development of a PD syndrome, leading to the widely accepted hypothesis that a gain of function in α-synuclein contributes to the pathogenesis of PD. A possible role for α-synuclein in sporadic PD is corroborated by a series of genome-wide association studies carried out on ethnically diverse populations of PD patients. The single-nucleotide

polymorphisms conferring the strongest risk are in the SNCA gene (Simon-Sanchez et al. 2009). Although SNCA is the strongest risk gene, polymorphisms within this gene only increase the risk 2–3 times above the low prevalence of about 0.14 % but serve to corroborate a mechanistic link between the development of sporadic PD and α-synuclein.

3 Structure of α-Synuclein

α-Synuclein is a small 140-amino acid protein, which was first identified as associated with purified synaptic vesicles from the electric ray *Torpedo californica* (Maroteaux et al. 1988). It is one of three synuclein family members (α, β, and γ) identified in humans. The α-synuclein protein can be divided into two regions: (i) the N-terminal region (residues 1–95), which contains seven imperfect repeats with a highly conserved hexameric motif (KTKEGV) and the aggregation-prone non-amyloid component (NAC) sequence (residues 60–95), and (ii) the unstructured C-terminal region (96–140), which is rich in acidic residues (Ueda et al. 1993; George et al. 1995).

The heat-stable nature of human α-synuclein was already described in 1994 (Jakes et al. 1994), and it was ascribed to its natively unfolded structure that possesses an ability to form α-helical structures in apolar solvents (Weinreb et al. 1996) and upon interactions with acidic phospholipid membranes (Clayton and George 1998). When analyzed by gel filtration, the unfolded structure caused the 14.4 kDa α-synuclein peptide to elute corresponding to an approximately 55 kDa globular protein. Moreover, certain dimers appearing on denaturing SDS-polyacrylamide gels were due to misincorporation of a cysteine residue in some preparations of bacterially expressed human α-synuclein (Masuda et al. 2006). Despite its unfolded nature, purified α-synuclein has been demonstrated to exhibit dynamic long-range interactions between the C-terminus and the central NAC domain and even further toward the N-terminus (Cho et al. 2009). Moreover, it undergoes a dramatic structural change upon binding to acidic liposomes where the N-terminal repeat regions fold into two α-helices (Bussell and Eliezer 2003; Chandra et al. 2003).

α-Synuclein aggregates into amyloid fibrils by a nucleation-dependent mechanism and the disease-causing mutations as well as C-terminal truncations favor the consumption of monomers into aggregates. The transformation from a monomeric state to an amyloid state proceeds through soluble oligomeric intermediates (Wood et al. 1999). Several lines of evidence suggest that the oligomers are the most toxic species. First, the disease-causing A30P mutation accelerates the initial oligomerization of α-synuclein in vitro, whereas it retards the formation of mature filamentous aggregates (Conway et al. 2000). Second, α-synuclein oligomers bind synthetic vesicles and cause transient permeabilization representing a potential mechanism of neurotoxicity (Lashuel et al. 2002). This was corroborated when oligomeric forms of α-synuclein applied extracellularly were shown to trigger

calcium entry and toxicity in a neuronal cell line (Danzer et al. 2007). Third, cytotoxicity in cellular models is usually seen without heavily aggregated α-synuclein (Xu et al. 2002; Outeiro et al. 2008; Tetzlaff et al. 2008; Kragh et al. 2009). The protofibrils formed in vitro from purified α-synuclein represent a diverse group generated from simple aggregation of monomers in buffer to induction by various agents and conditions, e.g., apolar solvents, lyophilization, and oxidative cross-linking by, e.g., metals and oxidation products from lipid peroxidation (Sharon et al. 2001; Nasstrom et al. 2011). Immunological characterization of α-synuclein oligomers has been feasible using two different antibodies: the pan-oligomeric A11 antibody recognizing soluble precursors of many types of amyloid-type fibrils (Kayed et al. 2007) and the FILA-1 antibody recognizing α-synuclein-specific epitopes shared between filaments and soluble oligomers. The FILA-1 antibody recognizes elevated levels of soluble α-synuclein aggregates in DLB brain extracts (Paleologou et al. 2009) indicating commonalities between in vitro-formed oligomers and those present in vivo. At present, the structural relation between in vitro-formed α-synuclein protofibrils and those found in cells and brain tissue is unclear and structure-specific imaging agents are in high demand.

Two recent reports challenge the dogma of α-synuclein as a natively unfolded protein and show evidence supporting native tetramers rich in α-helical structure. Both reports indicate that α-synuclein from red blood cells and recombinantly expressed in *Escherichia coli* with a short N-terminal extension can be purified as folded tetramers that irreversibly dissociate into monomers (Bartels et al. 2011; Wang et al. 2011). Interestingly, the tetramers are unable to aggregate and amyloid-type aggregation is only possible after dissociation into monomers. Importantly, the PD-causing missense mutations destabilize the tetramers and thus favor aggregation. These findings are controversial but potentially important as they hypothesize that a stabilization of the native tetrameric state could be a fruitful therapeutic strategy.

4 Posttranslational Modifications

α-Synuclein is subject to different posttranslational modifications. It can be phosphorylated at Ser87, Ser129, and Tyr125 (Ellis et al. 2001; Anderson et al. 2006; Paleologou et al. 2010) in human brain tissue. Though predominantly found in a non-phosphorylated state in vivo, α-synuclein is phosphorylated at Ser129 in inclusions in all α-synucleinopathies (Fujiwara et al. 2002; Anderson et al. 2006). Both Ser129-phosphorylation and ubiquitination are highly enriched in α-synuclein aggregates in diseased brain, whereas other less abundant modifications occur in both soluble and insoluble fractions.

The role of Ser129-phosphorylation in promoting toxic α-synuclein aggregation is unclear as in vitro phosphorylation of this residue has been demonstrated to prevent amyloid-type insoluble aggregation (Fujiwara et al. 2002;

Paleologou et al. 2008). α-Synuclein phosphorylation at Ser129 accelerates inclusion formation and/or toxicity in cell culture models (Smith et al. 2005b; Sugeno et al. 2008; Kragh et al. 2009) and correlates with pathology in transgenic *Drosophila* (Chen and Feany 2005). Studies in rats expressing the phosphomimic form of α-synuclein (S129D) using adeno-associated virus (AAV) have yielded conflicting results (see section on "Animal Models"). However, S129D may not be a suitable phosphomimicking variant of α-synuclein as it was demonstrated that S129D does not reproduce the effects of phosphorylation on structural and aggregation properties of α-synuclein in vitro (Paleologou et al. 2008). Mechanistically, Ser129-P may contribute functionally by conferring novel ligand-binding properties as demonstrated using a phosphopeptide co-immunoprecipitation approach (McFarland et al. 2008). Phosphorylation of tyrosines also occurs in α-synuclein (Ellis et al. 2001) and Tyr125-phosphorylation decreases with age in human brain tissue (Chen et al. 2009). Deletion of the tyrosine residues in α-synuclein increased toxicity in a *Drosophila* model (Chen et al. 2009) indicating a protective role for tyrosine phosphorylation that may be lost with aging. To fully understand the consequence of α-synuclein phosphorylation, it is important to identify the involved kinase(s). Casein kinase II, GRK2/5, and polo-like kinases 2/3 have been shown to phosphorylate α-synuclein in vitro, in cell culture, *Drosophila*, and mice (Pronin et al. 2000; Chen and Feany 2005; Arawaka et al. 2006; Inglis et al. 2009; Mbefo et al. 2010) (for a review see Oueslati et al. 2010).

Proteolytic C-terminal truncations after residues 115, 119, 122, 133, and 135 have been demonstrated in human brain tissue (Anderson et al. 2006), and such modifications increase α-synuclein aggregation in vitro (Crowther et al. 1998). Interestingly, α-synuclein is a substrate for cytoplasmic calpains, and cleaved α-synuclein is found in PD and DLB brain (Mishizen-Eberz et al. 2005; Kim et al. 2006; Dufty et al. 2007), suggesting that calpain may generate truncated toxic species of α-synuclein.

The early focus on dopaminergic neurons in substantia nigra in PD prior to the description of the Braak hypothesis and the use of L-dopa for therapy forms the basis for a large series of experiments on dopamine effects on α-synuclein and vice versa (for a review Leong et al. 2009). Key findings are that dopamine stimulates the formation of soluble off-pathway α-synuclein oligomers in vitro that do not aggregate into fibrils (Conway et al. 2001) and the dopamine oxidation product dopaminochrome promotes the formation of spherical oligomers (Norris et al. 2005). These observations were corroborated in cellular experiments where the dopamine level was modulated by expression of tyrosine hydroxylase (TH) forms (Mazzulli et al. 2006). In addition, α-synuclein has been related to different key proteins of the dopaminergic system. α-Synuclein influences dopamine synthesis by decreasing TH and/or aromatic amino acid decarboxylase activity via changes in the phosphorylation status of these two enzymes (Perez et al. 2002; Tehranian et al. 2006; Lou et al. 2010). Mutated α-synuclein A53T leads to low levels of VMAT2 and consequently to accumulation of dopamine in neurons (Lotharius et al. 2002). α-Synuclein levels have also been implicated in regulating the activity of the

dopamine transporter (DAT), although controversies exist. Hence, direct binding between transgenic hα-synuclein and DAT in cells enhanced dopamine uptake and dopamine-dependent cell death (Lee et al. 2001). However, a later study coexpressing the two proteins identified a negative effect on dopamine uptake (Wersinger and Sidhu 2003). Finally, using the opposite approach by silencing endogenously expressed α-synuclein showed a decreased sensitivity to the DAT-dependent toxin MPP+ and decreased dopamine uptake (Fountaine and Wade-Martins 2007). Hence, the definitive role of dopamine-dependent α-synuclein modifications in vivo and the effect of α-synuclein levels and modifications on dopamine metabolism are still enigmatic.

5 Cellular Metabolism

α-Synuclein is synthesized as a cytosolic protein but it can exist in various subcompartments, e.g., membrane associated or encapsulated with various post-translational modifications. Natively unmodified α-synuclein is readily degraded by the 20S proteasome (Liu et al. 2003) and by chaperone-mediated autophagy in lysosomes in vitro (Cuervo et al. 2004). How α-synuclein is degraded in the brain is less clear, and studies using cell lines and primary neuronal cultures have yielded different results (reviewed in (Xilouri and Stefanis 2011)). Neurons in culture appear to degrade the bulk of α-synuclein by chaperone-mediated autophagy and macroautophagy (Vogiatzi et al. 2008). A recent study using transgenic mice expressing human α-synuclein or α-synuclein-eGFP analyzed the levels of α-synuclein after topical application of inhibitors of the 20S proteasome and the autophagy-lysosome pathway (Ebrahimi-Fakhari et al. 2011). Using cranial window access to the brain surface combined with live imaging and tissue extraction, it was elegantly demonstrated that the basal α-synuclein turnover is mediated by the proteasome system and this process appears even more important with aging. Upon stress mediated by increased α-synuclein expression or compromised proteasomal activity, the autophagy-lysosome pathway is activated (Ebrahimi-Fakhari et al. 2011). The importance of lysosomal dysfunction in relation to Lewy body (LB) formation has been corroborated by the presence of LB pathology in Gaucher disease that is caused by dysfunctional lysosomal hydrolases (Vitner et al. 2010). Exocytosis of vesicle-bound α-synuclein was demonstrated in 2005 (Lee et al. 2005), and direct neuron-neuron transfer of α-synuclein was later confirmed using cell transplantation (Desplats et al. 2009). This formed the important experimental frame for testing whether α-synuclein by a prion-like mechanism causes the neurodegenerative progression described by the Braak hypothesis (Braak et al. 2004) ranging from development of LBs in fetal human dopaminergic neurons transplanted into PD patients (Kordower et al. 2008; Li et al. 2008) to Lewy-like pathology ascending to the brainstem after oral toxin-based generation of α-synuclein pathology in the gut (Pan-Montojo et al. 2010) and to neuronal uptake of preformed α-synuclein aggregates in vitro (Volpicelli-Daley et al. 2011).

6 Normal Functions

The normal function of α-synuclein has been difficult to pinpoint although its effective axonal transport (Jensen et al. 1999) and selective localization in nerve terminals (Maroteaux et al. 1988) suggest a function in this structure. Although it is expressed at high levels in the brain, it is also found in other tissues, e.g., hematopoietic cells (Miller et al. 2004). Reduction of α-synuclein in cultured neurons reduced the distal pool of synaptic vesicles (Murphy et al. 2000), and knockout mice corroborated a presynaptic function as evidenced by reduced striatal dopamine content and an increased neurotransmitter release upon paired stimuli (Abeliovich et al. 2000). However, the phenotype was surprisingly mild with the most striking feature being a resistance to the toxin MPTP (Dauer et al. 2002). Although knockout of α-synuclein alone or α-synuclein and β-synuclein together has minimal effects on neuronal function (Chandra et al. 2004), triple knockout mice lacking all synuclein members demonstrate a robust phenotype with gross reduction in presynaptic size and premature death (Greten-Harrison et al. 2010). These mice display deficits in assembly of SNARE complexes that are required for fusion of synaptic vesicles to the plasma membrane. Interestingly, it was recently shown that the synucleins act as chaperones for presynaptic SNARE proteins (Burre et al. 2010).

Transgenic expression of α-synuclein has been considered a rational approach for most studies of pathological α-synuclein effects because of the dominant inheritance of PD and DLB associated to mutations in the SNCA gene. Hence, wild-type and mutated human α-synuclein has been expressed in a range of cell lines, primary brain cells, yeast cells, and organisms ranging from *Caenorhabditis elegans* and *Drosophila* to mice, rats, and monkeys with variable results. Initially the criteria for success were generation of a LB-like structure and this frustrated many researchers. End points have later changed toward neuronal degeneration and cell death because it was realized that LBs may take months to years to develop in human neurons.

7 Pathogenic Functions

Mechanisms by which abnormal accumulation of α-synuclein disrupts cellular functions have been investigated intensively in several different model systems. Yeast studies have demonstrated that α-synuclein accumulation leads to a blockade of vesicular transport from the endoplasmatic reticulum to the Golgi apparatus and hereby inhibits the viability of the cells (Cooper et al. 2006). Moreover, activation of an unfolded protein response has been demonstrated in SHSY5Y cells overexpressing α-synuclein (Sugeno et al. 2008) and in postmortem brain tissue from PD patients (Hoozemans et al. 2007). However, it became clear in the yeast study that the toxic response was dependent on the α-synuclein dose as revealed by comparing high and low expressers (Su et al. 2010). A different approach has been

taken in mammalian cells that tolerate expression of α-synuclein but where cytotoxicity was induced by coexpressing proteins known to stimulate α-synuclein aggregation like p25α and synphilin-1 (Engelender et al. 1999; Kragh et al. 2009). Using this approach it was demonstrated that p25α induced cellular degeneration that could be attenuated by inhibitors of α-synuclein aggregation as well as of Ser129-phosphorylation (Kragh et al. 2009). The approach of including inhibitors of α-synuclein aggregation may be readily applicable to other models and thereby allow phenotypes caused by mere overexpression of α-synuclein to be more clearly distinguished from those forming aggregations.

It has previously been difficult to identify soluble α-synuclein aggregates in cellular models. Nevertheless, they have been detected by Western blotting in mesencephalic neurons exposed to polyunsaturated fatty acids (Sharon et al. 2003) and in a dopaminergic cell line overexpressing A53T α-synuclein (Zhou and Freed 2005). Moreover, a fluorescence lifetime imaging-based technique to probe the organization of α-synuclein within the cell has demonstrated the formation of small oligomers (Klucken et al. 2006). Toxic effects have also been demonstrated by applying α-synuclein from culture media to cells, but the toxic pathways elicited by internal versus external α-synuclein are still unclear. An elegant approach was used to test whether mature aggregates or insoluble protofibrils are responsible for toxic effects caused by overexpression of α-synuclein. In this study, α-synuclein mutants designed to favor generation of soluble protofibrils exhibited increased toxic potential compared to wild-type α-synuclein in cell and animal models (Karpinar et al. 2009).

Several lines of evidence implicate a dysfunction of the ubiquitin-proteasome system in the pathogenesis of α-synucleinopathies. Studies show that LBs contain ubiquitinated proteins (Chung et al. 2001; McNaught and Jenner 2001) and that the proteasomal activity is decreased in the substantia nigra of PD patients (McNaught and Jenner 2001; McNaught et al. 2003). Moreover, aggregated α-synuclein has been demonstrated to inhibit the activity of the proteasome in vitro (Snyder et al. 2003; Lindersson et al. 2004; Emmanouilidou et al. 2010), and this may have significant effects on proteasomal-dependent degradation of short-lived proteins.

Mitochondrial dysfunction as a consequence of α-synuclein accumulation has also been a major area of interest. Abnormalities of mitochondrial function and increased free radical-mediated damage were described in PD brain before the first PD-associated gene mutations were discovered. α-Synuclein can localize to mitochondria (Hsu et al. 2000; Tanaka et al. 2001; Smith et al. 2005a) and α-synuclein expression increases cellular sensitivity to rotenone, a mitochondrial complex I inhibitor (Orth et al. 2003; Ved et al. 2005). α-Synuclein has also been shown to reduce ATP synthesis and mitochondrial membrane potential (Kamp et al. 2010). Moreover, structural abnormalities of mitochondria have been observed in transgenic mice overexpressing mutant α-synuclein (Martin et al. 2006).

The existence of cellular α-synuclein excretion and uptake by neighboring neurons as previously described may form a path for spreading of the disease process in the nervous system. The potential of such mechanisms has been corroborated by the dramatic neuronal spread of α-synuclein pathology in transgenic mice

expressing human A53T mutant α-synuclein (Luk et al. 2012). Unilateral injection of homogenates from old symptomatic M83 mice into the brains of young mice caused a rapid propagation of aggregated and hyperphosphorylated α-synuclein ranging from the olfactory bulb to the spinal cord that also traversed the corpus callosum to the contralateral hemisphere. The propagation required the recruitment of endogenous mouse α-synuclein. Surprisingly, similar pathology was induced upon injection of in vitro-formed insoluble aggregates of a fusion protein consisting of C-terminally truncated human α-synuclein (1–120) and a Myc epitope tag that lacked the S129 phosphorylation site. Hence, insoluble α-synuclein aggregates may possess "prion-like" seeding activity (Luk et al. 2012).

8 Human Pathology

PD is diagnosed by two separate abnormalities in the absence of sufficient neuropathology for an alternative neurodegenerative disorder (Dickson et al. 2009). A moderate to substantial loss of the dopaminergic neurons in the substantia nigra is required, a finding that also occurs in the majority of movement disorders, and the presence of hallmark neuronal LBs in brainstem predilection sites is also required (Fig. 1a). LBs are also the hallmark pathology of DLB with the two conditions separated mainly on clinical rather than pathological grounds, particularly at end stage (McKeith et al. 2005). PD patients have a dominant slowly progressive movement disorder with cognitive impairment often intervening very late in the disease when LBs infiltrate into the cortex (Dickson et al. 2009). DLB patients have a dominant more rapidly progressive dementia disorder and have cortical LBs often with coexisting Alzheimer's disease (Halliday et al. 2011a). Although α-synuclein forms abnormal aggregates in all synucleinopathies, neuronal LBs only occur in PD and DLB with α-synuclein inclusions in alternate cell types (oligodendroglia) or cellular locations (axonal spheroids) in other synucleinopathies (Halliday et al. 2011a). The reason for the different locations and mechanisms of neurodegeneration involving α-synuclein remain largely unexplored.

In addition to PD and DLB, LBs occur in a small proportion of asymptomatic elderly, a finding which has allowed the development of a hypothesized pathological staging scheme for PD (Braak et al. 2003) and one for both PD and DLB (Beach et al. 2009). Braak hypothesis for the progression of pathology in PD states that the pathology starts peripherally in the olfactory and autonomic nervous systems and progresses through connected regions into the spinal cord and medulla oblongata (stage 1), progressing over time to the pons (stage 2) and midbrain (stage 3), to the basal ganglia (stage 4) and limbic systems (stage 5), and finally to association cortices (stage 6), largely sparing the somatosensory and motor system (Braak et al. 2003) (Fig. 1b). The alternate hypothesis for the progression of LB pathology across PD and DLB by Beach and colleagues (Beach et al. 2009) states that pathology begins in the olfactory bulb (stage 1) and then progresses in two different directions depending on clinical phenotype – for the motor phenotype (PD) LBs

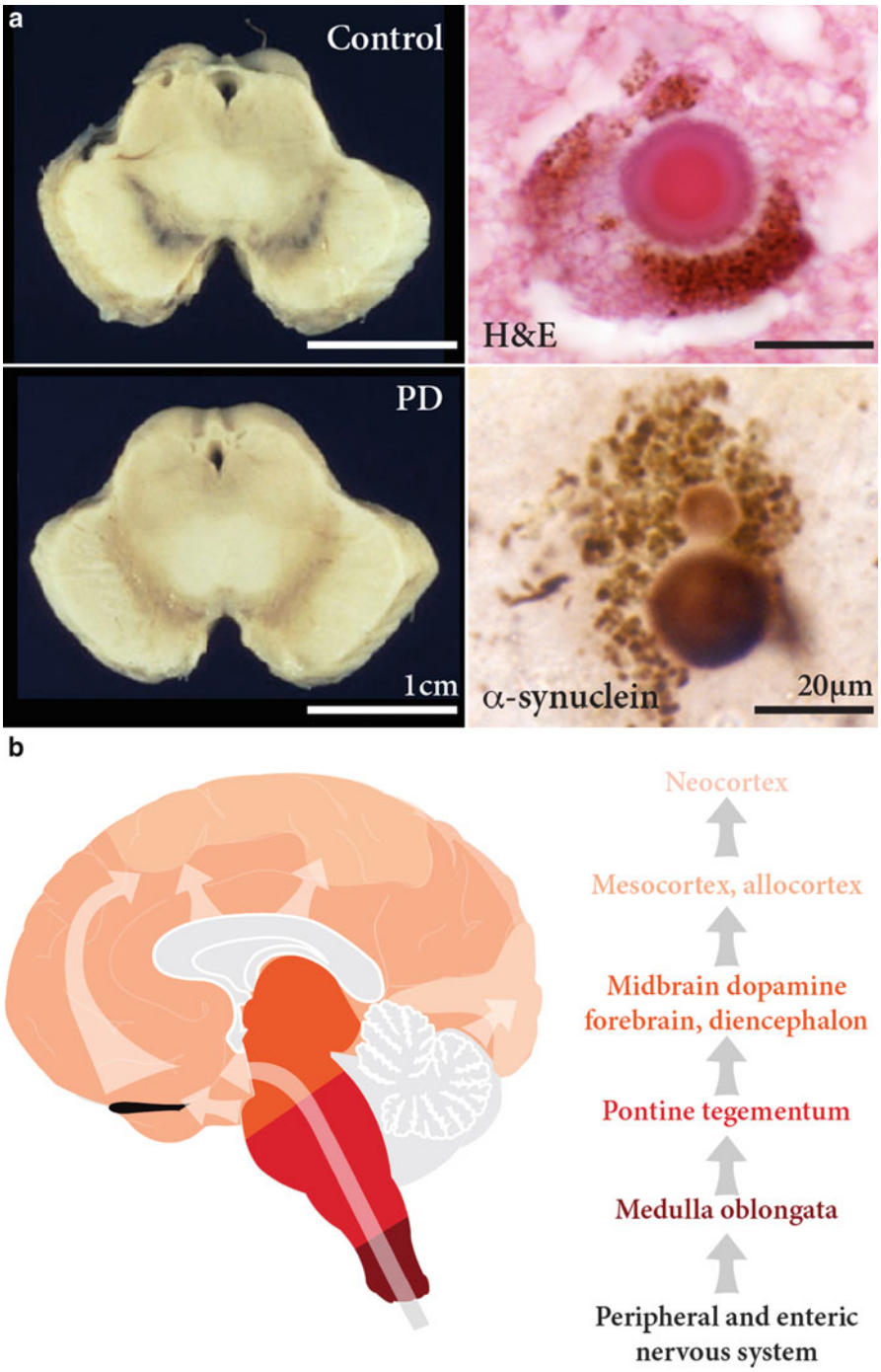

Fig. 1 (continued)

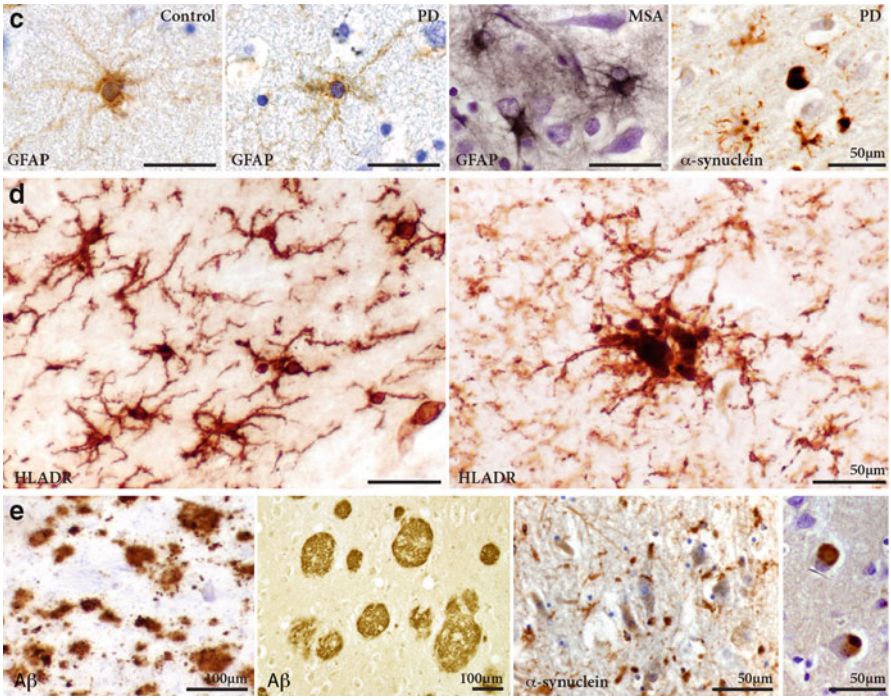

Fig. 1 Neuropathology found in patients with α-synucleinopathies. (**a**) The two diagnostic pathologies required for PD are the loss of pigmented dopaminergic neurons in the midbrain substantia nigra (shown macroscopically at *left*) and LB formation in remaining brainstem pigmented neurons (shown at *right*). Macroscopic photos of transverse sections through the midbrain from a control and a patient with PD demonstrate the loss of the dark neuromelanin pigment in the ventral midbrain. LBs within remaining pigmented neurons in patients with PD can be visualized as large round intracellular inclusions observed with hematoxylin and eosin (H&E) or α-synuclein immunohistochemistry. (**b**) Diagrammatic representation of the Braak staging scheme for the progression of PD from the olfactory and peripheral nervous systems to invade the brain in a slow hierarchical fashion from the lower brainstem to the upper brainstem and forebrain to the cortex. (**c**) Photomicrographs of the typical astrocytic changes that occur in PD versus MSA. While reactive astrogliosis is typical in MSA (observed as an increase in GFAP and hypertrophy of their cell bodies compared to controls), this reaction is not typically observed in end-stage cases of PD. Rather, in PD a large number of astrocytes abnormally accumulate α-synuclein throughout their cytoplasm, a finding associated with the formation of the large round intracytoplasmic LBs in nearby neurons. (**d**) Photomicrographs of the microglial changes observed in PD. Amoebic, phagocytic microglia aggregate only in regions with neuronal loss like substantia nigra (*right*), while in areas abnormally accumulating α-synuclein, the microglia display more HLA-DR reactivity in association with an increased branching morphology (*left*). (**e**) Pathologies associated with each other in patients with the more rapidly progressing DLB and later onset PD with early dementia. The density of amyloid plaques in both the striatum (*left*) and cortex (*middle left*) is related to each other and to the severity of dementia in these patients. Lewy neurites in the hippocampus (*middle right*) are also related to the severity of plaque and LB pathology (at *right*) in such cases

infiltrate the brainstem (stage 2a) and for the dementia phenotype (DLB) LBs infiltrate the limbic system (stage 2b). Finally, for both phenotypes, LBs are found in both the brainstem and limbic system (stage 3) prior to infiltrating cortical regions (stage 4) (Beach et al. 2009).

The Braak staging of PD pathology has been controversial due to discrepancies in a number of validation studies and also due to some conceptual difficulties, while the alternate staging scheme has not been independently evaluated. However, it should be noted that the majority of longitudinally followed cases with motor-dominant PD fit the Braak staging [see review (Halliday and McCann 2010)]. The most obvious difficulty with the Braak staging scheme is using asymptomatic cases with LBs to only stage the progression of PD and not DLB. The more restricted number of stages in the alternate hypothesis incorporating DLB makes this staging scheme of limited current utility. It should be noted that in ~10 % of asymptomatic elderly, LBs occur only in cortical regions (Zaccai et al. 2008). Additionally, the clinical progression of PD relates to the severity of dopaminergic cell loss (Greffard et al. 2006). This may not be surprising if LBs mark neurons destined to die with all LB debris removed following cell death. However, if this was the case, then as the disease progresses, there should be a reduction of LB pathology as cell death is enhanced and affects more regions, rather than the concept of pathology progressing to accumulate in more and more neurons and cells still viable in the brain. Empirical data shows that even in regions thought to be heavily affected with pathology, only 4–5 % of neurons contain LBs in PD (Harding et al. 2002; Greffard et al. 2010). Furthermore, there is no widespread brain tissue loss in PD (Weintraub et al. 2011), and the pattern and severity of cell loss does not reflect the demonstrated pattern and severity of LB formation. Definitive proof of the progression of LB pathology will only occur with the development of biomarkers capable of identifying this pathology.

In addition to the cell loss and LB pathology required for diagnosis, glial changes are obvious in PD (Halliday and Stevens 2011). Astrocytes in PD disengage from neurons leaving the neuronal membranes more exposed (Knott et al. 1999). They undergo PD-specific changes in that they accumulate non-fibrillar α-synuclein and lack the typical reactive astrogliosis that usually accompanies neurodegeneration (Song et al. 2009) (Fig. 1c). In particular, there is often no obvious hypertrophy of astrocytes or upregulation of their glial fibrillary acidic protein (GFAP) or any substantial proliferation, a finding that differs significantly from reactive astrogliosis observed in MSA (Song et al. 2009) (Fig. 1c). This may suggest that any reactive trigger resolves or is removed over time in PD, possibly by cellular uptake. In contrast, the astrocytes in patients with MSA do not accumulate α-synuclein but have the typical reactivity observed in chronic neurodegenerative conditions with obvious cell hypertrophy and upregulation of GFAP (Song et al. 2009) (Fig. 1c), changes that are associated with long-lasting reorganization of tissue architecture. The accumulation of α-synuclein in astrocytes in PD is related to the severity of LB infiltration and synaptic degeneration (Braak et al. 2007),

affecting more astrocytes (~45 %; (Song et al. 2009)) compared with neurons (~4–5 %; Harding et al. 2002; Greffard et al. 2010).

α-Synuclein in astrocytes causes an upregulation of inflammatory mediators (Klegeris et al. 2006; Lee et al. 2010) as well as neuroprotective factors (Power et al. 2002; Ishida et al. 2006; Durrenberger et al. 2009; Michael et al. 2011). The increase in α-synuclein deposition also correlates with an increase in activation of microglia (Croisier et al. 2005), and with the removal of astroglial processes from neurons, the vacated perineuronal space becomes occupied by amoeboid phagocytic microglia (Knott et al. 1999) (Fig. 1d). While activated microglia are well described as a feature of PD (see review Hirsch et al. 2012), phagocytic microglia are only found in regions of cell loss and express scavenger and high-affinity IgG receptor Fcγ (Orr et al. 2005) as well as macrophage markers (Croisier et al. 2005). These data suggest that the relatively large number of astrocytes affected by α-synuclein plays a significant role in the disease process.

PD is one of the age-associated neurodegenerative conditions and occurs often in association with other age-related neuropathologies (Compta et al. 2011). In particular, the pathology most often associated with age-related dementia is amyloid plaque accumulation (Matthews et al. 2009), which also contributes to dementia in older cases of PD (Compta et al. 2011) (Fig. 1e). It should be noted that patients with dementia and sufficient neurofibrillary tangle pathology for a diagnosis of Alzheimer's disease are not considered to have a LB syndrome that contributes to their dementia, due to their substantially greater Alzheimer-type pathology (Dickson et al. 2009). However, amyloid plaque pathology is known to occur in nearly all patients with DLB, being much less frequent in younger PD patients, even those with dementia (Halliday et al. 2011b). This confirms in vivo PiB imaging studies in patients with PD (Gomperts et al. 2008) and suggests that, in such typical patients, the α-synuclein cortical LB pathology is sufficient for their late end-stage dementia. Therefore, there is considerable clinical heterogeneity in patients with LBs that associates with other age-related pathologies – patients with a younger onset often have a slow disease course and more pure LB pathology at end stage, while patients with an older onset are more likely to have additional age-related pathologies and a more rapid and complex clinical course, the most rapid course occurring in patients with DLB (Compta et al. 2011).

9 α-Synuclein in Human Fluids as a Biomarker for PD Progression

Aggregated α-synuclein accumulates in degenerating neurons in PD making measurements of α-synuclein levels a rational biomarker for diagnosis and prediction of disease susceptibility. α-Synuclein has been detected in blood, CSF, and saliva at low nanogram/ml levels (e.g., (Fjorback et al. 2007; Devic et al. 2011; Shi et al. 2011)). Most investigations have focused on detecting the total amount of

α-synuclein but have also investigated the presence of polymers using identical monoclonal anti-α-synuclein antibodies as catching and detecting antibody (Tokuda et al. 2010). Future developments will likely aim at detecting specific α-synuclein isoforms, e.g., misfolded species as detected in brain extracts, and specific phosphorylations to allow for better stratification. For a review on α-synuclein in CSF, please see (Mollenhauer et al. 2010).

10 Modeling PD

Different organisms from flies and worms to rodents and primates have been used to model PD based on expression of wild-type or mutated human α-synuclein. In this section, we will highlight the discoveries related to mammals and refer readers to a recent review about other approaches (Pienaar et al. 2010). There are numerous human α-synuclein transgenic mice although the associated phenotypes are not always consistent probably due to the varying levels and areas of transgene expression as a consequence of the promoters used (for further reading see (Magen and Chesselet 2010; Chesselet and Richter 2011)). Disease modeling has focused on the following points: neurodegeneration in general and dopaminergic neurodegeneration in particular, basal ganglia-associated motor behavior and signs of abnormal α-synuclein folding and handling (see Table 1 for overview).

10.1 Dopaminergic Neurodegeneration

Mice lines using the dopaminergic TH promoter have been rather disappointing in modeling PD (Matsuoka et al. 2001; Rathke-Hartlieb et al. 2001) and only double-mutated α-synuclein (A30P and A53T; a condition not found in humans) has been able to induce dopaminergic failure (Richfield et al. 2002), and this was even obtained with the pan-neuronal Thy1 promoter (Ono et al. 2009). Interestingly, expressing C-terminal-truncated α-synuclein (1–120 or 1–130 α-synuclein) under the TH promoter induced dopaminergic cell death, although not progressively and related to developmental defects (Tofaris et al. 2006; Wakamatsu et al. 2007; Wakamatsu et al. 2008). Using the rat AAV model, the ability of truncated α-synuclein to promote pathological accumulation of wild-type α-synuclein was corroborated (Ulusoy et al. 2010). More widely expressing promoters like Thy1 and PDGF have also been used to model dopaminergic degeneration and subtle phenotypes have been obtained (Masliah et al. 2000; Rockenstein et al. 2002; Lam et al. 2011).

An alternative to the classic transgenic mice lines is adult transgenesis using viral vectors to induce overexpression of wild-type or mutated forms of α-synuclein in nigrostriatal neurons. This leads to progressive and selective neurodegeneration and dopaminergic loss in the basal ganglia (Kirik et al. 2002; Lo Bianco et al. 2002; Yamada et al. 2004; Maingay et al. 2006). These models have shown characteristics of α-synuclein misfolding (Lo Bianco et al. 2004;

Table 1 List of the better characterized and/or most representative transgenic human α-synuclein mice lines

Promoter	Human α-syn	a-Syn in SN	Dopaminergic system	Pathological α-syn	Behavioral phenotype	Remarks	References
Rat-TH	120 Trunc	+	Striatal TH terminal loss	Filamentous	Motor	Microgliosis	Tofaris et al. (2006)
			↓DA	Insoluble (WB)			
	130 Trunc	+	Nigral and striatal TH neuronal loss	?	Motor		Wakamatsu et al. (2008)
	A30P or A53T	+	No change	?			Matsuoka et al. (2001), Manning-Bog et al. (2003), Yu et al. (2008)
			↑MPTP susceptibility in the A53T				
			↓ Paraquat susceptibility				
	A30P and A53T	+	Nigral and striatal TH neuronal loss	Ubiquitin (−)	Motor	Microgliosis preceding cell death	Richfield et al. (2002), Su et al. (2009)
			↑Susceptibility to MPTP or maned and paraquat	Congo red (−)		Long-lasting increase on proinflammatory cytokines	
	A30P	+	No change	?			Rathke-Hartlieb et al. (2001)
			Unchanged MPTP susceptibility				

(*continued*)

Table 1 (continued)

Promoter	Human α-syn	a-Syn in SN	Dopaminergic system	Pathological α-syn	Behavioral phenotype	Remarks	References
Mouse prion	E46K	+		Lewy body-like	Motor correlated with spinal cord-brainstem pathology	Microgliosis	Emmer et al. (2011)
				Fibrillar			
				PSer129 α-syn			
				Insoluble (WB)			
				Nitrated			
				Tau inclusions			
	A30P	SN?	↓ Capacity of the DA storage pool and evoked release	?	Abnormal motor response to L-dopa	Changes in NE neurotransmission	Yavich et al. (2004, 2005, 2006), Oksman et al. (2009)
		Str+	No change in TH staining in Str or SN				
	A53T	+	?	Not aggregated	Motor	Lamp 1+ (if null background)	Gispert et al. (2003), Cabin et al. (2005)
	A53T		↑ D1 receptor in SN	Ubiquitin (+)	Hyperactivity associated with DA	Degenerated mitochondria	Lee et al. (2002), Martin et al. (2006), Unger et al. (2006)
				ThioF S (+)			
				Insoluble (WB) Oligomeric			
				Truncated	Motor defect associated with spinal cord-brainstem neurodegeneration	↓Complex IV	
			↓ DA reuptake and DAT in Str	Nitrated			
				Spheroids		Altered expression on inflammation-related genes	
				Eosinophilic			

	A53T	+	No Change	Oligomeric and insoluble (WB)	Severe associated to motor neurons	If crossed with DJ1 KO no change	Giasson et al. (2002), von Coelln et al. (2006), Norris et al. (2007), Tsika et al. (2010), Gao et al. (2011)
			DAergic hypersensitivity to LPS-induced inflammation	Oxidized Filamentous Nitrated		Parkin KO no change	
				Regional different oligomeric a-syn		↑Susceptibility (maned + paraquat)	
Thy1	Y39C	+	No change	Ubiquitin (+) PSer129 α-syn	Motor and cognitive		Zhou et al. (2008)
				Oligomeric soluble and insoluble (WB)			
	WT	+/−	↑DA release at 6 months	Prot. K resistant PSer129 a-syn	Sensorimotor	Microgliosis Early electrophysiological Changes in Str	Rockenstein et al. (2002), Fleming et al. (2004), Fleming and Chesselet (2006), Fernagut et al. (2007), Fleming et al. (2008)
			18 moths ↓ DA in Str	Oligomeric (WB)	Motor Olfactory		
				Insoluble (WB)	Colonic motility		

(*continued*)

Table 1 (continued)

Promoter	Human α-syn	a-Syn in SN	Dopaminergic system	Pathological α-syn	Behavioral phenotype	Remarks	References
	A30P	+	Normal DA content at all ages	Prot K resistant	Motor and cognitive	Oxidation of metabolic enzymes in mitochondria	Kahle et al. (2001), Neumann et al. (2002), Poon et al. (2005), Freichel et al. (2007), Schell et al. (2009)
				Insoluble α-syn			
				PSer129 α-syn			
				ThioF S (+)			
				Ubiquitin (+)			
	A30P and A53T	+	Nigral and striatal TH neuronal loss	PSer 129 α-syn	Motor		Ono et al. (2009)
PDGF	WT	+/−	Striatal TH terminal loss	Non-fibrillar a-syn	Motor	↓Neurodegenerative changes upon immunization (active or passive)	Masliah et al. (2000), Masliah et al. (2005), Koob et al. (2010), Haggerty et al. (2011)
				Oxidized a-syn			
			↓DA	Hyperphosp. TAU			
				Ubiquitin (+)			
Hamster prion	A30P	+	No changes	Oligomeric and truncated soluble (WB)	Motor	Microgliosis	Gomez-Isla et al. (2003), Nieto et al. (2006)
			↑ Susceptibility to MPTP				

DA dopamine, *DAergic* dopaminergic, *TH* tyrosine hydroxylase, *Str* striatum, *SN* substantia nigra, *Prot. K* proteinase K, *PSer129 α-syn* phosphorylated human α-synuclein at Ser129, *WB* western blot, *ThioF S* thioflavin S, *?* not tested/reported

Eslamboli et al. 2007), markers of dysfunctional synapses, and neuroinflammation (Theodore et al. 2008; Chung et al. 2009; Sanchez-Guajardo et al. 2010). So far, this is the only approach that has allowed transgenesis in nonhuman primates to successfully model several of the cardinal symptoms of the disease (Eslamboli et al. 2007; Yasuda et al. 2007).

10.2 Non-dopaminergic Neurodegeneration and Braak

The Braak hypothesis posits that α-synuclein degeneration also occurs in non-dopaminergic neurons (Fig. 1b). Indeed enteric nervous system anomalies and colonic problems have been demonstrated in α-synuclein mice (Wang et al. 2008; Kuo et al. 2010). Olfactory problems being early symptoms in the disease have also been observed early in mice along with concomitant α-synuclein pathology in the olfactory bulb (Fleming et al. 2004, 2008; Wang et al. 2008). Very pronounced brainstem and spinal cord degeneration has been observed in the lines using the prion promoter (Giasson et al. 2002; Lee et al. 2002) with up to 75 % of motor neuron cell death (Gispert et al. 2003).

10.3 Pathological α-Synuclein In Vivo

Several α-synuclein transgenic mice models have shown ubiquitin-positive intracellular inclusions (Masliah et al. 2000; Kahle et al. 2001; Lee et al. 2002; Eslamboli et al. 2007; Zhou et al. 2008) and characteristics of aggregation, like resistance to proteinase K treatment (Magen and Chesselet 2010). Early dysfunctional or plastic changes have been observed in the tissue before pathological deposition of α-synuclein can be detected that appears to correlate over time with the severity of neurodegeneration (Gispert et al. 2003). The putative role of α-synuclein Ser129-phosphorylation has been seen in different models (Magen and Chesselet 2010). Phosphorylation of Ser129 is a prerequisite for dopaminergic cell death in *Drosophila* (Chen and Feany 2005), but this has not been corroborated in mammalian models. Three studies have been conducted comparing the neurotoxicity of expressing wild-type and pseudo-phosphorylated S129D mutant human α-synuclein. In two studies wild-type α-synuclein was more toxic than S129D, while the third reported no difference between the two α-synuclein species (Gorbatyuk et al. 2008; Azeredo da Silveira et al. 2009; McFarland et al. 2009). However, it should be kept in mind that S129D α-synuclein structurally is a poor mimic of Ser129-phosphorylated α-synuclein (Paleologou et al. 2008). Indirect approaches for increasing Ser129-phosphorylation using overexpression of phosphoprotein phosphatase 2A activity in a Thy1 transgenic mice line (Lee et al. 2011) or the α-synuclein directed kinase GRK6 using viral vectors in rats (Sato et al. 2011) report increased toxicity, but these approaches also affect the phosphorylation status of other proteins. Noteworthy, phosphorylation at Ser129 is not necessary for α-synuclein

transgene-dependent cell death to occur, as demonstrated in animals overexpressing α-synuclein C-terminally truncated before the Ser129 residue (Wakamatsu et al. 2008; Ulusoy et al. 2010).

10.4 Inflammation and Microglia in the Disease

Microglia activation and neuroinflammation in PD is recapitulated in several models: changes in microglia numbers or morphology, the cytokine pattern, or the gene expression (van der Putten et al. 2000; Neumann et al. 2002; Gomez-Isla et al. 2003; Tofaris et al. 2006; Su et al. 2008; Emmer et al. 2011). The microglial activation patterns correlate with the degree of α-synuclein-dependent neuropathology in the rat viral vector PD model (Sanchez-Guajardo et al. 2010), and pro-degenerative synergism exists between the proinflammatory environment induced by local or peripheral LPS treatment and α-synuclein in transgenic models (Gao et al. 2008; Gao et al. 2011). Neuroprotection observed by both passive and active vaccination in an α-synuclein transgenic mice line demonstrates that the peripheral immune system may be harnessed therapeutically and such an approach is already in a Phase 1 trial (Masliah et al. 2005; Haggerty et al. 2011).

10.5 Interactions with Other Brain Disease-Related Genes

Regarding the possible interactions with other proteins and genes associated with PD, coexpression of A53T α-synuclein and LRRK2 (wild-type, mutated G2019S, or KD) resulted in synergistic toxicity enhancing and accelerating the α-synuclein-induced neurodegeneration (Lin et al. 2009). However, no difference in toxicity was observed in A53T α-synuclein transgenic mice when crossed with DJ1-deficient (Ramsey et al. 2010) or parkin null mice (von Coelln et al. 2006). Transgenic lines coexpressing wild-type α-synuclein and β-amyloid peptides showed more severe anomalies than single transgenics, suggesting a shared pathogenic event between both proteins (Masliah et al. 2001). In parallel, the role of α-synuclein in Huntington's disease has also been highlighted by a decrease in neurodegenerative changes in a knock-in mouse model of Huntington's disease with an α-synuclein knockout background (Tomas-Zapico et al. 2011).

11 Conclusion

There is still considerable doubt over the species of α-synuclein that contributes most to neurotoxicity (Fig. 2). Currently there is a huge gap between data obtained through in vitro versus in vivo methods. This is highlighted by recent data that suggests the native state of α-synuclein is a folded tetramer (Bartels et al. 2011; Wang et al. 2011). If this concept proves true, it has fundamental

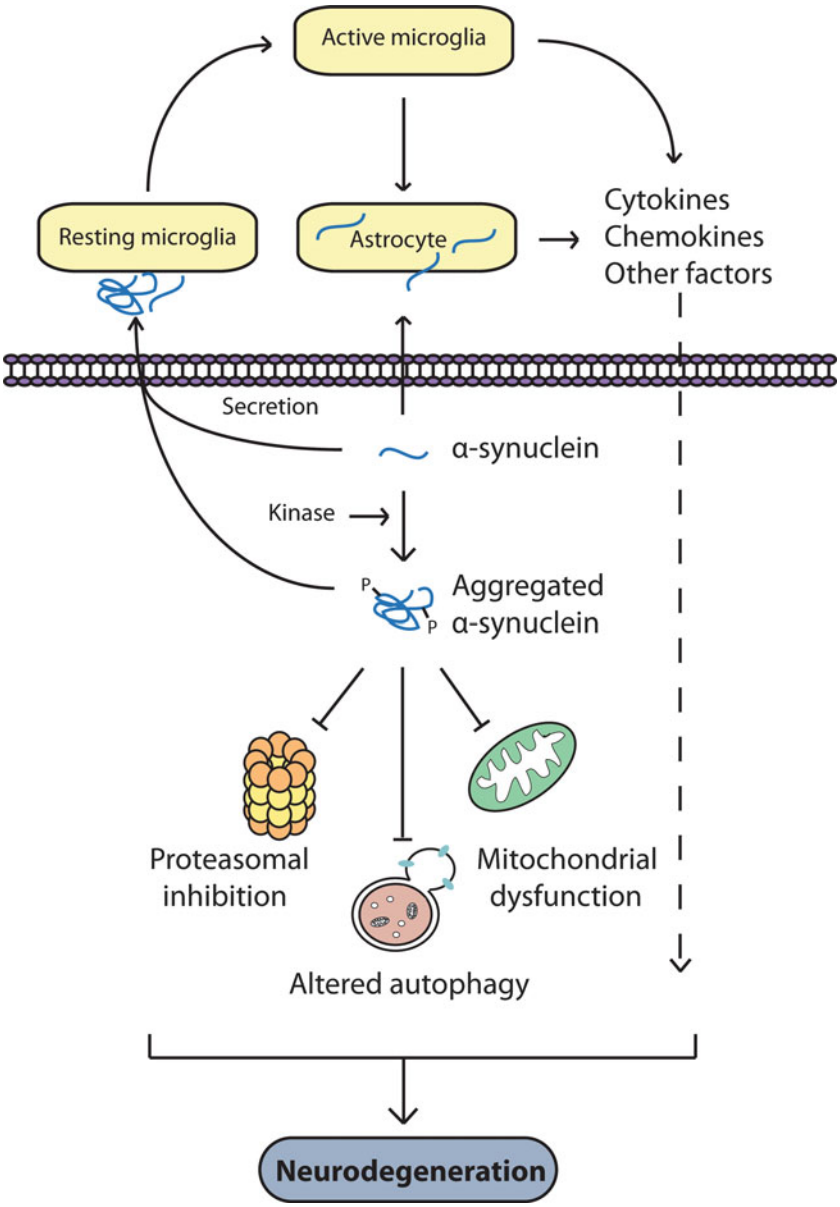

Fig. 2 Proposed mechanisms of neurodegeneration in PD. There are multiple potential pathways of neurodegeneration in PD. α-Synuclein aggregation and accumulation (e.g., of phosphorylated α-synuclein) can cause proteasomal, lysosomal, and mitochondrial impairment leading to neurodegeneration. In addition, α-synuclein can be secreted from neurons and taken up by microglia and astrocytes. Proinflammatory mediators such as cytokines and chemokines produced by activated microglia activate astrocytes, and the products released by activated microglia and astrocytes may exert neurotoxic effects

implications for conceptualizing the potential molecular mechanisms involved in α-synucleinopathies. How Ser129-phosphorylation contributes to these structures or to pathogenesis needs to be determined, as Ser129-phosphorylation is tightly linked to human pathological lesions. To date the functional significance of Ser129-phosphorylation in cell and in vivo models has been inconclusive, findings that may be related to its structural state. Development of novel structure-specific imaging tools, like antibodies and small molecules, is likely to assist with understanding of both the structural and posttranslational changes in α-synuclein that occur in α-synucleinopathies. It is important to clarify the significance of Ser129-phosphorylation of different α-synuclein structural species to determine their role in disease initiation and progression because kinases hold great potential as drug targets.

The novel hypothesis that the disease is spread through the uptake of extracellular α-synuclein has significant implications currently driving considerable research activity. The observation that astrocytes seem to be more prone to such uptake in PD now needs to be understood fully. Particularly, the functional significance of the early and pervasive astrocytic accumulation of α-synuclein needs to be determined. Does such behavior play a paralytic role in the neurodegeneration in PD? Regarding uptake of extracellular α-synuclein, research questions currently pursued include the following: How is α-synuclein externalized during aging and disease and in which forms? How does external α-synuclein affect cells at the cell surface and upon internalization? The answers to these questions will be important for the further development of the currently promising in vivo α-synuclein immunotherapies, as externalized α-synuclein is more amenable to such therapies. Whether there are specific α-synuclein species and/or cell types that are optimal for antibody-dependent targeting needs to be determined. Furthermore, any immunotherapy successes require replication in multiple models.

Aging is known to be the greatest risk factor for PD and LB pathology, but the mechanisms affected by aging still need to be identified. While the importance of mitochondrial function with aging needs to be considered, the specificity that mitochondrial aging precipitates only α-synucleinopathies and not other neurodegenerative conditions requires further explanation. Although speculative, Tyr125-phosphorylation may represent a α-synuclein-dependent cytoprotective mechanism associated with tetramer stability that is lost upon aging. The identification of the toxic species of α-synuclein and changes with aging will be important to determine. The identification that α-synuclein can be catabolized through autophagy and recent in vivo modeling suggests that disruption in the cross talk between proteasomal and autophagic α-synuclein catabolism may be important, particularly as the catabolism of α-synuclein changes during brain development and aging. Another significant factor may be the coexisting pathologies that accumulate with aging. Cellular defenses against misfolded proteins have been characterized in model organisms, and their role in PD needs to be clarified. Whether concentrating on α-synuclein therapeutic interventions alone is the best strategy needs to be clarified.

References

Abeliovich, A., Schmitz, Y., Farinas, I., Choi-Lundberg, D., Ho, W. H., Castillo, P. E., Shinsky, N., Verdugo, J. M., Armanini, M., Ryan, A., Hynes, M., Phillips, H., Sulzer, D., & Rosenthal, A. (2000). Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron, 25*, 239–252.

Anderson, J. P., Walker, D. E., Goldstein, J. M., de Laat, R., Banducci, K., Caccavello, R. J., Barbour, R., Huang, J., Kling, K., Lee, M., Diep, L., Keim, P. S., Shen, X., Chataway, T., Schlossmacher, M. G., Seubert, P., Schenk, D., Sinha, S., Gai, W. P., & Chilcote, T. J. (2006). Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *The Journal of Biological Chemistry, 281*, 29739–29752.

Arawaka, S., Wada, M., Goto, S., Karube, H., Sakamoto, M., Ren, C. H., Koyama, S., Nagasawa, H., Kimura, H., Kawanami, T., Kurita, K., Tajima, K., Daimon, M., Baba, M., Kido, T., Saino, S., Goto, K., Asao, H., Kitanaka, C., Takashita, E., Hongo, S., Nakamura, T., Kayama, T., Suzuki, Y., Kobayashi, K., Katagiri, T., Kurokawa, K., Kurimura, M., Toyoshima, I., Niizato, K., Tsuchiya, K., Iwatsubo, T., Muramatsu, M., Matsumine, H., & Kato, T. (2006). The role of G-protein-coupled receptor kinase 5 in pathogenesis of sporadic Parkinson's disease. *The Journal of Neuroscience, 26*, 9227–9238.

Azeredo da Silveira, S., Schneider, B. L., Cifuentes-Diaz, C., Sage, D., Abbas-Terki, T., Iwatsubo, T., Unser, M., & Aebischer, P. (2009). Phosphorylation does not prompt, nor prevent, the formation of alpha-synuclein toxic species in a rat model of Parkinson's disease. *Human Molecular Genetics, 18*, 872–887.

Bartels, T., Choi, J. G., & Selkoe, D. J. (2011). Alpha-synuclein occurs physiologically as a helically folded tetramer that resists aggregation. *Nature, 477*, 107–110.

Beach, T. G., Adler, C. H., Lue, L., Sue, L. I., Bachalakuri, J., Henry-Watson, J., Sasse, J., Boyer, S., Shirohi, S., Brooks, R., Eschbacher, J., White, C. L., 3rd, Akiyama, H., Caviness, J., Shill, H. A., Connor, D. J., Sabbagh, M. N., & Walker, D. G. (2009). Unified staging system for Lewy body disorders: Correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. *Acta Neuropathologica, 117*, 613–634.

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging, 24*, 197–211.

Braak, H., Ghebremedhin, E., Rub, U., Bratzke, H., & Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell and Tissue Research, 318*, 121–134.

Braak, H., Sastre, M., & Del Tredici, K. (2007). Development of alpha-synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. *Acta Neuropathologica, 114*, 231–241.

Burre, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R., & Sudhof, T. C. (2010). Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science, 329*, 1663–1667.

Bussell, R., Jr., & Eliezer, D. (2003). A structural and functional role for 11-mer repeats in alpha-synuclein and other exchangeable lipid binding proteins. *Journal of Molecular Biology, 329*, 763–778.

Cabin, D. E., Gispert-Sanchez, S., Murphy, D., Auburger, G., Myers, R. R., & Nussbaum, R. L. (2005). Exacerbated synucleinopathy in mice expressing A53T SNCA on a Snca null background. *Neurobiology of Aging, 26*, 25–35.

Chandra, S., Chen, X., Rizo, J., Jahn, R., & Sudhof, T. C. (2003). A broken alpha-helix in folded alpha-synuclein. *The Journal of Biological Chemistry, 278*, 15313–15318.

Chandra, S., Fornai, F., Kwon, H. B., Yazdani, U., Atasoy, D., Liu, X., Hammer, R. E., Battaglia, G., German, D. C., Castillo, P. E., & Sudhof, T. C. (2004). Double-knockout mice for alpha- and beta-synucleins: Effect on synaptic functions. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 14966–14971.

Chen, L., & Feany, M. B. (2005). Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a *Drosophila* model of Parkinson disease. *Nature Neuroscience, 8*, 657–663.

Chen, L., Periquet, M., Wang, X., Negro, A., McLean, P. J., Hyman, B. T., & Feany, M. B. (2009). Tyrosine and serine phosphorylation of alpha-synuclein have opposing effects on neurotoxicity and soluble oligomer formation. *The Journal of Clinical Investigation, 119*, 3257–3265.

Chesselet, M. F., & Richter, F. (2011). Modelling of Parkinson's disease in mice. *Lancet Neurology, 10*, 1108–1118.

Cho, M. K., Nodet, G., Kim, H. Y., Jensen, M. R., Bernado, P., Fernandez, C. O., Becker, S., Blackledge, M., & Zweckstetter, M. (2009). Structural characterization of alpha-synuclein in an aggregation prone state. *Protein Science: A Publication of the Protein Society, 18*, 1840–1846.

Chung, K. K., Dawson, V. L., & Dawson, T. M. (2001). The role of the ubiquitin-proteasomal pathway in Parkinson's disease and other neurodegenerative disorders. *Trends in Neurosciences, 24*, S7–S14.

Chung, C. Y., Koprich, J. B., Siddiqi, H., & Isacson, O. (2009). Dynamic changes in presynaptic and axonal transport proteins combined with striatal neuroinflammation precede dopaminergic neuronal loss in a rat model of AAV alpha-synucleinopathy. *The Journal of Neuroscience, 29*, 3365–3373.

Clayton, D. F., & George, J. M. (1998). The synucleins: A family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *Trends in Neurosciences, 21*, 249–254.

Compta, Y., Parkkinen, L., O'Sullivan, S. S., Vandrovcova, J., Holton, J. L., Collins, C., Lashley, T., Kallis, C., Williams, D. R., de Silva, R., Lees, A. J., & Revesz, T. (2011). Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: Which is more important? *Brain: A Journal of Neurology, 134*, 1493–1505.

Conway, K. A., Lee, S. J., Rochet, J. C., Ding, T. T., Williamson, R. E., & Lansbury, P. T., Jr. (2000). Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: Implications for pathogenesis and therapy. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 571–576.

Conway, K. A., Rochet, J. C., Bieganski, R. M., & Lansbury, P. T., Jr. (2001). Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. *Science, 294*, 1346–1349.

Cooper, A. A., Gitler, A. D., Cashikar, A., Haynes, C. M., Hill, K. J., Bhullar, B., Liu, K., Xu, K., Strathearn, K. E., Liu, F., Cao, S., Caldwell, K. A., Caldwell, G. A., Marsischky, G., Kolodner, R. D., Labaer, J., Rochet, J. C., Bonini, N. M., & Lindquist, S. (2006). Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science, 313*, 324–328.

Croisier, E., Moran, L. B., Dexter, D. T., Pearce, R. K., & Graeber, M. B. (2005). Microglial inflammation in the parkinsonian substantia nigra: Relationship to alpha-synuclein deposition. *Journal of Neuroinflammation, 2*, 14.

Crowther, R. A., Jakes, R., Spillantini, M. G., & Goedert, M. (1998). Synthetic filaments assembled from C-terminally truncated alpha-synuclein. *FEBS Letters, 436*, 309–312.

Cuervo, A. M., Stefanis, L., Fredenburg, R., Lansbury, P. T., & Sulzer, D. (2004). Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science, 305*, 1292–1295.

Danzer, K. M., Haasen, D., Karow, A. R., Moussaud, S., Habeck, M., Giese, A., Kretzschmar, H., Hengerer, B., & Kostka, M. (2007). Different species of alpha-synuclein oligomers induce calcium influx and seeding. *The Journal of Neuroscience, 27*, 9220–9232.

Dauer, W., Kholodilov, N., Vila, M., Trillat, A. C., Goodchild, R., Larsen, K. E., Staal, R., Tieu, K., Schmitz, Y., Yuan, C. A., Rocha, M., Jackson-Lewis, V., Hersch, S., Sulzer, D., Przedborski, S., Burke, R. E., & Hen, R. (2002). Resistance of alpha-synuclein null mice to the parkinsonian neurotoxin MPTP. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 14524–14529.

Desplats, P., Lee, H. J., Bae, E. J., Patrick, C., Rockenstein, E., Crews, L., Spencer, B., Masliah, E., & Lee, S. J. (2009). Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 13010–13015.

Devic, I., Hwang, H., Edgar, J. S., Izutsu, K., Presland, R., Pan, C., Goodlett, D. R., Wang, Y., Armaly, J., Tumas, V., Zabetian, C. P., Leverenz, J. B., Shi, M., & Zhang, J. (2011). Salivary alpha-synuclein and DJ-1: Potential biomarkers for Parkinson's disease. *Brain: A Journal of Neurology, 134*, e178.

Dickson, D. W., Braak, H., Duda, J. E., Duyckaerts, C., Gasser, T., Halliday, G. M., Hardy, J., Leverenz, J. B., Del Tredici, K., Wszolek, Z. K., & Litvan, I. (2009). Neuropathological assessment of Parkinson's disease: Refining the diagnostic criteria. *Lancet Neurology, 8*, 1150–1157.

Dufty, B. M., Warner, L. R., Hou, S. T., Jiang, S. X., Gomez-Isla, T., Leenhouts, K. M., Oxford, J. T., Feany, M. B., Masliah, E., & Rohn, T. T. (2007). Calpain-cleavage of {alpha}-synuclein: Connecting proteolytic processing to disease-linked aggregation. *The American Journal of Pathology, 170*, 1725–1738.

Durrenberger, P. F., Filiou, M. D., Moran, L. B., Michael, G. J., Novoselov, S., Cheetham, M. E., Clark, P., Pearce, R. K., & Graeber, M. B. (2009). DnaJB6 is present in the core of Lewy bodies and is highly up-regulated in parkinsonian astrocytes. *Journal of Neuroscience Research, 87*, 238–245.

Ebrahimi-Fakhari, D., Cantuti-Castelvetri, I., Fan, Z., Rockenstein, E., Masliah, E., Hyman, B. T., McLean, P. J., & Unni, V. K. (2011). Distinct roles in vivo for the ubiquitin-proteasome system and the autophagy-lysosomal pathway in the degradation of alpha-synuclein. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 31*, 14508–14520.

Ellis, C. E., Schwartzberg, P. L., Grider, T. L., Fink, D. W., & Nussbaum, R. L. (2001). Alpha-synuclein is phosphorylated by members of the Src family of protein-tyrosine kinases. *The Journal of Biological Chemistry, 276*, 3879–3884.

Emmanouilidou, E., Stefanis, L., & Vekrellis, K. (2010). Cell-produced alpha-synuclein oligomers are targeted to, and impair, the 26S proteasome. *Neurobiology of Aging, 31*, 953–968.

Emmer, K. L., Waxman, E. A., Covy, J. P., & Giasson, B. I. (2011). E46K human alpha-synuclein transgenic mice develop Lewy-like and tau pathology associated with age-dependent, detrimental motor impairment. *The Journal of Biological Chemistry, 286*, 35104–35118.

Engelender, S., Kaminsky, Z., Guo, X., Sharp, A. H., Amaravi, R. K., Kleiderlein, J. J., Margolis, R. L., Troncoso, J. C., Lanahan, A. A., Worley, P. F., Dawson, V. L., Dwson, T. M., & Ross, C. A. (1999). Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. *Nature Genetics, 22*, 110–114.

Eslamboli, A., Romero-Ramos, M., Burger, C., Bjorklund, T., Muzyczka, N., Mandel, R. J., Baker, H., Ridley, R. M., & Kirik, D. (2007). Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain. *Brain, 130*, 799–815.

Fjorback, A. W., Varming, K., & Jensen, P. H. (2007). Determination of alpha-synuclein concentration in human plasma using ELISA. *Scandinavian Journal of Clinical and Laboratory Investigation, 67*, 431–435.

Fernagut, et al. (2007). Behavioral and histopathological consequences of paraquat intoxication in mice: effects of alpha-synuclein over-expression. In: Fernagut, P. O., Hutson, C. B., Fleming, S. M., Tetreaut, N. A., Salcedo, J., Masliah, E., & Chesselet, M. F. *Synapse*, 61(12):991–1001.

Fleming, S. M., & Chesselet, M. F. (2006). Behavioral phenotypes and pharmacology in genetic mouse models of Parkinsonism. *Behavioural Pharmacology*, *17*, 383–391.

Fleming, S. M., Salcedo, J., Fernagut, P. O., Rockenstein, E., Masliah, E., Levine, M. S., & Chesselet, M. F. (2004). Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. *The Journal of Neuroscience, 24*, 9434–9440.

Fleming, S. M., Tetreault, N. A., Mulligan, C. K., Hutson, C. B., Masliah, E., & Chesselet, M. F. (2008). Olfactory deficits in mice overexpressing human wildtype alpha-synuclein. *The European Journal of Neuroscience, 28*, 247–256.

Fountaine, T. M., & Wade-Martins, R. (2007). RNA interference-mediated knockdown of alpha-synuclein protects human dopaminergic neuroblastoma cells from MPP(+) toxicity and reduces dopamine transport. *Journal of Neuroscience Research, 85*, 351–363.

Freichel, C., Neumann, M., Ballard, T., Muller, V., Woolley, M., Ozmen, L., Borroni, E., Kretzschmar, H. A., Haass, C., Spooren, W., & Kahle, P. J. (2007). Age-dependent cognitive decline and amygdala pathology in alpha-synuclein transgenic mice. *Neurobiology of Aging*, *28*, 1421–1435.

Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M. S., Shen, J., Takio, K., & Iwatsubo, T. (2002). Alpha-synuclein is phosphorylated in synucleinopathy lesions. *Nature Cell Biology, 4*, 160–164.

Gai, W., Power, J., Blumberg, P., & Blessing, W. (1998). Multiple-system atrophy: A new α-synuclein disease? *Lancet, 352*, 547–548.

Gao, H. M., Kotzbauer, P. T., Uryu, K., Leight, S., Trojanowski, J. Q., & Lee, V. M. (2008). Neuroinflammation and oxidation/nitration of alpha-synuclein linked to dopaminergic neurodegeneration. *The Journal of Neuroscience, 28*, 7687–7698.

Gao, H. M., Zhang, F., Zhou, H., Kam, W., Wilson, B., & Hong, J. S. (2011). Neuroinflammation and alpha-synuclein dysfunction potentiate each other, driving chronic progression of neurodegeneration in a mouse model of Parkinson's disease. *Environmental Health Perspectives, 119*, 807–814.

George, J., Jin, H., Woods, W., & Clayton, D. (1995). Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. *Neuron, 15*, 361–372.

Giasson, B. I., Duda, J. E., Quinn, S. M., Zhang, B., Trojanowski, J. Q., & Lee, V. M. (2002). Neuronal alpha-Synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. *Neuron, 34*, 521–533.

Gispert, S., Del Turco, D., Garrett, L., Chen, A., Bernard, D. J., Hamm-Clement, J., Korf, H. W., Deller, T., Braak, H., Auburger, G., & Nussbaum, R. L. (2003). Transgenic mice expressing mutant A53T human alpha-synuclein show neuronal dysfunction in the absence of aggregate formation. *Molecular and Cellular Neurosciences, 24*, 419–429.

Gomez-Isla, T., Irizarry, M. C., Mariash, A., Cheung, B., Soto, O., Schrump, S., Sondel, J., Kotilinek, L., Day, J., Schwarzschild, M. A., Cha, J. H., Newell, K., Miller, D. W., Ueda, K., Young, A. B., Hyman, B. T., & Ashe, K. H. (2003). Motor dysfunction and gliosis with preserved dopaminergic markers in human alpha-synuclein A30P transgenic mice. *Neurobiology of Aging, 24*, 245–258.

Gomperts, S. N., Rentz, D. M., Moran, E., Becker, J. A., Locascio, J. J., Klunk, W. E., Mathis, C. A., Elmaleh, D. R., Shoup, T., Fischman, A. J., Hyman, B. T., Growdon, J. H., & Johnson, K. A. (2008). Imaging amyloid deposition in Lewy body diseases. *Neurology, 71*, 903–910.

Gorbatyuk, O. S., Li, S., Sullivan, L. F., Chen, W., Kondrikova, G., Manfredsson, F. P., Mandel, R. J., & Muzyczka, N. (2008). The phosphorylation state of Ser-129 in human alpha-synuclein determines neurodegeneration in a rat model of Parkinson disease. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 763–768.

Greffard, S., Verny, M., Bonnet, A. M., Beinis, J. Y., Gallinari, C., Meaume, S., Piette, F., Hauw, J. J., & Duyckaerts, C. (2006). Motor score of the Unified Parkinson Disease Rating Scale as a good predictor of Lewy body-associated neuronal loss in the substantia nigra. *Archives of Neurology, 63*, 584–588.

Greffard, S., Verny, M., Bonnet, A. M., Seilhean, D., Hauw, J. J., & Duyckaerts, C. (2010). A stable proportion of Lewy body bearing neurons in the substantia nigra suggests a model in which the Lewy body causes neuronal death. *Neurobiology of Aging, 31*, 99–103.

Greten-Harrison, B., Polydoro, M., Morimoto-Tomita, M., Diao, L., Williams, A. M., Nie, E. H., Makani, S., Tian, N., Castillo, P. E., Buchman, V. L., & Chandra, S. S. (2010). Alphabetagamma-synuclein triple knockout mice reveal age-dependent neuronal dysfunction. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 19573–19578.

Haggerty, T., Credle, J., Rodriguez, O., Wills, J., Oaks, A. W., Masliah, E., & Sidhu, A. (2011). Hyperphosphorylated Tau in an alpha-synuclein-overexpressing transgenic model of Parkinson's disease. *The European Journal of Neuroscience, 33*, 1598–1610.

Halliday, G. M., & McCann, H. (2010). The progression of pathology in Parkinson's disease. *Annals of the New York Academy of Sciences, 1184*, 188–195.

Halliday, G. M., & Stevens, C. H. (2011). Glia: Initiators and progressors of pathology in Parkinson's disease. *Movement Disorders, 26*, 6–17.

Halliday, G. M., Holton, J. L., Revesz, T., & Dickson, D. W. (2011a). Neuropathology underlying clinical variability in patients with synucleinopathies. *Acta Neuropathologica, 122*, 187–204.

Halliday, G. M., Song, Y. J., & Harding, A. J. (2011b). Striatal beta-amyloid in dementia with Lewy bodies but not Parkinson's disease. *Journal of Neural Transmission, 118*, 713–719.

Harding, A. J., Stimson, E., Henderson, J. M., & Halliday, G. M. (2002). Clinical correlates of selective pathology in the amygdala of patients with Parkinson's disease. *Brain: A Journal of Neurology, 125*, 2431–2445.

Hirsch, E. C., Vyas, S., & Hunot, S. (2012). Neuroinflammation in Parkinson's disease. *Parkinsonism & Related Disorders, 18*(Suppl 1), S210–S212.

Hoozemans, J. J., van Haastert, E. S., Eikelenboom, P., de Vos, R. A., Rozemuller, J. M., & Scheper, W. (2007). Activation of the unfolded protein response in Parkinson's disease. *Biochemical and Biophysical Research Communications, 354*, 707–711.

Hsu, L. J., Sagara, Y., Arroyo, A., Rockenstein, E., Sisk, A., Mallory, M., Wong, J., Takenouchi, T., Hashimoto, M., & Masliah, E. (2000). Alpha-synuclein promotes mitochondrial deficit and oxidative stress. *The American Journal of Pathology, 157*, 401–410.

Huang, Y., Cheung, L., Rowe, D., & Halliday, G. (2004). Genetic contributions to Parkinson's disease. *Brain Research. Brain Research Reviews, 46*, 44–70.

Inglis, K. J., Chereau, D., Brigham, E. F., Chiou, S. S., Schobel, S., Frigon, N. L., Yu, M., Caccavello, R. J., Nelson, S., Motter, R., Wright, S., Chian, D., Santiago, P., Soriano, F., Ramos, C., Powell, K., Goldstein, J. M., Babcock, M., Yednock, T., Bard, F., Basi, G. S., Sham, H., Chilcote, T. J., McConlogue, L., Griswold-Prenner, I., & Anderson, J. P. (2009). Polo-like kinase 2 (PLK2) phosphorylates alpha-synuclein at serine 129 in central nervous system. *The Journal of Biological Chemistry, 284*, 2598–2602.

Ishida, Y., Nagai, A., Kobayashi, S., & Kim, S. U. (2006). Upregulation of protease-activated receptor-1 in astrocytes in Parkinson disease: Astrocyte-mediated neuroprotection through increased levels of glutathione peroxidase. *Journal of Neuropathology and Experimental Neurology, 65*, 66–77.

Jakes, R., Spillantini, M. G., & Goedert, M. (1994). Identification of two distinct synucleins from human brain. *FEBS Letters, 345*, 27–32.

Jensen, P., Li, J.-Y., Dahlstrom, A., & Dotti, C. (1999). Axonal transport of synucleins is mediated by all rate components. *Europian Journal of Neuroscience, 11*, 3369–3376.

Kahle, P. J., Neumann, M., Ozmen, L., Muller, V., Odoy, S., Okamoto, N., Jacobsen, H., Iwatsubo, T., Trojanowski, J. Q., Takahashi, H., Wakabayashi, K., Bogdanovic, N., Riederer, P., Kretzschmar, H. A., & Haass, C. (2001). Selective insolubility of alpha-synuclein in human Lewy body diseases is recapitulated in a transgenic mouse model. *The American Journal of Pathology, 159*, 2215–2225.

Kamp, F., Exner, N., Lutz, A. K., Wender, N., Hegermann, J., Brunner, B., Nuscher, B., Bartels, T., Giese, A., Beyer, K., Eimer, S., Winklhofer, K. F., & Haass, C. (2010). Inhibition of mitochondrial fusion by alpha-synuclein is rescued by PINK1, Parkin and DJ-1. *The EMBO Journal, 29*, 3571–3589.

Karpinar, D. P., Balija, M. B., Kugler, S., Opazo, F., Rezaei-Ghaleh, N., Wender, N., Kim, H. Y., Taschenberger, G., Falkenburger, B. H., Heise, H., Kumar, A., Riedel, D., Fichtner, L., Voigt, A., Braus, G. H., Giller, K., Becker, S., Herzig, A., Baldus, M., Jackle, H., Eimer, S., Schulz, J. B., Griesinger, C., & Zweckstetter, M. (2009). Pre-fibrillar alpha-synuclein variants with impaired beta-structure increase neurotoxicity in Parkinson's disease models. *The EMBO Journal, 28*, 3256–3268.

Kayed, R., Head, E., Sarsoza, F., Saing, T., Cotman, C. W., Necula, M., Margol, L., Wu, J., Breydo, L., Thompson, J. L., Rasool, S., Gurlo, T., Butler, P., & Glabe, C. G. (2007). Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers. *Molecular Neurodegeneration, 2*, 18.

Kim, H. J., Lee, D., Lee, C. H., Chung, K. C., Kim, J., & Paik, S. R. (2006). Calpain-resistant fragment(s) of alpha-synuclein regulates the synuclein-cleaving activity of 20S proteasome. *Archives of Biochemistry and Biophysics, 455*, 40–47.

Kirik, D., Rosenblad, C., Burger, C., Lundberg, C., Johansen, T. E., Muzyczka, N., Mandel, R. J., & Bjorklund, A. (2002). Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. *The Journal of Neuroscience, 22*, 2780–2791.

Klegeris, A., Giasson, B. I., Zhang, H., Maguire, J., Pelech, S., & McGeer, P. L. (2006). Alpha-synuclein and its disease-causing mutants induce ICAM-1 and IL-6 in human astrocytes and astrocytoma cells. *The FASEB Journal: Official publication of the Federation of American Societies for Experimental Biology, 20*, 2000–2008.

Klucken, J., Outeiro, T. F., Nguyen, P., McLean, P. J., & Hyman, B. T. (2006). Detection of novel intracellular alpha-synuclein oligomeric species by fluorescence lifetime imaging. *The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 20*, 2050–2057.

Knott, C., Wilkin, G. P., & Stern, G. (1999). Astrocytes and microglia in the substantia nigra and caudate-putamen in Parkinson's disease. *Parkinsonism & Related Disorders, 5*, 115–122.

Koob, A. O., Ubhi, K., Paulsson, J. F., Kelly, J., Rockenstein, E., Mante, M., Adame, A., & Masliah, E. (2010). Lovastatin ameliorates alpha-synuclein accumulation and oxidation in transgenic mouse models of alpha-synucleinopathies. *Experimental Neurology*, *221*, 267–274.

Kordower, J. H., Chu, Y., Hauser, R. A., Freeman, T. B., & Olanow, C. W. (2008). Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nature Medicine, 14*, 504–506.

Kragh, C. L., Lund, L. B., Febbraro, F., Hansen, H. D., Gai, W. P., El-Agnaf, O., Richter-Landsberg, C., & Jensen, P. H. (2009). {alpha}-synuclein aggregation and Ser-129 phosphorylation-dependent cell death in oligodendroglial cells. *The Journal of Biological Chemistry, 284*, 10211–10222.

Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J., Schols, L., & Reiss, O. (1998). Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease. *Nature Genetics, 18*, 106–108.

Kuo, Y. M., Li, Z., Jiao, Y., Gaborit, N., Pani, A. K., Orrison, B. M., Bruneau, B. G., Giasson, B. I., Smeyne, R. J., Gershon, M. D., & Nussbaum, R. L. (2010). Extensive enteric nervous system abnormalities in mice transgenic for artificial chromosomes containing Parkinson disease-associated alpha-synuclein gene mutations precede central nervous system changes. *Human Molecular Genetics, 19*, 1633–1650.

Lam, H. A., Wu, N., Cely, I., Kelly, R. L., Hean, S., Richter, F., Magen, I., Cepeda, C., Ackerson, L. C., Walwyn, W., Masliah, E., Chesselet, M. F., Levine, M. S., & Maidment, N. T. (2011). Elevated tonic extracellular dopamine concentration and altered dopamine modulation of synaptic activity precede dopamine loss in the striatum of mice overexpressing human alpha-synuclein. *Journal of Neuroscience Research, 89*, 1091–1102.

Lashuel, H. A., Petre, B. M., Wall, J., Simon, M., Nowak, R. J., Walz, T., & Lansbury, P. T., Jr. (2002). Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. *Journal of Molecular Biology, 322*, 1089–1102.

Lee, F. J., Liu, F., Pristupa, Z. B., & Niznik, H. B. (2001). Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 15*, 916–926.

Lee, M. K., Stirling, W., Xu, Y., Xu, X., Qui, D., Mandir, A. S., Dawson, T. M., Copeland, N. G., Jenkins, N. A., & Price, D. L. (2002). Human alpha-synuclein-harboring familial Parkinson's

disease-linked Ala-53 –> Thr mutation causes neurodegenerative disease with alpha-synuclein aggregation in transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 8968–8973.

Lee, H. J., Patel, S., & Lee, S. J. (2005). Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *The Journal of Neuroscience, 25*, 6016–6024.

Lee, H. J., Suk, J. E., Patrick, C., Bae, E. J., Cho, J. H., Rho, S., Hwang, D., Masliah, E., & Lee, S. J. (2010). Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. *The Journal of Biological Chemistry, 285*, 9262–9272.

Lee, K. W., Chen, W., Junn, E., Im, J. Y., Grosso, H., Sonsalla, P. K., Feng, X., Ray, N., Fernandez, J. R., Chao, Y., Masliah, E., Voronkov, M., Braithwaite, S. P., Stock, J. B., & Mouradian, M. M. (2011). Enhanced phosphatase activity attenuates alpha-synucleinopathy in a mouse model. *The Journal of Neuroscience, 31*, 6963–6971.

Leong, S. L., Cappai, R., Barnham, K. J., & Pham, C. L. (2009). Modulation of alpha-synuclein aggregation by dopamine: A review. *Neurochemical Research, 34*, 1838–1846.

Li, J. Y., Englund, E., Holton, J. L., Soulet, D., Hagell, P., Lees, A. J., Lashley, T., Quinn, N. P., Rehncrona, S., Bjorklund, A., Widner, H., Revesz, T., Lindvall, O., & Brundin, P. (2008). Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nature Medicine, 14*, 501–503.

Lin, X., Parisiadou, L., Gu, X. L., Wang, L., Shim, H., Sun, L., Xie, C., Long, C. X., Yang, W. J., Ding, J., Chen, Z. Z., Gallant, P. E., Tao-Cheng, J. H., Rudow, G., Troncoso, J. C., Liu, Z., Li, Z., & Cai, H. (2009). Leucine-rich repeat kinase 2 regulates the progression of neuropathology induced by Parkinson's-disease-related mutant alpha-synuclein. *Neuron, 64*, 807–827.

Lindersson, E., Beedholm, R., Hojrup, P., Moos, T., Gai, W., Hendil, K. B., & Jensen, P. H. (2004). Proteasomal inhibition by alpha-synuclein filaments and oligomers. *The Journal of Biological Chemistry, 279*, 12924–12934.

Liu, C. W., Corboy, M. J., DeMartino, G. N., & Thomas, P. J. (2003). Endoproteolytic activity of the proteasome. *Science, 299*, 408–411.

Lo Bianco, C., Ridet, J. L., Schneider, B. L., Deglon, N., & Aebischer, P. (2002). Alpha-synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 10813–10818.

Lo Bianco, C., Schneider, B. L., Bauer, M., Sajadi, A., Brice, A., Iwatsubo, T., & Aebischer, P. (2004). Lentiviral vector delivery of parkin prevents dopaminergic degeneration in an alpha-synuclein rat model of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 17510–17515.

Lotharius, J., Barg, S., Wiekop, P., Lundberg, C., Raymon, H. K., & Brundin, P. (2002). Effect of mutant alpha-synuclein on dopamine homeostasis in a new human mesencephalic cell line. *The Journal of Biological Chemistry, 277*, 38884–38894.

Lou, H., Montoya, S. E., Alerte, T. N., Wang, J., Wu, J., Peng, X., Hong, C. S., Friedrich, E. E., Mader, S. A., Pedersen, C. J., Marcus, B. S., McCormack, A. L., Di Monte, D. A., Daubner, S. C., & Perez, R. G. (2010). Serine 129 phosphorylation reduces the ability of alpha-synuclein to regulate tyrosine hydroxylase and protein phosphatase 2A in vitro and in vivo. *The Journal of Biological Chemistry, 285*, 17648–17661.

Luk, K. C., Kehm, V. M., Zhang, B., O' Brien, P., Trojanowski, J. Q., & Lee, V. M. (2012). Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. *The Journal of Experimental Medicine, 209*(5), 975–986.

Magen, I., & Chesselet, M. F. (2010). Genetic mouse models of Parkinson's disease The state of the art. *Progress in Brain Research, 184*, 53–87.

Maingay, M., Romero-Ramos, M., Carta, M., & Kirik, D. (2006). Ventral tegmental area dopamine neurons are resistant to human mutant alpha-synuclein overexpression. *Neurobiology of Disease*, *23*, 522–532.

Manning-Bog, A. B., McCormack, A. L., Purisai, M. G., Bolin, L. M., & Di Monte, D. A. (2003). Alpha-synuclein overexpression protects against paraquat-induced neurodegeneration. *Journal of Neuroscience*, *23*, 3095–3099.

Maroteaux, L., Campanelli, J., & Scheller, R. (1988). Synuclein: A neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 8*, 2804–2815.

Martin, L. J., Pan, Y., Price, A. C., Sterling, W., Copeland, N. G., Jenkins, N. A., Price, D. L., & Lee, M. K. (2006). Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. *The Journal of Neuroscience, 26*, 41–50.

Masliah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., Sagara, Y., Sisk, A., & Mucke, L. (2000). Dopaminergic loss and inclusion body formation in alpha-synuclein mice: Implications for neurodegenerative disorders. *Science, 287*, 1265–1269.

Masliah, E., Rockenstein, E., Veinbergs, I., Sagara, Y., Mallory, M., Hashimoto, M., & Mucke, L. (2001). Beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 12245–12250.

Masliah, E., Rockenstein, E., Adame, A., Alford, M., Crews, L., Hashimoto, M., Seubert, P., Lee, M., Goldstein, J., Chilcote, T., Games, D., & Schenk, D. (2005). Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron, 46*, 857–868.

Masuda, M., Dohmae, N., Nonaka, T., Oikawa, T., Hisanaga, S., Goedert, M., & Hasegawa, M. (2006). Cysteine misincorporation in bacterially expressed human alpha-synuclein. *FEBS Letters, 580*, 1775–1779.

Matsuoka, Y., Vila, M., Lincoln, S., McCormack, A., Picciano, M., LaFrancois, J., Yu, X., Dickson, D., Langston, W. J., McGowan, E., Farrer, M., Hardy, J., Duff, K., Przedborski, S., & Di Monte, D. A. (2001). Lack of nigral pathology in transgenic mice expressing human alpha-synuclein driven by the tyrosine hydroxylase promoter. *Neurobiology of Disease, 8*, 535–539.

Matthews, F. E., Brayne, C., Lowe, J., McKeith, I., Wharton, S. B., & Ince, P. (2009). Epidemiological pathology of dementia: Attributable-risks at death in the Medical Research Council Cognitive Function and Ageing study. *PLoS Medicine, 6*, e1000180.

Mazzulli, J. R., Mishizen, A. J., Giasson, B. I., Lynch, D. R., Thomas, S. A., Nakashima, A., Nagatsu, T., Ota, A., & Ischiropoulos, H. (2006). Cytosolic catechols inhibit alpha-synuclein aggregation and facilitate the formation of intracellular soluble oligomeric intermediates. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 26*, 10068–10078.

Mbefo, M. K., Paleologou, K. E., Boucharaba, A., Oueslati, A., Schell, H., Fournier, M., Olschewski, D., Yin, G., Zweckstetter, M., Masliah, E., Kahle, P. J., Hirling, H., & Lashuel, H. A. (2010). Phosphorylation of synucleins by members of the Polo-like kinase family. *The Journal of Biological Chemistry, 285*, 2807–2822.

McFarland, M. A., Ellis, C. E., Markey, S. P., & Nussbaum, R. L. (2008). Proteomics analysis identifies phosphorylation-dependent alpha-synuclein protein interactions. *Molecular & Cellular Proteomics, 7*, 2123–2137.

McFarland, N. R., Fan, Z., Xu, K., Schwarzschild, M. A., Feany, M. B., Hyman, B. T., & McLean, P. J. (2009). Alpha-synuclein S129 phosphorylation mutants do not alter nigrostriatal toxicity in a rat model of Parkinson disease. *Journal of Neuropathology and Experimental Neurology, 68*, 515–524.

McKeith, I. G., Dickson, D. W., Lowe, J., Emre, M., O'Brien, J. T., Feldman, H., Cummings, J., Duda, J. E., Lippa, C., Perry, E. K., Aarsland, D., Arai, H., Ballard, C. G., Boeve, B., Burn, D. J., Costa, D., Del Ser, T., Dubois, B., Galasko, D., Gauthier, S., Goetz, C. G., Gomez-Tortosa, E., Halliday, G., Hansen, L. A., Hardy, J., Iwatsubo, T., Kalaria, R. N., Kaufer, D., Kenny, R. A., Korczyn, A., Kosaka, K., Lee, V. M., Lees, A., Litvan, I., Londos, E., Lopez, O. L., Minoshima, S., Mizuno, Y., Molina, J. A., Mukaetova-Ladinska, E. B., Pasquier, F., Perry, R. H., Schulz, J. B., Trojanowski, J. Q., & Yamada, M. (2005). Diagnosis and management of dementia with Lewy bodies: Third report of the DLB Consortium. *Neurology, 65*, 1863–1872.

McNaught, K., & Jenner, P. (2001). Proteasomal function is impaired in substantial nigra in Parkinson's disease. *Neuroscience Letters, 297*, 191–194.

McNaught, K. S., Belizaire, R., Isacson, O., Jenner, P., & Olanow, C. W. (2003). Altered proteasomal function in sporadic Parkinson's disease. *Experimental Neurology, 179*, 38–46.

Michael, G. J., Esmailzadeh, S., Moran, L. B., Christian, L., Pearce, R. K., & Graeber, M. B. (2011). Up-regulation of metallothionein gene expression in Parkinsonian astrocytes. *Neurogenetics, 12*, 295–305.

Miller, D. W., Hague, S. M., Clarimon, J., Baptista, M., Gwinn-Hardy, K., Cookson, M. R., & Singleton, A. B. (2004). Alpha-synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. *Neurology, 62*, 1835–1838.

Mishizen-Eberz, A. J., Norris, E. H., Giasson, B. I., Hodara, R., Ischiropoulos, H., Lee, V. M., Trojanowski, J. Q., & Lynch, D. R. (2005). Cleavage of alpha-synuclein by calpain: Potential role in degradation of fibrillized and nitrated species of alpha-synuclein. *Biochemistry, 44*, 7818–7829.

Mollenhauer, B., El-Agnaf, O. M., Marcus, K., Trenkwalder, C., & Schlossmacher, M. G. (2010). Quantification of alpha-synuclein in cerebrospinal fluid as a biomarker candidate: Review of the literature and considerations for future studies. *Biomarkers in Medicine, 4*, 683–699.

Murphy, D. D., Rueter, S. M., Trojanowski, J. Q., & Lee, V. M. (2000). Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *The Journal of Neuroscience, 20*, 3214–3220.

Nasstrom, T., Fagerqvist, T., Barbu, M., Karlsson, M., Nikolajeff, F., Kasrayan, A., Ekberg, M., Lannfelt, L., Ingelsson, M., & Bergstrom, J. (2011). The lipid peroxidation products 4-oxo-2-nonenal and 4-hydroxy-2-nonenal promote the formation of alpha-synuclein oligomers with distinct biochemical, morphological, and functional properties. *Free Radical Biology & Medicine, 50*, 428–437.

Neumann, M., Kahle, P. J., Giasson, B. I., Ozmen, L., Borroni, E., Spooren, W., Muller, V., Odoy, S., Fujiwara, H., Hasegawa, M., Iwatsubo, T., Trojanowski, J. Q., Kretzschmar, H. A., & Haass, C. (2002). Misfolded proteinase K-resistant hyperphosphorylated alpha-synuclein in aged transgenic mice with locomotor deterioration and in human alpha-synucleinopathies. *The Journal of Clinical Investigation, 110*, 1429–1439.

Nieto, M., Gil-Bea, F. J., Dalfo, E., Cuadrado, M., Cabodevilla, F., Sanchez, B., Catena, S., Sesma, T., Ribe, E., Ferrer, I., Ramirez, M. J., & Gomez-Isla, T. (2006). Increased sensitivity to MPTP in human alpha-synuclein A30P transgenic mice. *Neurobiology of Aging*, *27*, 848–856.

Norris, E. H., Giasson, B. I., Hodara, R., Xu, S., Trojanowski, J. Q., Ischiropoulos, H., & Lee, V. M. (2005). Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome-mediated conformational alterations. *The Journal of Biological Chemistry, 280*, 21212–21219.

Norris, E. H., Uryu, K., Leight, S., Giasson, B. I., Trojanowski, J. Q., & Lee, V. M. (2007). Pesticide exposure exacerbates alpha-synucleinopathy in an A53T transgenic mouse model. *American Journal of Pathology*, *170*, 658–666.

Oksman, M., Tanila, H., & Yavich, L. (2009). Behavioural and neurochemical response of alpha-synuclein A30P transgenic mice to the effects of L-DOPA. *Neuropharmacology*, *56*, 647–652.

Ono, K., Ikemoto, M., Kawarabayashi, T., Ikeda, M., Nishinakagawa, T., Hosokawa, M., Shoji, M., Takahashi, M., & Nakashima, M. (2009). A chemical chaperone, sodium 4-phenylbutyric acid, attenuates the pathogenic potency in human alpha-synuclein A30P+ A53T transgenic mice. *Parkinsonism & Related Disorders, 15*, 649–654.

Orr, C. F., Rowe, D. B., Mizuno, Y., Mori, H., & Halliday, G. M. (2005). A possible role for humoral immunity in the pathogenesis of Parkinson's disease. *Brain: A Journal of Neurology, 128*, 2665–2674.

Orth, M., Tabrizi, S. J., Schapira, A. H., & Cooper, J. M. (2003). Alpha-synuclein expression in HEK293 cells enhances the mitochondrial sensitivity to rotenone. *Neuroscience Letters, 351*, 29–32.

Oueslati, A., Fournier, M., & Lashuel, H. A. (2010). Role of post-translational modifications in modulating the structure, function and toxicity of alpha-synuclein: Implications for Parkinson's disease pathogenesis and therapies. *Progress in Brain Research, 183*, 115–145.

Outeiro, T. F., Putcha, P., Tetzlaff, J. E., Spoelgen, R., Koker, M., Carvalho, F., Hyman, B. T., & McLean, P. J. (2008). Formation of toxic oligomeric alpha-synuclein species in living cells. *PLoS One, 3*, e1867.

Paleologou, K. E., Schmid, A. W., Rospigliosi, C. C., Kim, H. Y., Lamberto, G. R., Fredenburg, R. A., Lansbury, P. T., Jr., Fernandez, C. O., Eliezer, D., Zweckstetter, M., & Lashuel, H. A. (2008). Phosphorylation at Ser-129 but not the phosphomimics S129E/D inhibits the fibrillation of alpha-synuclein. *The Journal of Biological Chemistry, 283*, 16895–16905.

Paleologou, K. E., Kragh, C. L., Mann, D. M., Salem, S. A., Al-Shami, R., Allsop, D., Hassan, A. H., Jensen, P. H., & El-Agnaf, O. M. (2009). Detection of elevated levels of soluble alpha-synuclein oligomers in post-mortem brain extracts from patients with dementia with Lewy bodies. *Brain: A Journal of Neurology, 132*, 1093–1101.

Paleologou, K. E., Oueslati, A., Shakked, G., Rospigliosi, C. C., Kim, H. Y., Lamberto, G. R., Fernandez, C. O., Schmid, A., Chegini, F., Gai, W. P., Chiappe, D., Moniatte, M., Schneider, B. L., Aebischer, P., Eliezer, D., Zweckstetter, M., Masliah, E., & Lashuel, H. A. (2010). Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 30*, 3184–3198.

Pan-Montojo, F., Anichtchik, O., Dening, Y., Knels, L., Pursche, S., Jung, R., Jackson, S., Gille, G., Spillantini, M. G., Reichmann, H., & Funk, R. H. (2010). Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. *PLoS One, 5*, e8762.

Perez, R. G., Waymire, J. C., Lin, E., Liu, J. J., Guo, F., & Zigmond, M. J. (2002). A role for alpha-synuclein in the regulation of dopamine biosynthesis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 22*, 3090–3099.

Pienaar, I. S., Gotz, J., & Feany, M. B. (2010). Parkinson's disease: Insights from non-traditional model organisms. *Progress in Neurobiology, 92*, 558–571.

Polymeropoulos, M., Lavedan, C., Leroy, E., Ide, S., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E., Chandrasekharappa, S., Athanassiadou, A., Papapetropulos, T., Johnson, W., Lazzarini, A., Duvoisin, R., Di Iorio, G., Golbe, L., & Nussbaum, R. (1997). Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science, 276*, 2045–2047.

Poon, H. F., Frasier, M., Shreve, N., Calabrese, V., Wolozin, B., & Butterfield, D. A. (2005). Mitochondrial associated metabolic proteins are selectively oxidized in A30P alpha-synuclein transgenic mice–a model of familial Parkinson's disease. *Neurobiology of Disease*, *18*, 492–498.

Power, J. H., Shannon, J. M., Blumbergs, P. C., & Gai, W. P. (2002). Nonselenium glutathione peroxidase in human brain: Elevated levels in Parkinson's disease and dementia with lewy bodies. *The American Journal of Pathology, 161*, 885–894.

Pronin, A. N., Morris, A. J., Surguchov, A., & Benovic, J. L. (2000). Synucleins are a novel class of substrates for G protein-coupled receptor kinases. *The Journal of Biological Chemistry, 275*, 26515–26522.

Ramsey, C. P., Tsika, E., Ischiropoulos, H., & Giasson, B. I. (2010). DJ-1 deficient mice demonstrate similar vulnerability to pathogenic Ala53Thr human alpha-syn toxicity. *Human Molecular Genetics, 19*, 1425–1437.

Rathke-Hartlieb, S., Kahle, P. J., Neumann, M., Ozmen, L., Haid, S., Okochi, M., Haass, C., & Schulz, J. B. (2001). Sensitivity to MPTP is not increased in Parkinson's disease-associated mutant alpha-synuclein transgenic mice. *Journal of Neurochemistry, 77*, 1181–1184.

Richfield, E. K., Thiruchelvam, M. J., Cory-Slechta, D. A., Wuertzer, C., Gainetdinov, R. R., Caron, M. G., Di Monte, D. A., & Federoff, H. J. (2002). Behavioral and neurochemical effects of wild-type and mutated human alpha-synuclein in transgenic mice. *Experimental Neurology, 175*, 35–48.

Rockenstein, E., Mallory, M., Hashimoto, M., Song, D., Shults, C. W., Lang, I., & Masliah, E. (2002). Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters. *Journal of Neuroscience Research, 68*, 568–578.

Sanchez-Guajardo, V., Febbraro, F., Kirik, D., & Romero-Ramos, M. (2010). Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. *PLoS One, 5*, e8784.

Sato, H., Arawaka, S., Hara, S., Fukushima, S., Koga, K., Koyama, S., & Kato, T. (2011). Authentically phosphorylated alpha-synuclein at Ser129 accelerates neurodegeneration in a rat model of familial Parkinson's disease. *The Journal of Neuroscience, 31*, 16884–16894.

Schell, H., Hasegawa, T., Neumann, M., & Kahle, P. J. (2009). Nuclear and neuritic distribution of serine-129 phosphorylated alpha-synuclein in transgenic mice. *Neuroscience*, *160*, 796–804.

Sharon, R., Goldberg, M. S., Bar-Josef, I., Betensky, R. A., Shen, J., & Selkoe, D. J. (2001). Alpha-synuclein occurs in lipid-rich high molecular weight complexes, binds fatty acids, and shows homology to the fatty acid-binding proteins. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 9110–9115.

Sharon, R., Bar-Joseph, I., Frosch, M. P., Walsh, D. M., Hamilton, J. A., & Selkoe, D. J. (2003). The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. *Neuron, 37*, 583–595.

Shi, M., Bradner, J., Hancock, A. M., Chung, K. A., Quinn, J. F., Peskind, E. R., Galasko, D., Jankovic, J., Zabetian, C. P., Kim, H. M., Leverenz, J. B., Montine, T. J., Ginghina, C., Kang, U. J., Cain, K. C., Wang, Y., Aasly, J., Goldstein, D., & Zhang, J. (2011). Cerebrospinal fluid biomarkers for Parkinson disease diagnosis and progression. *Annals of Neurology, 69*, 570–580.

Simon-Sanchez, J., Schulte, C., Bras, J. M., Sharma, M., Gibbs, J. R., Berg, D., Paisan-Ruiz, C., Lichtner, P., Scholz, S. W., Hernandez, D. G., Kruger, R., Federoff, M., Klein, C., Goate, A., Perlmutter, J., Bonin, M., Nalls, M. A., Illig, T., Gieger, C., Houlden, H., Steffens, M., Okun, M. S., Racette, B. A., Cookson, M. R., Foote, K. D., Fernandez, H. H., Traynor, B. J., Schreiber, S., Arepalli, S., Zonozi, R., Gwinn, K., van der Brug, M., Lopez, G., Chanock, S. J., Schatzkin, A., Park, Y., Hollenbeck, A., Gao, J., Huang, X., Wood, N. W., Lorenz, D., Deuschl, G., Chen, H., Riess, O., Hardy, J. A., Singleton, A. B., & Gasser, T. (2009). Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nature Genetics, 41*, 1308–1312.

Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M. R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J., & Gwinn-Hardy, K. (2003). Alpha-synuclein locus triplication causes Parkinson's disease. *Science, 302*, 841.

Smith, W. W., Jiang, H., Pei, Z., Tanaka, Y., Morita, H., Sawa, A., Dawson, V. L., Dawson, T. M., & Ross, C. A. (2005a). Endoplasmic reticulum stress and mitochondrial cell death pathways mediate A53T mutant alpha-synuclein-induced toxicity. *Human Molecular Genetics, 14*, 3801–3811.

Smith, W. W., Margolis, R. L., Li, X., Troncoso, J. C., Lee, M. K., Dawson, V. L., Dawson, T. M., Iwatsubo, T., & Ross, C. A. (2005b). Alpha-synuclein phosphorylation enhances eosinophilic cytoplasmic inclusion formation in SH-SY5Y cells. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 25*, 5544–5552.

Snyder, H., Mensah, K., Theisler, C., Lee, J. M., Matouschek, A., & Wolozin, B. (2003). Aggregated and monomeric alpha-synuclein bind to the S6' proteasomal protein and inhibit proteasomal function. *The Journal of Biological Chemistry, 278*(14), 11753–11759.

Song, Y. J., Halliday, G. M., Holton, J. L., Lashley, T., O'Sullivan, S. S., McCann, H., Lees, A. J., Ozawa, T., Williams, D. R., Lockhart, P. J., & Revesz, T. R. (2009). Degeneration in different parkinsonian syndromes relates to astrocyte type and astrocyte protein expression. *Journal of Neuropathology and Experimental Neurology, 68*, 1073–1083.

Spillantini, M. G., & Goedert, M. (2000). The alpha-synucleinopathies: Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. *Annals of the New York Academy of Sciences, 920*, 16–27.

Su, X., Federoff, H. J., & Maguire-Zeiss, K. A. (2009). Mutant alpha-synuclein overexpression mediates early proinflammatory activity. *Neurotoxicity Research*, *16*(3), 238–254.

Su, X., Maguire-Zeiss, K. A., Giuliano, R., Prifti, L., Venkatesh, K., & Federoff, H. J. (2008). Synuclein activates microglia in a model of Parkinson's disease. *Neurobiology of Aging, 29*, 1690–1701.

Su, L. J., Auluck, P. K., Outeiro, T. F., Yeger-Lotem, E., Kritzer, J. A., Tardiff, D. F., Strathearn, K. E., Liu, F., Cao, S., Hamamichi, S., Hill, K. J., Caldwell, K. A., Bell, G. W., Fraenkel, E., Cooper, A. A., Caldwell, G. A., McCaffery, J. M., Rochet, J. C., & Lindquist, S. (2010). Compounds from an unbiased chemical screen reverse both ER-to-Golgi trafficking defects and mitochondrial dysfunction in Parkinson's disease models. *Disease Models & Mechanisms, 3*, 194–208.

Sugeno, N., Takeda, A., Hasegawa, T., Kobayashi, M., Kikuchi, A., Mori, F., Wakabayashi, K., & Itoyama, Y. (2008). Serine 129 phosphorylation of alpha-synuclein induces unfolded protein response-mediated cell death. *The Journal of Biological Chemistry, 283*, 23179–23188.

Tanaka, Y., Engelender, S., Igarashi, S., Rao, R. K., Wanner, T., Tanzi, R., Sawa, A. L., Dawson, V., Dawson, T. M., & Ross, C. A. (2001). Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. *Human Molecular Genetics, 10*, 919–992.

Tehranian, R., Montoya, S. E., Van Laar, A. D., Hastings, T. G., & Perez, R. G. (2006). Alpha-synuclein inhibits aromatic amino acid decarboxylase activity in dopaminergic cells. *Journal of Neurochemistry, 99*, 1188–1196.

Tetzlaff, J. E., Putcha, P., Outeiro, T. F., Ivanov, A., Berezovska, O., Hyman, B. T., & McLean, P. J. (2008). CHIP targets toxic alpha-synuclein oligomers for degradation. *The Journal of Biological Chemistry, 283*, 17962–17968.

Theodore, S., Cao, S., McLean, P. J., & Standaert, D. G. (2008). Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. *Journal of Neuropathology and Experimental Neurology, 67*, 1149–1158.

Tofaris, G. K., Garcia Reitbock, P., Humby, T., Lambourne, S. L., O'Connell, M., Ghetti, B., Gossage, H., Emson, P. C., Wilkinson, L. S., Goedert, M., & Spillantini, M. G. (2006). Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1–120): Implications for Lewy body disorders. *The Journal of Neuroscience, 26*, 3942–3950.

Tokuda, T., Qureshi, M. M., Ardah, M. T., Varghese, S., Shehab, S. A., Kasai, T., Ishigami, N., Tamaoka, A., Nakagawa, M., & El-Agnaf, O. M. (2010). Detection of elevated levels of alpha-synuclein oligomers in CSF from patients with Parkinson disease. *Neurology, 75*, 1766–1772.

Tomas-Zapico, C., Diez-Zaera, M., Ferrer, I., Gomez-Ramos, P., Moran, M. A., Miras-Portugal, M. T., Diaz-Hernandez, M., & Lucas, J. J. (2011). Alpha-synuclein accumulates in huntingtin inclusions but forms independent filaments and its deficiency attenuates early phenotype in a mouse model of Huntington's disease. *Human Molecular Genetics, 21*(3), 495–510.

Tsika, E., Moysidou, M., Guo, J., Cushman, M., Gannon, P., Sandaltzopoulos, R., Giasson, B. I., Krainc, D., Ischiropoulos, H., & Mazzulli, J. R. (2010). Distinct region-specific alpha-synuclein oligomers in A53T transgenic mice: Implications for neurodegeneration. *Journal of Neuroscience*, *30*, 3409–3418.

Ubhi, K., Low, P., & Masliah, E. (2011). Multiple system atrophy: A clinical and neuropathological perspective. *Trends in Neurosciences, 34*, 581–590.

Ueda, K., Fukushima, H., Masliah, E., Xia, Y., Iwai, A., Yoshimoto, M., Otero, D. A., Kondo, J., Ihara, Y., & Saitoh, T. (1993). Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 11282–11286.

Ulusoy, A., Febbraro, F., Jensen, P. H., Kirik, D., & Romero-Ramos, M. (2010). Co-expression of C-terminal truncated alpha-synuclein enhances full-length alpha-synuclein-induced pathology. *The European Journal of Neuroscience, 32*, 409–422.

van der Putten, H., Wiederhold, K. H., Probst, A., Barbieri, S., Mistl, C., Danner, S., Kauffmann, S., Hofele, K., Spooren, W. P., Ruegg, M. A., Lin, S., Caroni, P., Sommer, B., Tolnay, M., & Bilbe, G. (2000). Neuropathology in mice expressing human alpha-synuclein. *The Journal of Neuroscience, 20*, 6021–6029.

Ved, R., Saha, S., Westlund, B., Perier, C., Burnam, L., Sluder, A., Hoener, M., Rodrigues, C. M., Alfonso, A., Steer, C., Liu, L., Przedborski, S., & Wolozin, B. (2005). Similar patterns of mitochondrial vulnerability and rescue induced by genetic modification of alpha-synuclein, parkin, and DJ-1 in *Caenorhabditis elegans*. *The Journal of Biological Chemistry, 280*, 42655–42668.

Vitner, E. B., Platt, F. M., & Futerman, A. H. (2010). Common and uncommon pathogenic cascades in lysosomal storage diseases. *The Journal of Biological Chemistry, 285*, 20423–20427.

Vogiatzi, T., Xilouri, M., Vekrellis, K., & Stefanis, L. (2008). Wild type alpha-synuclein is degraded by chaperone-mediated autophagy and macroautophagy in neuronal cells. *The Journal of Biological Chemistry, 283*, 23542–23556.

Volpicelli-Daley, L. A., Luk, K. C., Patel, T. P., Tanik, S. A., Riddle, D. M., Stieber, A., Meaney, D. F., Trojanowski, J. Q., & Lee, V. M. (2011). Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron, 72*, 57–71.

von Coelln, R., Thomas, B., Andrabi, S. A., Lim, K. L., Savitt, J. M., Saffary, R., Stirling, W., Bruno, K., Hess, E. J., Lee, M. K., Dawson, V. L., & Dawson, T. M. (2006). Inclusion body formation and neurodegeneration are parkin independent in a mouse model of alpha-synucleinopathy. *The Journal of Neuroscience, 26*, 3685–3696.

Wakamatsu, M., Ishii, A., Ukai, Y., Sakagami, J., Iwata, S., Ono, M., Matsumoto, K., Nakamura, A., Tada, N., Kobayashi, K., Iwatsubo, T., & Yoshimoto, M. (2007). Accumulation of phosphorylated alpha-synuclein in dopaminergic neurons of transgenic mice that express human alpha-synuclein. *Journal of Neuroscience Research, 85*, 1819–1825.

Wakamatsu, M., Ishii, A., Iwata, S., Sakagami, J., Ukai, Y., Ono, M., Kanbe, D., Muramatsu, S., Kobayashi, K., Iwatsubo, T., & Yoshimoto, M. (2008). Selective loss of nigral dopamine neurons induced by overexpression of truncated human alpha-synuclein in mice. *Neurobiology of Aging, 29*, 574–585.

Wang, L., Fleming, S. M., Chesselet, M. F., & Tache, Y. (2008). Abnormal colonic motility in mice overexpressing human wild-type alpha-synuclein. *NeuroReport, 19*, 873–876.

Wang, W., Perovic, I., Chittuluru, J., Kaganovich, A., Nguyen, L. T., Liao, J., Auclair, J. R., Johnson, D., Landeru, A., Simorellis, A. K., Ju, S., Cookson, M. R., Asturias, F. J., Agar, J. N., Webb, B. N., Kang, C., Ringe, D., Petsko, G. A., Pochapsky, T. C., & Hoang, Q. Q. (2011). A soluble alpha-synuclein construct forms a dynamic tetramer. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 17797–17802.

Weinreb, P., Zhen, W., Poon, A., Conway, K., & Lansbury, P. J. (1996). NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry, 35*, 13709–13715.

Weintraub, D., Doshi, J., Koka, D., Davatzikos, C., Siderowf, A. D., Duda, J. E., Wolk, D. A., Moberg, P. J., Xie, S. X., & Clark, C. M. (2011). Neurodegeneration across stages of cognitive decline in Parkinson disease. *Archives of Neurology, 68*, 1562–1568.

Wersinger, C., & Sidhu, A. (2003). Attenuation of dopamine transporter activity by alpha-synuclein. *Neuroscience Letters, 340*, 189–192.

Wood, S. J., Wypych, J., Steavenson, S., Louis, J. C., Citron, M., & Biere, A. L. (1999). Alpha-synuclein fibrillogenesis is nucleation dependent. Implications for the pathogenesis of Parkinson's disease. *The Journal of Biological Chemistry, 274*, 19509–19512.

Xilouri, M., & Stefanis, L. (2011). Autophagic pathways in Parkinson disease and related disorders. *Expert Reviews in Molecular Medicine, 13*, e8.

Xu, J., Kao, S. Y., Lee, F. J., Song, W., Jin, L. W., & Yankner, B. A. (2002). Dopamine-dependent neurotoxicity of alpha-synuclein: A mechanism for selective neurodegeneration in Parkinson disease. *Nature Medicine, 8*, 600–606.

Yamada, M., Iwatsubo, T., Mizuno, Y., & Mochizuki, H. (2004). Overexpression of alpha-synuclein in rat substantia nigra results in loss of dopaminergic neurons, phosphorylation of alpha-synuclein and activation of caspase-9: Resemblance to pathogenetic changes in Parkinson's disease. *Journal of Neurochemistry, 91*, 451–461.

Yasuda, T., Miyachi, S., Kitagawa, R., Wada, K., Nihira, T., Ren, Y. R., Hirai, Y., Ageyama, N., Terao, K., Shimada, T., Takada, M., Mizuno, Y., & Mochizuki, H. (2007). Neuronal specificity of alpha-synuclein toxicity and effect of Parkin co-expression in primates. *Neuroscience, 144*, 743–753.

Yavich, L., Jakala, P., & Tanila, H. (2006). Abnormal compartmentalization of norepinephrine in mouse dentate gyrus in alpha-synuclein knockout and A30P transgenic mice. *Journal of Neurochemistry*, *99*, 724–732.

Yavich, L., Oksman, M., Tanila, H., Kerokoski, P., Hiltunen, M., van Groen, T., Puolivali, J., Mannisto, P. T., Garcia-Horsman, A., MacDonald, E., Beyreuther, K., Hartmann, T., & Jakala, P. (2005). Locomotor activity and evoked dopamine release are reduced in mice overexpressing A30P-mutated human alpha-synuclein. *Neurobiology of Disease*, *20*, 303–313.

Yavich, L., Tanila, H., Vepsalainen, S., & Jakala, P. (2004). Role of alpha-synuclein in presynaptic dopamine recruitment. *Journal of Neuroscience*, *24*, 11165–11170.

Yu, W. H., Matsuoka, Y., Sziraki, I., Hashim, A., Lafrancois, J., Sershen, H., & Duff, K. E. (2008). Increased dopaminergic neuron sensitivity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in transgenic mice expressing mutant A53T alpha-synuclein. *Neurochemical Research*, *33*, 902–911.

Zaccai, J., Brayne, C., McKeith, I., Matthews, F., & Ince, P. G. (2008). Patterns and stages of alpha-synucleinopathy: Relevance in a population-based cohort. *Neurology, 70*, 1042–1048.

Zarranz, J. J., Alegre, J., Gomez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atares, B., Llorens, V., Gomez Tortosa, E., del Ser, T., Munoz, D. G., & de Yebenes, J. G. (2004). The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Annals of Neurology, 55*, 164–173.

Zhou, W., & Freed, C. R. (2005). DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T alpha-synuclein toxicity. *The Journal of Biological Chemistry, 280*, 43150–43158.

Zhou, W., Milder, J. B., & Freed, C. R. (2008). Transgenic mice overexpressing tyrosine-to-cysteine mutant human alpha-synuclein: A progressive neurodegenerative model of diffuse Lewy body disease. *The Journal of Biological Chemistry, 283*, 9863–9870.

Autophagic Pathways and Parkinson Disease

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Abstract

Autophagy is responsible for the degradation and recycling of intracellular material including organelles, cytosolic proteins, and accumulated misfolded proteins. Increasing evidence implicates autophagy dysfunction in several neurodegenerative disorders including Parkinson's disease (PD). In this chapter, we analyze recent studies that provide new links between genes associated to PD and the autophagy process. Mutations in PD-associated proteins like α-synuclein, parkin, PINK1, LRRK2, DJ-1, UCH-L1, ATP12A3, and GBA have been recently directly or indirectly linked to alterations in the autophagic system, thereby contributing to the understanding of the role of this system in the pathogenesis of Parkinson's disease.

Keywords

α-synuclein • ATP12A3 • Autophagy • DJ-1 • GBA • LRRK2 • Parkin • Parkinson's disease • PINK1 • UCH-L1

List of Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
CMA	Chaperone-mediated autophagy
GBase	Glucocerebrosidase protein
GD	Gaucher's disease
HD	Huntington's disease
LB	Lewy bodies
LIR	LC3-interacting region
LMP	Lysosomal membrane permeabilization
LSD	Lysosomal storage disorders
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MTOC	Microtubule-organizing center
PD	Parkinson's disease
ROS	Reactive oxygen species
SNpc	Substantia nigra pars compacta
UBA domain	Ubiquitin-associated domain
UPS	Ubiquitin-proteasome system
WT	Wild type

1 Introduction

In the last few years, autophagy malfunction had been profusely linked to a growing number of human disorders (Levine and Kroemer 2008; Mizushima et al. 2008), including several neurodegenerative diseases such as Parkinson's disease (Crews et al. 2010; Dagda et al. 2009; Levine and Kroemer 2008;

Narendra et al. 2008; Pan et al. 2008a; Stefanis et al. 2001; Vives-Bauza et al. 2009; Webb et al. 2003; Dehay et al. 2010; Vila et al. 2011), Alzheimer's disease (AD) (Boland et al. 2008; Ling and Salvaterra 2009; Nixon et al. 2005; Pickford et al. 2008), Huntington's disease (HD) (Kegel et al. 2000; Nagata et al. 2004; Petersen et al. 2001; Ravikumar et al. 2004), and amyotrophic lateral sclerosis (ALS) (Morimoto et al. 2007).

The first evidence that the lysosomal system could be altered in neurodegenerative disorders came from the observed accumulation of autophagosomes in brain samples from patients and animal models of these diseases (Anglade et al. 1997; Kegel et al. 2000; Nixon et al. 2005; Sapp et al. 1997; Dehay et al. 2010). This increase in autophagosomes was originally attributed to an activation of the autophagic process aimed at eliminating the abnormal accumulation of proteins occurring in these diseases. However, now we know that accumulation of autophagosomes can also be secondary to a failure in the completion of the autophagic process. After some years of abundant research in the field, a better understanding of the role of autophagy in the intracellular quality control system, particularly in neurons, is now providing new evidences to identify the specific autophagic pathways and steps affected in each of the neurodegenerative pathologies. All the accumulated information may provide new therapeutic approaches aimed at modulating autophagy to attenuate, halt, or prevent neurodegeneration.

2 Types of Autophagy

Autophagy, from the Greek "auto" (self) and "phagy" (eating), refers to the delivery of intracellular components to *lysosomes* for degradation. Lysosomes are intracellular organelles which contain acid hydrolases able to break up all type of intracellular and extracellular components and macromolecules (Bohley and Seglen 1992; Dice 2000; Mullins and Bonifacino 2001). In mammalian cells, there are various types of autophagy (Fig. 1) – macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) – differing in their mechanisms, their specificity of the substrate to be degraded, their regulation, and their function (Cuervo 2006; He and Klionsky 2006; Klionsky 2005; Levine and Kroemer 2008). Although each type of autophagy displays particular features, they all share some common components and are highly interconnected, resulting in a cross talk between the different autophagic mechanisms as well as between other non-lysosomal proteolytic systems, such as the ubiquitin-proteasome system (UPS). This connection between the different proteolytic systems in the cell allows the activation of compensatory mechanisms, when necessary (Kaushik et al. 2008; Massey et al. 2006a; Nedelsky et al. 2008; Pandey et al. 2007).

In *chaperone-mediated autophagy* (CMA), specific cytosolic proteins are directly translocated into the lysosomes through the lysosomal membrane without any vesicular trafficking (Cuervo and Dice 1996; Majeski and Dice 2004; Massey et al. 2004) (Fig. 1). All CMA substrates contain a sequence biochemically related to the pentapeptide KFERQ (Dice 1990), which targets the substrate to the

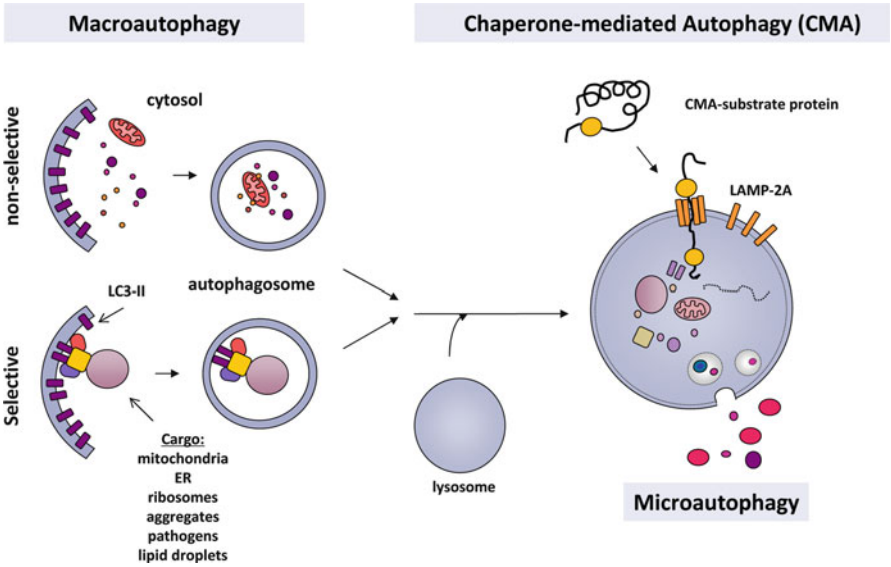

Fig. 1 *Autophagy pathways*. Three types of autophagy occur in mammalian cells. In chaperone-mediated autophagy (CMA), specific cytosolic proteins containing the CMA-targeting motif are recognized by a chaperone complex and sent to lysosome where they bind the membrane receptor LAMP-2A and directly are translocated into the lysosomes for degradation. Microautophagy involves the sequestration and degradation of complete regions of the cytosol (including proteins and organelles) through invaginations and tubulations of the lysosomal membrane. In macroautophagy intracellular components are enclosed in a double-membrane vesicle called autophagosome. This vesicle is then fused with lysosomes, which provides the hydrolytic enzymes required for the total degradation of the sequestered material. Macroautophagy can be a nonselective process engulfing in-bulk portions of the cytosol but also can be a selective mechanism where specific substrates are recognized by different autophagic adapters and sent selectively to degradation

lysosome membrane with the help of some cytosolic chaperones and binding to receptor membrane protein LAMP-2A (Agarraberes and Dice 2001; Cuervo and Dice 1996). The substrate protein is then translocated into the lysosomal matrix where it is rapidly degraded by the hydrolytic enzymes. CMA is activated by nutrient stress (Cuervo et al. 1995) but also in response to some other stress conditions like oxidative stress or exposure to toxic compounds (Kiffin et al. 2004).

Microautophagy involves the sequestration and degradation of complete regions of the cytosol by the lysosomal system, including cytosolic proteins and organelles, through the engulfment of the lysosomal membrane itself (Ahlberg and Glaumann 1985; Marzella et al. 1982; Mortimore et al. 1988; Sakai et al. 1998). The lysosomal membrane can form invaginations or fingerlike protrusions to form vesicles loaded with cytoplasmic components. Once the vesicle is inside the lysosome, the membrane disappears and the sequestered material is degraded (Fig. 1). To date, the mechanisms of induction or regulation of microautophagy in mammalian cells remain unknown, and it is assumed that microautophagy participates in the continuous basal turnover of cellular components under normal cellular conditions.

Macroautophagy is the best-characterized form of autophagy and is frequently referred just as "autophagy." Macroautophagy is responsible for the degradation of most intracellular components as a major mechanism to maintain cellular homeostasis and appropriate balance between protein synthesis and degradation. During macroautophagy, a portion of cytosol, including entire organelles, is surrounded by an intracellular membrane to form a double-membrane organelle called *autophagosome*, which contains the sequestered components (Wang and Klionsky 2003). This vesicle fusions later with lysosomes, which provides the hydrolytic enzymes required for the total degradation of the sequestered material (Fig. 1). Under stress conditions, autophagy can be activated as part of the cellular stress-response mechanism, as it occurs, for instance, in early stages of nutrient deprivation in order to provide amino acids and other essential basic elements (He and Klionsky 2009; Ravikumar et al. 2010). In addition to this well-known role of autophagy, this pathway also participates in other cell functions such as cell differentiation, tissue remodeling, development, host-to-pathogen response, and removal of toxic or abnormal intracellular components (Mizushima et al. 2008).

The demand for autophagy activity varies among different tissues and cell types. In most tissues, macroautophagy is mainly induced upon stress conditions. Consequently, a failure in macroautophagy can result in an increased cell susceptibility to stress, since the cell is no longer able to respond properly to stress situations, but under basal conditions cell survival may not be compromised. In contrast, postmitotic cells like neurons require an effective and constitutive macroautophagy activity to maintain cell homeostasis. Consequently, any disruption of autophagy in neurons results in an accumulation of abnormal proteins within the cell and leads to neurodegeneration, even in the absence of any aggravating event such as the presence of mutant or prone-to-aggregate proteins (Hara et al. 2006; Komatsu et al. 2006; Wang and Klionsky 2003).

Macroautophagy is a complex mechanism that can be divided into different steps: induction, elongation of the limiting membrane or phagophore, cargo recognition of the substrate to be degraded, fusion of the autophagosome with lysosomes, and clearance of the cargo (Fig. 1). While macroautophagy was originally described as a nonselective step in the autophagy process, recent studies demonstrated the selectivity of the autophagic degradation process. As a result, new types of autophagy have been described according to the selective substrate involved, including *mitophagy* (i.e., selective degradation of mitochondria by autophagy) (Kim et al. 2007), pexophagy (i.e., selective degradation of peroxisomes by autophagy) (Dunn et al. 2005; Sakai et al. 1998), reticulophagy (i.e., selective degradation of endoplasmic reticulum portions by autophagy) (Bernales et al. 2007), ribophagy (i.e., selective degradation of ribosomes by autophagy) (Kraft et al. 2008), lipomacrophagy (i.e., selective degradation of lipid droplets portions by autophagy) (Singh et al. 2009), xenophagy (i.e., selective degradation of intracellular pathogens by autophagy) (Levine 2005), and aggregophagy (i.e., selective autophagic degradation of ubiquitinated aggregates).

The intraneuronal accumulation of autophagosomes and aberrant or misfolded proteins that eventually aggregate forming inclusion bodies is observed in affected

brain regions of several neurodegenerative disorders and represent one of the hallmarks of these diseases, thus emphasizing the role of the autophagy system in the pathogenesis of these disorders. However, in each neurodegenerative disorders, autophagy can be affected at different step/s of the process, and therefore, the potential therapeutic approaches aimed to reestablish normal degradation activity in these diseases should differ depending on the autophagy step that is impaired.

3 Role of Autophagy in Neurodegenerative Disorders

Growing evidence indicates a role of autophagy failure in neurodegenerative disorders (Martinez-Vicente and Cuervo 2007; Mizushima et al. 2008; Wong and Cuervo 2010). When the intracellular degradative systems become compromised, the protein quality control of the cell fails, and as a consequence, many altered proteins (including mutant, posttranslational modified, or damaged proteins) often have the tendency to organize into complex structures like oligomers, protofibrils, and small aggregates that may be toxic to the cells. As part of the protein quality control system, cells have developed mechanisms to fight against protein misfolding, first trying to identify and repair altered proteins with chaperones (Voisine et al. 2010) and then, if necessary, eliminating the aberrant misfolded proteins through UPS or autophagy. Defects in either molecular chaperones or proteolytic systems have been identified in different neurodegenerative disorders, which have been proposed to contribute to the pathogenesis of these diseases. A failure in macroautophagy, in particular, can be dramatic for the cell since it is the only degradative mechanism able to eliminate the toxic protofibrils and aggregates once these have become insoluble. Furthermore, autophagy also contributes to the selective turnover of organelles, such as mitochondria, through the selective macroautophagy machinery. Therefore, an impairment in autophagy like the one observed in some neurodegenerative disease can lead to the accumulation of old or damaged mitochondria that become a new source of reactive oxygen species (ROS), thereby contributing to oxidative stress and aggravating the consequences of the pathologic process. Further supporting a role for autophagy in neurodegeneration, it has been reported that pharmacological or genetic inhibition of autophagy worsens the pathology and increases the number of aggregates in different cellular and animal models of neurodegeneration, while activation of macroautophagy with drugs like rapamycin can reduce the number of intracellular aggregates and ameliorates the neurodegenerative process in these experimental systems (Bove et al. 2011).

4 Autophagy and Parkinson's Disease

Parkinson's disease is a common neurodegenerative disorder of unknown origin mainly characterized by the loss of dopaminergic neurons from the substantia nigra pars compacta (SNpc) and the presence, in the affected brain

regions, of ubiquitinated intraneuronal proteinaceous cytoplasmic inclusions called *Lewy bodies* (LB). The presence of Lewy bodies in PD suggests that defective protein handling may contribute to the pathogenesis of the disease. The first evidence linking autophagy with PD involved the observation of accumulated autophagosomes in the SNpc of postmortem brain samples from PD patients (Anglade et al. 1997; Voisine et al. 2010; Zhu et al. 2003). Recent studies confirmed this observation (Chu et al. 2009; Dehay et al. 2010). More recently, a pathogenic autophagy impairment was observed in the MPTP mouse model of PD. MPTP-treated mice exhibit accumulations of undegraded autophagosomes and reductions in the number of functional lysosomes in SNpc dopaminergic neurons that precede cell death in this model. Lysosomal deficiency in this model was attributed to abnormal lysosomal membrane permeabilization (LMP) mediated by mitochondria-derived oxidative attack. Following LMP, the release of hydrolytic lysosomal enzymes to the cytosol can, among other consequences, abolish the lysosomal capacity of the cell, which results in an accumulation of undegraded autophagosomes that cannot be cleared by dysfunctional lysosomes (Dehay et al. 2010). Pharmacological reactivation of lysosomal-mediated degradation by *rapamycin* in MPTP-treated mice can restore autophagosome clearance and attenuate SNpc dopaminergic cell death in this model of PD (Dehay et al. 2010). Rapamycin also provides neuroprotection in other in vivo models of PD, such as 6-OHDA-treated mice (Santini et al. 2009), lactacystin-microinjected mice (Pan et al. 2008b), transgenic mice overexpressing wild-type (WT) α-synuclein (Crews et al. 2010), or paraquat-treated drosophila (Bjedov et al. 2010). The neuroprotective effects of rapamycin in all these models were attributed, at least in part, to the activation of autophagy.

5 PD-Related Proteins Involved in Autophagy

Genetic studies have provided important insights into the pathological mechanism underlying PD. Among all the genes linked to familial forms of PD, many of them appear now related to autophagy pathways, thereby providing new evidences and mechanisms to potentially explain the link between autophagic failure and PD. In this section, we will discuss some of the findings linking PD-related genes/proteins with the autophagy system.

5.1 α-Synuclein

α-*Synuclein* is a small protein encoded by the SNCA gene. Three missense point mutations have been linked to autosomal dominant forms of PD (A30P, A53T, and E46K), as well as duplications and triplications of the gene (Chartier-Harlin et al. 2004; Ibanez et al. 2004; Kruger et al. 1998; Polymeropoulos et al. 1997; Singleton et al. 2003; Zarranz et al. 2004). α-Synuclein is mostly found in neuronal presynaptic terminals, and although its exact functions remain unknown, it has

been proposed to be involved in the regulation of synaptic vesicles, neurotransmitter release, molecular chaperone activity in the SNARE complexes, and vesicle trafficking (Bonini and Giasson 2005; Burre et al. 2010; Cooper et al. 2006). α-Synuclein is a major component of LB and is normally degraded by different proteolytic systems, including the UPS (Bennett et al. 1999; Imai et al. 2003), macroautophagy, and CMA (Cuervo et al. 2004; Vogiatzi et al. 2008; Webb et al. 2003; Xilouri et al. 2009). As PD progresses, alterations in UPS and autophagy systems may impair the capacity of the cell to properly handle and execute α-synuclein turnover and thus contribute to the formation of protein inclusions like LB. The presence of aberrant or mutated forms of α-synuclein can induce the failure of one of these proteolytic pathways and interfere with the rest of proteolytic mechanisms, given the important cross talk between all the proteolytic systems of the cell.

α-Synuclein contains the CMA-targeting motif needed for the recognition by the hsc70 chaperone, which delivers the substrate to the lysosomal membrane receptor LAMP-2A in order to be translocated into the lysosomal lumen for its degradation. The two main point mutations in the α-synuclein gene (A30P and A53T) that lead to familial forms of PD can affect CMA activity. When one of these α-synuclein mutants is recognized through its CMA-motif, it is targeted to lysosomes and binds LAMP-2A receptor with higher affinity than the WT protein. However, the translocation of mutant α-synuclein inside the lysosome is impaired, and therefore, it cannot be degraded by CMA. In addition, mutant α-synuclein remains bound to the CMA translocation complex, thereby blocking not only their own degradation but also the degradation of other CMA substrates (Cuervo et al. 2004). The pathogenic effect of mutant α-synuclein on CMA activity was also observed in neuronal cell cultures (Vogiatzi et al. 2008; Xilouri et al. 2009). Additionally, CMA failure linked to α-synuclein pathogenic mutations is followed by a compensatory induction of macroautophagy, as it has been observed in other situations (Massey et al. 2006b).

Abnormal high levels of WT α-synuclein are also associated with PD. In cellular models, overexpression of WT α-synuclein impairs CMA activity and contributes to cell death through an effect on CMA-substrate MEF2D (Yang et al. 2009). MEF2D is a transcriptional factor required for neuronal survival that is shuttled from the neuronal nucleus to the cytoplasm, where it interacts with chaperone protein hsc70 before being targeted to lysosomes for degradation. High levels of α-synuclein (like those observed in PD patients with triplications of the α-synuclein gene) in α-synuclein-overexpressing cellular and animal models impair CMA-mediated degradation of MEF2D, leading to the accumulation of MEF2D in the cytoplasm and subsequent neuronal death (Yang et al. 2009).

In addition to the rare α-synuclein mutations and duplications/triplications of the gene (which occur in less than 2 % of patients from familial PD), the most common posttranslational modifications of α-synuclein have been described in sporadic PD patients and experimental PD models (Anderson et al. 2006; McFarlane et al. 2005; Paxinou et al. 2001; Smith et al. 2005; Uversky et al. 2005; Volles and Lansbury 2003). These modifications include phosphorylation, ubiquitination, nitration, and

oxidation of α-synuclein, as well as α-synuclein modifications caused by its interaction with oxidized dopamine (Norris et al. 2005). Some of these posttranslational modifications of WT α-synuclein can promote the formation of oligomeric protofibril intermediates, which usually evolve into insoluble α-synuclein fibrils, the main component of LB (Chen and Feany 2005; Conway et al. 2000; Rochet et al. 2004; Uversky et al. 2005). The ability of the modified α-synuclein forms to be degraded by CMA depends on the type of modification (Martinez-Vicente et al. 2008). For instance, phosphorylation, nitration, and oxidation of α-synuclein decrease the capacity of α-synuclein to be translocated and eliminated through the CMA pathway, thereby increasing the amount of these α-synuclein soluble forms into the cytosol and thus the possibility to interact with other molecules and become oligomers or aggregates. In contrast, dopamine-modified α-synuclein behaves as the A30P and A53T mutant α-synuclein, thereby remaining bound to the CMA translocation complex and blocking the degradation of other CMA substrates. The origin of this blockage seems to reside in the ability of dopamine-modified α-synuclein to form oligomers on the lysosomal surface after binding as a monomer to the CMA translocation complex. The formation of these clusters on the lysosomal membrane seems to prevent the binding and translocation of other proteins. The consequences of this inability to degrade modified forms of α-synuclein result in increased cytosolic concentrations of the protein, thus favoring its oligomerization. On the other hand, normal substrate proteins for CMA can no longer be turned over through this pathway and also end up accumulating inside the affected cells.

Overexpression of WT α-synuclein can also inhibit macroautophagy activity, as it has been recently showed in PD models (Winslow et al. 2010). In particular, the overexpression of α-synuclein causes the loss of function of Rab1, a protein involved in secretory pathways. Inhibition of Rab1a activity, in turn, causes mislocalization of Atg9, an autophagy protein essential for autophagosome formation. As a consequence, autophagosome formation is impaired, thereby resulting in an accumulation of autophagic substrates.

5.2 LRRK2

*LRRK*2 is a large protein with several domains including, among others, a ROC domain (with GTPase activity), a COR (C-terminal of ROC) domain, a leucine-rich repeat domain (LRR), and a serine/threonine kinase domain (Gloeckner et al. 2006; Greggio et al. 2006, 2008; Greggio and Cookson 2009; Smith et al. 2006; West et al. 2005). Several mutations in this protein cause an autosomal dominant form of PD (Paisan-Ruiz et al. 2004) with pathological and clinical features very similar to late-onset sporadic PD, including the presence, in most cases, of LB. The majority of these pathological mutations are localized in the GTPase or the kinase domain, being G2019S the most common mutation present in PD cases (Dachsel et al. 2010; Paisan-Ruiz et al. 2004, 2008; Zimprich et al. 2004). Many different physiological roles for LRRK2 have been proposed, but the putative substrate/s that LRRK2

might phosphorylate in vivo remains controversial. So far, it is known that the kinase activity is required for its GTPase activity and this kinase activity depends on the formation of LRRK2 dimers. It has also been shown that LRRK2 kinase activity is required for neurodegeneration, and therefore, inhibition of its kinase activity has neuroprotective effects in PD models (Greggio et al. 2006; Smith et al. 2006).

In the last few years, LRRK2 has been suggested to play a role in numerous different cellular mechanisms. Some of the suggested functions of LRRK2 include modulation of protein translation through the direct phosphorylation of the translation initiation factor 4E-BP1 (Imai et al. 2008) and the interaction with microRNAs that regulate protein translation (Gehrke et al. 2010). In cellular models, apoptosis also seems to be regulated by LRRK2 through the direct interaction of LRRK2 with the death adaptor FADD (Ho et al. 2009). Different groups have shown that LRRK2 can also be involved in neuronal morphogenesis (MacLeod et al. 2006; Parisiadou et al. 2009; Plowey et al. 2008; Wang et al. 2008). Relevant to this chapter, other authors have implicated LRRK2 in autophagy and the lysosomal system.

While the role of LRRK2 on autophagy is not fully understood, some authors propose that this function could be due, in part, to the role of LRRK2 on cytoskeleton dynamics and its interaction with tubulins, microtubules, and other proteins involved in trafficking and endocytosis. LRRK2 loss-of-function mutations would impair the trafficking and the cytoskeleton dynamics, thereby affecting, among other mechanisms, the lysosomal system (Dzamko et al. 2010; Gandhi et al. 2008; Gillardon 2009a, b; Sakaguchi-Nakashima et al. 2007; Shin et al. 2008). At the same time, other authors used different cellular models expressing mutant LRRK2 or LRRK2 knockdown animals to confirm the hypothesis that LRRK2 is involved in the autophagy process. These cellular and animal models exhibited an LRRK2-dependent impairment of the autophagy process, thereby confirming the role of this protein in the correct functioning of autophagy (Alegre-Abarrategui et al. 2009; Ferree et al. 2011; Gomez-Suaga et al. 2012; Ramonet et al. 2011; Tong et al. 2010, 2012). More recently, a novel PD cellular model based on the generation of dopaminergic neurons derived from induced pluripotent stem cells (iPSC) obtained from fibroblasts of patients carrying the G2019S LRRK2 mutation exhibited the typical autophagic alterations observed in postmortem brain samples from PD patients, such as an accumulation of autophagic vacuoles and defects in autophagosome clearance (Sanchez-Danes et al. 2012). Taken together, these data confirm the implication of LRRK2 in the autophagy process. However, the mechanisms underlying this effect and the consequences of PD-linked mutations of LRRK2 on this function remain unknown.

5.3 Parkin and PINK1

Autophagic failure in PD has recently been confirmed through the newly identified roles of parkin and *PINK*1 proteins in maintaining mitochondria homeostasis by mediating mitochondrial trafficking and autophagic clearance of dysfunctional

mitochondria. *Parkin* is a component of a multiprotein E3 ubiquitin ligase and PINK1 is a serine/threonine protein kinase that localizes in the mitochondria and is involved in stress-induced mitochondrial dysfunction. Recently, both proteins have been proposed to belong to the mitochondrial quality control system, working together to promote degradation of dysfunctional mitochondria by mitophagy (Dagda et al. 2009; Narendra et al. 2008; Vives-Bauza et al. 2009; Whitworth and Pallanck 2009).

Loss-of-function mutations in PINK1 or parkin are associated to autosomal recessive familial PD. These mutations result in mitochondrial dysfunction with impaired mitochondrial turnover that leads to cell death. Indeed, it has recently been shown that PD-linked mutations in parkin can cause defects in the mitophagy process (Geisler et al. 2010; Lee et al. 2010b; Matsuda et al. 2010; Narendra et al. 2010b; Vives-Bauza et al. 2010). In addition, PD-linked mutations in PINK1 cause defective parkin recruitment to mitochondria and, consequently, mitophagy efficiency is affected (Geisler et al. 2010; Narendra et al. 2010b; Ziviani and Whitworth 2010; Vives-Bauza et al. 2010). PINK1is localized in mitochondria where it is cleaved to a shorter form that is no longer retained in mitochondria and is released to the cytosol, resulting in low mitochondrial levels of full-length PINK. This PINK1 cleavage is membrane-voltage dependent, and after depolarization of the mitochondrial membrane, the cleavage is inhibited, resulting in an accumulation of full-length PINK1in the mitochondria (Narendra et al. 2010b) (Fig. 2). The retention of PINK1 in damaged mitochondria recruits parkin to mitochondria which, through its E3 ubiquitin ligase activity, can polyubiquitinate different membrane proteins like VDAC1 and mitofusin (Geisler et al. 2010; Narendra et al., 2010b; Ziviani et al. 2010; Gegg et al. 2010). Polyubiquitination of mitofusin inhibits mitochondrial fusion (Tanaka et al. 2010; Ziviani and Whitworth 2010), thereby preventing the fusion of damaged mitochondria with healthy ones, while polyubiquitination of VDAC1 is required for mitochondrial clearance by mitophagy (Fig. 2). After VDAC1 polyubiquitination by parkin, depolarized mitochondria is tagged and autophagic adapters can bind the mitochondria to be sent to the autophagosome, where it will be degraded after fusion with lysosomes. A potential role for the autophagic adapter *p*62 in this parkin-mediated mitophagy process is still controversial. p62 can bind both ubiquitin proteins, by its ubiquitin-associated (UBA) domain, and also *LC*3, through its LC3-interacting region (LIR) domain, thus acting as a recruiter of ubiquitin-tagged substrates that are sent to autophagic limiting membrane, where LC3 is one of the main components (Pankiv et al. 2007). The autophagosome is then formed and the substrate is eliminated by macroautophagy. Some authors have reported that this PINK/parkin-mediated process is p62-dependent and that the damaged mitochondria tagged by ubiquitination requires the binding of p62 to be eliminated (Ding et al. 2010; Geisler et al. 2010; Okatsu et al. 2010). However, other studies have found that mitophagy is not p62-dependent and probably other autophagic adapters can display this function (Lee et al. 2010a; Narendra et al. 2010a; Okatsu et al. 2010).

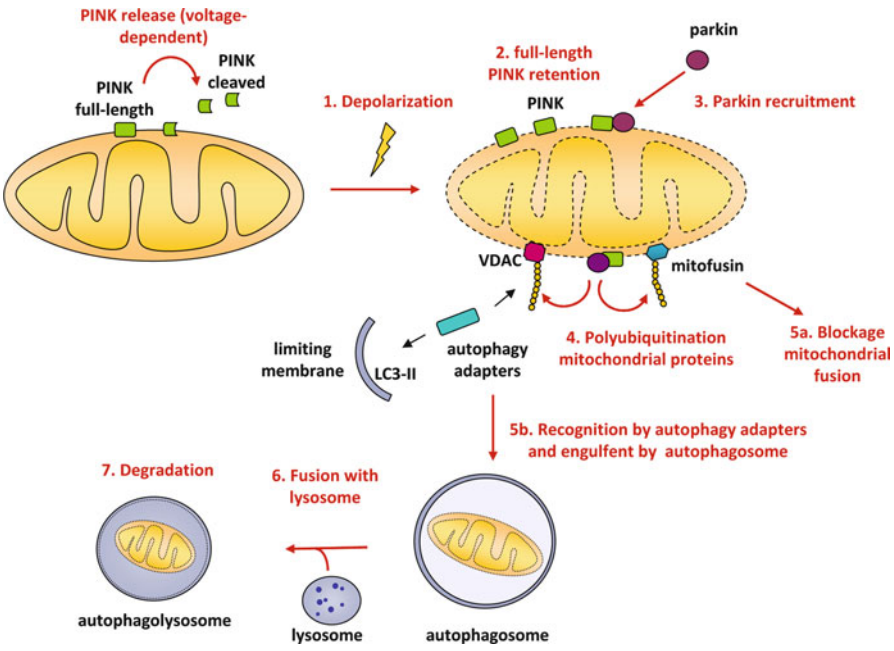

Fig. 2 *PINK/parkin-mediated mitophagy*. Upon mitochondria membrane depolarization, PINK1 full-length protein is retained in mitochondria membrane and recruits parkin protein. Once parkin is localized in the mitochondria membrane, they can polyubiquitinate different membrane proteins like VDAC1 and mitofusin. Polyubiquitinization of mitofusin inhibits future mitochondrial fusion with other healthy mitochondria, while polyubiquitinization of VDAC allows the interaction with autophagic adapters that will trigger the engulfment of the depolarized mitochondria by the autophagosome and its subsequent degradation inside the lysosomes

5.4 DJ-1

Mutations in the *DJ-1* gene result in a rare cause of autosomal recessive PD (Bonifati et al. 2003; Wong and Cuervo 2010). The physiological role of DJ-1 is still not fully understood, but it has been implicated in different biological functions like maintaining mitochondria integrity and acts as a redox-sensitive chaperone with antioxidant properties (Blackinton et al. 2009; Canet-Aviles et al. 2004; Irrcher et al. 2010; Junn et al. 2009; Kim et al. 2005; Meulener et al. 2005; Taira et al. 2004; Wong and Cuervo 2010). Most of these studies were performed in experimental in vivo and in vitro models of DJ-1 knockdown and showed that loss of DJ-1 function is linked to mitochondria depolarization and fragmentation, hypersensitivity to oxidative stress, and reduction of basal autophagy (Krebiehl et al. 2010). Recently, DJ-1 has also been linked to mitophagy by working together with parkin and PINK1 in the elimination of damaged mitochondria after oxidative stress (Thomas et al. 2011). The mechanism of action of DJ-1 on mitophagy remains unclear, but deficiency in DJ-1 leads to a similar mitochondrial phenotype as the one observed in

PINK1- and parkin-deficiency models (Thomas et al. 2011). In addition, DJ-1 overexpression is able to protect against oxidative stress in PINK1-deficient cells (Thomas et al. 2011). Overexpression of parkin or PINK1 can rescue the mitochondrial alterations occurring in DJ-1-knockdown cells, thus suggesting a potential role for DJ-1 in PINK1/parkin-mediated mitophagy.

5.5 UCH-L1

Another potential link between PD pathogenesis and autophagy is the role of UCH-L1 protein in the CMA pathway. *UCH-L1* (ubiquitin C-terminal hydrolase L1) is expressed in neurons and is associated with familial PD forms. This protein physically interacts with CMA receptor LAMP-2A at the lysosomal membrane. When the UCH-L1 protein carries the PD-linked mutation I93M or a posttranslational carbonyl modification reported in sporadic forms of PD, the interaction of the protein with the CMA translocation complex is increased, thereby preventing the CMA-mediated degradation of other substrates, as previously observed with some forms of α-synuclein (Kabuta et al. 2008). Interestingly, the UCH-L1 polymorphism S18Y may have opposite effects, being protective against the development of sporadic PD (Satoh and Kuroda 2001; Elbaz et al. 2003; Carmine et al. 2007). Experimental in vitro and in vivo PD models experimentally confirmed a neuroprotective effect of the S18Y polymorphism (Kyratzi et al. 2008; Xilouri et al. 2012). While the basis for this neuroprotective effect is still controversial, it may involve the antioxidant function of this UCH-L1 variant.

5.6 ATP13A2

The *ATP13A2*/*PARK9* gene encodes the protein *ATP13A2*, a lysosomal type 5 P-type ATPase present in lysosomal membranes, that is linked to autosomal recessive, levodopa responsive, nigrostriatal-pallidal-pyramidal neurodegeneration (Kufor-Rakeb Syndrome) as well as to some juvenile and young-onset forms of PD parkinsonism (Di Fonzo et al. 2007). The exact mechanism underlying the cellular functions of human ATP13A2 remains unknown, as well as its role in PD. Recently, it was shown that mutations/loss of ATP13A2 in human fibroblasts from patients with Kufor-Rakeb syndrome or in mouse primary neurons and dopaminergic cell lines leads to impaired lysosomal degradation capacity (Dehay et al. 2012; Usenovic et al. 2012a). ATP13A2 protein plays an important role in lysosomal membrane integrity, and mutations present in the *ATP13A2*/*PARK9* gene cause instability of the lysosomal membrane, impaired lysosomal acidification, decreased proteolytic capacity, and diminished lysosomal-mediated clearance of autophagosomes (Dehay et al. 2012). This lysosomal dysfunction results in accumulation of α-synuclein and toxicity in primary cortical neurons (Usenovic et al. 2012a). In addition, silencing of endogenous α-synuclein attenuated the toxicity in ATP13A2-depleted neurons, suggesting that loss of ATP13A2

mediates neurotoxicity at least in part via the accumulation of α-synuclein (Usenovic et al. 2012a). Identification of ATP13A2-interacting proteins by yeast two-hybrid system provide insights into ATP13A2 function and suggest a role in vesicular trafficking and fusion (Usenovic et al. 2012b). Loss of function of this protein could mediate α-synuclein aggregation and toxicity and explain the link between ATP13A2 mutations observed in patients with Kufor-Rakeb syndrome and neurodegeneration. In addition, loss of ATP13A2 function in cellular models was reported to disrupt autophagy activity and affect, in particular, mitochondrial quality control, thereby leading to impairment of mitochondrial turnover and increase of ROS production (Gusdon et al. 2012).

5.7 Glucocerebrosidase (GBA)

Loss-of-function mutations in the *GBA* gene are associated to *Gaucher's disease* (GD) and are also linked to an increased risk of PD and other related α-synucleinopathies (Neumann et al. 2009). *Glucocerebrosidase* protein (GCase) is a lysosomal enzyme responsible for the breakdown of the glycolipid glucocerebrosidase into glucose and ceramide inside lysosomes. GD is an autosomal recessive lysosomal storage disease caused by loss-of-function mutations of the GCase enzyme that results in accumulation of GCase substrates inside the lysosomes, especially in macrophages. GD is clinically classified in three groups: type 1 or non-neuropathic, type 2 or acute neuropathic, and type 3 or chronic neuropathic. Interestingly, patients with GD type 1, the non-neuropathic variant, have increased propensity to develop PD or related LB diseases (DePaolo et al. 2009). In turn, patients with PD have increased frequency of GBA mutations (DePaolo et al. 2009).

Although the mechanism underlying the association of GBA with PD remains unknown, several groups have proposed that impaired autophagy, aggregate formation, and α-synuclein metabolism may link GBA mutations with the pathogenesis of PD (Westbroek et al. 2011). In particular, some studies propose that alterations in the lysosomal system secondary to the presence of mutant GCase might connect PD and GD (Sun et al. 2010; Vitner et al. 2010). In cell culture models, the GBA mutants with gain-of-toxic function promote α-synuclein accumulation, an effect that can be reverted by autophagy induction with rapamycin (Cullen et al. 2011). Furthermore, patients with GD and PD exhibit LB positive for GCase, which suggests that α-synuclein oligomers may sequester mutant misfolded GCase within LB during the progression of the disease (Cullen et al. 2011). These results indicate that loss of GBA creates a positive feedback loop of reduced lysosomal function and α-synuclein accumulation that ultimately leads to neurodegeneration (Dawson and Dawson 2011; Mazzulli et al. 2011; Sardi et al. 2011).

Additional work is necessary to further identify the mechanisms underlying the connection between PD and GD, as other not-yet-identified factors or proteins may probably play a role since only a small percentage of GD patients and carriers with GBA mutations actually develop PD.

6 Conclusion

Mounting evidence indicate a role for autophagy dysfunction in neurodegenerative diseases, including PD. The potential involvement of autophagy alterations in PD has been reinforced in recent years by the finding that many of the mutated nuclear genes associated with familial forms of PD either directly or indirectly affect the normal activity of autophagy pathways. These findings might help to explain the pathogenesis of these diseases and most importantly lay the ground for the development of new therapeutic strategies based on the modulation of autophagy pathways for this group of devastating, currently incurable, diseases.

References

Agarraberes, F. A., & Dice, J. F. (2001). A molecular chaperone complex at the lysosomal membrane is required for protein translocation. *Journal of Cell Science, 114*, 2491–2499.

Ahlberg, J., & Glaumann, H. (1985). Uptake–microautophagy–and degradation of exogenous proteins by isolated rat liver lysosomes. Effects of pH, ATP, and inhibitors of proteolysis. *Experimental and Molecular Pathology, 42*, 78–88.

Alegre-Abarrategui, J., Christian, H., Lufino, M. M., Mutihac, R., Venda, L. L., Ansorge, O., & Wade-Martins, R. (2009). LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. *Human Molecular Genetics, 18*, 4022–4034.

Anderson, J. P., Walker, D. E., Goldstein, J. M., de Laat, R., Banducci, K., Caccavello, R. J., Barbour, R., Huang, J., Kling, K., Lee, M., Diep, L., Keim, P. S., Shen, X., Chataway, T., Schlossmacher, M. G., Seubert, P., Schenk, D., Sinha, S., Gai, W. P., & Chilcote, T. J. (2006). Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *The Journal of Biological Chemistry, 281*, 29739–29752.

Anglade, P., Vyas, S., Javoy-Agid, F., Herrero, M. T., Michel, P. P., Marquez, J., Mouatt-Prigent, A., Ruberg, M., Hirsch, E. C., & Agid, Y. (1997). Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histology and Histopathology, 12*, 25–31.

Bennett, M. C., Bishop, J. F., Leng, Y., Chock, P. B., Chase, T. N., & Mouradian, M. M. (1999). Degradation of alpha-synuclein by proteasome. *The Journal of Biological Chemistry, 274*, 33855–33858.

Bernales, S., Schuck, S., & Walter, P. (2007). ER-phagy: Selective autophagy of the endoplasmic reticulum. *Autophagy, 3*, 285–287.

Bjedov, I., Toivonen, J. M., Kerr, F., Slack, C., Jacobson, J., Foley, A., & Partridge, L. (2010). Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metabolism, 11*, 35–46.

Blackinton, J., Lakshminarasimhan, M., Thomas, K. J., Ahmad, R., Greggio, E., Raza, A. S., Cookson, M. R., & Wilson, M. A. (2009). Formation of a stabilized cysteine sulfinic acid is critical for the mitochondrial function of the parkinsonism protein DJ-1. *The Journal of Biological Chemistry, 284*, 6476–6485.

Bohley, P., & Seglen, P. O. (1992). Proteases and proteolysis in the lysosome. *Experientia, 48*, 151–157.

Boland, B., Kumar, A., Lee, S., Platt, F. M., Wegiel, J., Yu, W. H., & Nixon, R. A. (2008). Autophagy induction and autophagosome clearance in neurons: Relationship to autophagic pathology in Alzheimer's disease. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 28*, 6926–6937.

Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J. W., Vanacore, N., van Swieten, J. C.,

Brice, A., Meco, G., van Duijn, C. M., Oostra, B. A., & Heutink, P. (2003). Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science, 299*, 256–259.

Bonini, N. M., & Giasson, B. I. (2005). Snaring the function of alpha-synuclein. *Cell, 123*, 359–361.

Bove, J., Martinez-Vicente, M., & Vila, M. (2011). Fighting neurodegeneration with rapamycin: Mechanistic insights. *Nature Reviews. Neuroscience, 12*, 437–452.

Burre, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R., & Sudhof, T. C. (2010). Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science, 329*, 1663–1667.

Canet-Aviles, R. M., Wilson, M. A., Miller, D. W., Ahmad, R., McLendon, C., Bandyopadhyay, S., Baptista, M. J., Ringe, D., Petsko, G. A., & Cookson, M. R. (2004). The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 9103–9108.

Carmine, B. A., Westerlund, M., Bergman, O., Nissbrandt, H., Lind, C., Sydow, O., & Galter, D. (2007). S18Y in ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) associated with decreased risk of Parkinson's disease in Sweden. *Parkinsonism and Related Disorders, 13*, 295–298.

Chartier-Harlin, M. C., Kachergus, J., Roumier, C., Mouroux, V., Douay, X., Lincoln, S., Levecque, C., Larvor, L., Andrieux, J., Hulihan, M., Waucquier, N., Defebvre, L., Amouyel, P., Farrer, M., & Destee, A. (2004). Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet, 364*, 1167–1169.

Chen, L., & Feany, M. B. (2005). Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a *Drosophila* model of Parkinson disease. *Nature Neuroscience, 8*, 657–663.

Chu, Y., Dodiya, H., Aebischer, P., Olanow, C. W., & Kordower, J. H. (2009). Alterations in lysosomal and proteasomal markers in Parkinson's disease: Relationship to alpha-synuclein inclusions. *Neurobiology of Disease, 35*, 385–398.

Conway, K. A., Harper, J. D., & Lansbury, P. T., Jr. (2000). Fibrils formed in vitro from alpha-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. *Biochemistry, 39*, 2552–2563.

Cooper, A. A., Gitler, A. D., Cashikar, A., Haynes, C. M., Hill, K. J., Bhullar, B., Liu, K., Xu, K., Strathearn, K. E., Liu, F., Cao, S., Caldwell, K. A., Caldwell, G. A., Marsischky, G., Kolodner, R. D., Labaer, J., Rochet, J. C., Bonini, N. M., & Lindquist, S. (2006). Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science, 313*, 324–328.

Crews, L., Spencer, B., Desplats, P., Patrick, C., Paulino, A., Rockenstein, E., Hansen, L., Adame, A., Galasko, D., & Masliah, E. (2010). Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of alpha-synucleinopathy. *PLoS One, 5*, e9313.

Cuervo, A. M. (2006). Autophagy in neurons: It is not all about food. *Trends in Molecular Medicine, 12*, 461–464.

Cuervo, A. M., & Dice, J. F. (1996). A receptor for the selective uptake and degradation of proteins by lysosomes. *Science, 273*, 501–503.

Cuervo, A. M., Knecht, E., Terlecky, S. R., & Dice, J. F. (1995). Activation of a selective pathway of lysosomal proteolysis in rat liver by prolonged starvation. *American Journal of Physiology, 269*, 1200–1208.

Cuervo, A. M., Stefanis, L., Fredenburg, R., Lansbury, P. T., & Sulzer, D. (2004). Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science, 305*, 1292–1295.

Cullen, V., Sardi, S. P., Ng, J., Xu, Y. H., Sun, Y., Tomlinson, J. J., Kolodziej, P., Kahn, I., Saftig, P., Woulfe, J., Rochet, J. C., Glicksman, M. A., Cheng, S. H., Grabowski, G. A., Shihabuddin, L. S., & Schlossmacher, M. G. (2011). Acid beta-glucosidase mutants linked to Gaucher disease, Parkinson disease, and Lewy body dementia alter alpha-synuclein processing. *Annals of Neurology, 69*, 940–953.

Dachsel, J. C., Nishioka, K., Vilarino-Guell, C., Lincoln, S. J., Soto-Ortolaza, A. I., Kachergus, J., Hinkle, K. M., Heckman, M. G., Jasinska-Myga, B., Taylor, J. P., Dickson, D. W., Gibson, R. A., Hentati, F., Ross, O. A., & Farrer, M. J. (2010). Heterodimerization of Lrrk1-Lrrk2: Implications for LRRK2-associated Parkinson disease. *Mechanisms of Ageing and Development, 131*, 210–214.

Dagda, R. K., Cherra, S. J., 3rd, Kulich, S. M., Tandon, A., Park, D., & Chu, C. T. (2009). Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission. *The Journal of Biological Chemistry, 284*, 13843–13855.

Dawson, T. M., & Dawson, V. L. (2011). A lysosomal lair for a pathogenic protein pair. *Science Translational Medicine, 3*(91), 91ps28.

Dehay, B., Bove, J., Rodriguez-Muela, N., Perier, C., Recasens, A., Boya, P., & Vila, M. (2010). Pathogenic lysosomal depletion in Parkinson's disease. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 30*, 12535–12544.

Dehay, B., Ramirez, A., Martinez-Vicente, M., Perier, C., Canron, M. H., Doudnikoff, E., Vital, A., Vila, M., Klein, C., & Bezard, E. (2012). Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 9611–9616.

DePaolo, J., Goker-Alpan, O., Samaddar, T., Lopez, G., & Sidransky, E. (2009). The association between mutations in the lysosomal protein glucocerebrosidase and parkinsonism. *Movement Disorders, 24*, 1571–1578.

Di Fonzo, A., Chien, H. F., Socal, M., Giraudo, S., Tassorelli, C., Iliceto, G., Fabbrini, G., Marconi, R., Fincati, E., Abbruzzese, G., Marini, P., Squitieri, F., Horstink, M. W., Montagna, P., Libera, A. D., Stocchi, F., Goldwurm, S., Ferreira, J. J., Meco, G., Martignoni, E., Lopiano, L., Jardim, L. B., Oostra, B. A., Barbosa, E. R., & Bonifati, V. (2007). ATP13A2 missense mutations in juvenile parkinsonism and young onset Parkinson disease. *Neurology, 68*, 1557–1562.

Dice, J. (1990). Peptide sequences that target cytosolic proteins for lysosomal proteolysis. *Trends in Biochemical Sciences, 15*, 305–309.

Dice, J. (2000). *Lysosomal pathways of protein degradation*. Austin, TX: Landes Bioscience.

Ding, W. X., Ni, H. M., Li, M., Liao, Y., Chen, X., Stolz, D. B., Dorn, G. W., & Yin, X. M. (2010). Nix is critical to two distinct phases of mitophagy, reactive oxygen species-mediated autophagy induction and Parkin-ubiquitin-p62-mediated mitochondrial priming. *Journal of Biological Chemistry, 285*, 27879–27890.

Dunn, W. A., Jr., Cregg, J. M., Kiel, J. A., van der Klei, I. J., Oku, M., Sakai, Y., Sibirny, A. A., Stasyk, O. V., & Veenhuis, M. (2005). Pexophagy: The selective autophagy of peroxisomes. *Autophagy, 1*, 75–83.

Dzamko, N., Deak, M., Hentati, F., Reith, A. D., Prescott, A. R., Alessi, D. R., & Nichols, R. J. (2010). Inhibition of LRRK2 kinase activity leads to dephosphorylation of Ser(910)/Ser(935), disruption of 14-3-3 binding and altered cytoplasmic localization. *Biochemistry Journal, 430*, 405–413.

Elbaz, A., Levecque, C., Clavel, J., Vidal, J. S., Richard, F., Correze, J. R., Delemotte, B., Amouyel, P., Alperovitch, A., Chartier-Harlin, M. C., & Tzourio, C. (2003). S18Y polymorphism in the UCH-L1 gene and Parkinson's disease: Evidence for an age-dependent relationship. *Movement Disorders, 18*, 130–137.

Ferree, A., Guillily, M., Li, H., Smith, K., Takashima, A., Squillace, R., Weigele, M., Collins, J. J., & Wolozin, B. (2011). Regulation of physiologic actions of LRRK2: Focus on autophagy. *Neurodegenerative Diseases, 10*(1–4), 238–241.

Gandhi, P. N., Wang, X., Zhu, X., Chen, S. G., & Wilson-Delfosse, A. L. (2008). The Roc domain of leucine-rich repeat kinase 2 is sufficient for interaction with microtubules. *Journal of Neuroscience Research, 86*, 1711–1720.

Gegg, M. E., Cooper, J. M., Chau, K. Y., Rojo, M., Schapira, A. H., & Taanman, J. W. (2010). Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. *Human Molecular Genetics, 19*, 4861–4870.

Gehrke, S., Imai, Y., Sokol, N., & Lu, B. (2010). Pathogenic LRRK2 negatively regulates microRNA-mediated translational repression. *Nature, 466*, 637–641.

Geisler, S., Holmstrom, K. M., Skujat, D., Fiesel, F. C., Rothfuss, O. C., Kahle, P. J., & Springer, W. (2010). PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nature Cell Biology, 12*, 119–131.

Gillardon, F. (2009a). Interaction of elongation factor 1-alpha with leucine-rich repeat kinase 2 impairs kinase activity and microtubule bundling in vitro. *Neuroscience, 163*, 533–539.

Gillardon, F. (2009b). Leucine-rich repeat kinase 2 phosphorylates brain tubulin-beta isoforms and modulates microtubule stability – a point of convergence in parkinsonian neurodegeneration? *Journal of Neurochemistry, 110*, 1514–1522.

Gloeckner, C. J., Kinkl, N., Schumacher, A., Braun, R. J., O'Neill, E., Meitinger, T., Kolch, W., Prokisch, H., & Ueffing, M. (2006). The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. *Human Molecular Genetics, 15*, 223–232.

Gomez-Suaga, P., Luzon-Toro, B., Churamani, D., Zhang, L., Bloor-Young, D., Patel, S., Woodman, P. G., Churchill, G. C., & Hilfiker, S. (2012). Leucine-rich repeat kinase 2 regulates autophagy through a calcium-dependent pathway involving NAADP. *Human Molecular Genetics, 21*, 511–525.

Greggio, E., & Cookson, M. R. (2009). Leucine-rich repeat kinase 2 mutations and Parkinson's disease: Three questions. *ASN Neuro, 1*. doi:pii: e00002. 10.1042/AN20090007. Review.

Greggio, E., Jain, S., Kingsbury, A., Bandopadhyay, R., Lewis, P., Kaganovich, A., van der Brug, M. P., Beilina, A., Blackinton, J., Thomas, K. J., Ahmad, R., Miller, D. W., Kesavapany, S., Singleton, A., Lees, A., Harvey, R. J., Harvey, K., & Cookson, M. R. (2006). Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiology of Disease, 23*, 329–341.

Greggio, E., Zambrano, I., Kaganovich, A., Beilina, A., Taymans, J. M., Daniels, V., Lewis, P., Jain, S., Ding, J., Syed, A., Thomas, K. J., Baekelandt, V., & Cookson, M. R. (2008). The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. *The Journal of Biological Chemistry, 283*, 16906–16914.

Gusdon, A. M., Zhu, J., Van Houten, B., & Chu, C. T. (2012). ATP13A2 regulates mitochondrial bioenergetics through macroautophagy. *Neurobiology of Disease, 45*, 962–972.

Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., Yokoyama, M., Mishima, K., Saito, I., Okano, H., & Mizushima, N. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature, 441*, 885–889.

He, C., & Klionsky, D. J. (2006). Autophagy and neurodegeneration. *ACS Chemical Biology, 1*, 211–213.

He, C., & Klionsky, D. J. (2009). Regulation mechanisms and signaling pathways of autophagy. *Annual Review of Genetics, 43*, 67–93.

Ho, C. C., Rideout, H. J., Ribe, E., Troy, C. M., & Dauer, W. T. (2009). The Parkinson disease protein leucine-rich repeat kinase 2 transduces death signals via Fas-associated protein with death domain and caspase-8 in a cellular model of neurodegeneration. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 29*, 1011–1016.

Ibanez, P., Bonnet, A. M., Debarges, B., Lohmann, E., Tison, F., Pollak, P., Agid, Y., Durr, A., & Brice, A. (2004). Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. *Lancet, 364*, 1169–1171.

Imai, J., Yashiroda, H., Maruya, M., Yahara, I., & Tanaka, K. (2003). Proteasomes and molecular chaperones: Cellular machinery responsible for folding and destruction of unfolded proteins. *Cell Cycle, 2*, 585–590.

Imai, Y., Gehrke, S., Wang, H. Q., Takahashi, R., Hasegawa, K., Oota, E., & Lu, B. (2008). Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in Drosophila. *The EMBO Journal, 27*, 2432–2443.

Irrcher, I., Aleyasin, H., Seifert, E. L., Hewitt, S. J., Chhabra, S., Phillips, M., Lutz, A. K., Rousseaux, M. W., Bevilacqua, L., Jahani-Asl, A., Callaghan, S., MacLaurin, J. G.,

Winklhofer, K. F., Rizzu, P., Rippstein, P., Kim, R. H., Chen, C. X., Fon, E. A., Slack, R. S., Harper, M. E., McBride, H. M., Mak, T. W., & Park, D. S. (2010). Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics. *Human Molecular Genetics, 19*, 3734–3746.

Junn, E., Jang, W. H., Zhao, X., Jeong, B. S., & Mouradian, M. M. (2009). Mitochondrial localization of DJ-1 leads to enhanced neuroprotection. *Journal of Neuroscience Research, 87*, 123–129.

Kabuta, T., Setsuie, R., Mitsui, T., Kinugawa, A., Sakurai, M., Aoki, S., Uchida, K., & Wada, K. (2008). Aberrant molecular properties shared by familial Parkinson's disease-associated mutant UCH-L1 and carbonyl-modified UCH-L1. *Human Molecular Genetics, 17*, 1482–1496.

Kaushik, S., Massey, A. C., Mizushima, N., & Cuervo, A. M. (2008). Constitutive activation of chaperone-mediated autophagy in cells with impaired macroautophagy. *Molecular Biology of the Cell, 19*, 2179–2192.

Kegel, K. B., Kim, M., Sapp, E., McIntyre, C., Castano, J. G., Aronin, N., & DiFiglia, M. (2000). Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. *Journal of Neuroscience, 20*, 7268–7278.

Kiffin, R., Christian, C., Knecht, E., & Cuervo, A. M. (2004). Activation of chaperone-mediated autophagy during oxidative stress. *Molecular Biology of the Cell, 15*, 4829–4840.

Kim, R. H., Smith, P. D., Aleyasin, H., Hayley, S., Mount, M. P., Pownall, S., Wakeham, A., You, T., Kalia, S. K., Horne, P., Westaway, D., Lozano, A. M., Anisman, H., Park, D. S., & Mak, T. W. (2005). Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 5215–5220.

Kim, I., Rodriguez-Enriquez, S., & Lemasters, J. J. (2007). Selective degradation of mitochondria by mitophagy. *Archives of Biochemistry and Biophysics, 462*, 245–253.

Klionsky, D. J. (2005). The molecular machinery of autophagy: Unanswered questions. *Journal of Cell Science, 118*, 7–18.

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Koike, M., Uchiyama, Y., Kominami, E., & Tanaka, K. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature, 441*, 880–884.

Kraft, C., Deplazes, A., Sohrmann, M., & Peter, M. (2008). Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. *Nature Cell Biology, 10*, 602–610.

Krebiehl, G., Ruckerbauer, S., Burbulla, L. F., Kieper, N., Maurer, B., Waak, J., Wolburg, H., Gizatullina, Z., Gellerich, F. N., Woitalla, D., Riess, O., Kahle, P. J., Proikas-Cezanne, T., & Kruger, R. (2010). Reduced basal autophagy and impaired mitochondrial dynamics due to loss of Parkinson's disease-associated protein DJ-1. *PLoS One, 5*, e9367.

Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L., & Riess, O. (1998). Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nature Genetics, 18*, 106–108.

Kyratzi, E., Pavlaki, M., & Stefanis, L. (2008). The S18Y polymorphic variant of UCH-L1 confers an antioxidant function to neuronal cells. *Human Molecular Genetics, 17*, 2160–2171.

Lee, J. Y., Koga, H., Kawaguchi, Y., Tang, W., Wong, E., Gao, Y. S., Pandey, U. B., Kaushik, S., Tresse, E., Lu, J., Taylor, J. P., Cuervo, A. M., & Yao, T. P. (2010a). HDAC6 controls autophagosome maturation essential for ubiquitin-selective quality-control autophagy. *The EMBO Journal, 29*, 969–980.

Lee, J. Y., Nagano, Y., Taylor, J. P., Lim, K. L., & Yao, T. P. (2010b). Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. *The Journal of Cell Biology, 189*, 671–679.

Levine, B. (2005). Eating oneself and uninvited guests: Autophagy-related pathways in cellular defense. *Cell, 120*, 159–162.

Levine, B., & Kroemer, G. (2008). Autophagy in the pathogenesis of disease. *Cell, 132*, 27–42.

Ling, D., & Salvaterra, P. M. (2009). A central role for autophagy in Alzheimer-type neurodegeneration. *Autophagy, 5*, 738–740.

MacLeod, D., Dowman, J., Hammond, R., Leete, T., Inoue, K., & Abeliovich, A. (2006). The familial Parkinsonism gene LRRK2 regulates neurite process morphology. *Neuron, 52*, 587–593.

Majeski, A. E., & Dice, J. F. (2004). Mechanisms of chaperone-mediated autophagy. *The International Journal of Biochemistry & Cell Biology, 36*, 2435–2444.

Martinez-Vicente, M., & Cuervo, A. M. (2007). Autophagy and neurodegeneration: When the cleaning crew goes on strike. *Lancet Neurology, 6*, 352–361.

Martinez-Vicente, M., Talloczy, Z., Kaushik, S., Massey, A. C., Mazzulli, J., Mosharov, E. V., Hodara, R., Fredenburg, R., Wu, D. C., Follenzi, A., Dauer, W., Przedborski, S., Ischiropoulos, H., Lansbury, P. T., Sulzer, D., & Cuervo, A. M. (2008). Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *The Journal of Clinical Investigation, 118*, 777–788.

Marzella, L., Ahlberg, J., & Glaumann, H. (1982). Isolation of autophagic vacuoles from rat liver: Morphological and biochemical characterization. *The Journal of Cell Biology, 93*, 144–154.

Massey, A., Kiffin, R., & Cuervo, A. M. (2004). Pathophysiology of chaperone-mediated autophagy. *The International Journal of Biochemistry & Cell Biology, 36*, 2420–2434.

Massey, A. C., Kaushik, S., & Cuervo, A. M. (2006a). Lysosomal chat maintains the balance. *Autophagy, 2*, 325–327.

Massey, A. C., Kaushik, S., Sovak, G., Kiffin, R., & Cuervo, A. M. (2006b). Consequences of the selective blockage of chaperone-mediated autophagy. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 5805–5810.

Matsuda, N., Sato, S., Shiba, K., Okatsu, K., Saisho, K., Gautier, C. A., Sou, Y. S., Saiki, S., Kawajiri, S., Sato, F., Kimura, M., Komatsu, M., Hattori, N., & Tanaka, K. (2010). PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *The Journal of Cell Biology, 189*, 211–221.

Mazzulli, J. R., Xu, Y. H., Sun, Y., Knight, A. L., McLean, P. J., Caldwell, G. A., et al. (2011). Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell, 146*(1), 37–52.

McFarlane, D., Dybdal, N., Donaldson, M. T., Miller, L., & Cribb, A. E. (2005). Nitration and increased alpha-synuclein expression associated with dopaminergic neurodegeneration in equine pituitary pars intermedia dysfunction. *Journal of Neuroendocrinology, 17*, 73–80.

Meulener, M., Whitworth, A. J., Armstrong-Gold, C. E., Rizzu, P., Heutink, P., Wes, P. D., Pallanck, L. J., & Bonini, N. M. (2005). Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. *Current Biology, 15*, 1572–1577.

Mizushima, N., Levine, B., Cuervo, A. M., & Klionsky, D. J. (2008). Autophagy fights disease through cellular self-digestion. *Nature, 451*, 1069–1075.

Morimoto, N., Nagai, M., Ohta, Y., Miyazaki, K., Kurata, T., Morimoto, M., Murakami, T., Takehisa, Y., Ikeda, Y., Kamiya, T., & Abe, K. (2007). Increased autophagy in transgenic mice with a G93A mutant SOD1 gene. *Brain Research, 1167*, 112–117.

Mortimore, G., Lardeux, B. R., & Adams, C. E. (1988). Regulation of microautophagy and basal protein turnover in rat liver. Effects of short-term starvation. *Journal of Biological Chemistry, 263*, 2506–2512.

Mullins, C., & Bonifacino, J. S. (2001). The molecular machinery for lysosome biogenesis. *BioEssays, 23*, 333–343.

Nagata, E., Sawa, A., Ross, C. A., & Snyder, S. H. (2004). Autophagosome-like vacuole formation in Huntington's disease lymphoblasts. *NeuroReport, 15*, 1325–1328.

Narendra, D., Tanaka, A., Suen, D. F., & Youle, R. J. (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *The Journal of Cell Biology, 183*, 795–803.

Narendra, D., Kane, L. A., Hauser, D. N., Fearnley, I. M., & Youle, R. J. (2010a). p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy, 6*, 1090–1106.

Narendra, D. P., Jin, S. M., Tanaka, A., Suen, D. F., Gautier, C. A., Shen, J., Cookson, M. R., & Youle, R. J. (2010b). PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biology, 8*, e1000298.

Nedelsky, N. B., Todd, P. K., & Taylor, J. P. (2008). Autophagy and the ubiquitin-proteasome system: Collaborators in neuroprotection. *Biochimica et Biophysica Acta, 1782*, 691–699.

Neumann, J., Bras, J., Deas, E., O'Sullivan, S. S., Parkkinen, L., Lachmann, R. H., Li, A., Holton, J., Guerreiro, R., Paudel, R., Segarane, B., Singleton, A., Lees, A., Hardy, J., Houlden, H., Revesz, T., & Wood, N. W. (2009). Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain, 132*, 1783–1794.

Nixon, R. A., Wegiel, J., Kumar, A., Yu, W. H., Peterhoff, C., Cataldo, A., & Cuervo, A. M. (2005). Extensive involvement of autophagy in Alzheimer disease: An immuno-electron microscopy study. *Journal of Neuropathology and Experimental Neurology, 64*, 113–122.

Norris, E. H., Giasson, B. I., Hodara, R., Xu, S., Trojanowski, J. Q., Ischiropoulos, H., & Lee, V. M. (2005). Reversible inhibition of {alpha}-synuclein fibrillization by dopaminochrome-mediated conformational alterations. *The Journal of Biological Chemistry, 280*, 21212–21219.

Okatsu, K., Saisho, K., Shimanuki, M., Nakada, K., Shitara, H., Sou, Y. S., Kimura, M., Sato, S., Hattori, N., Komatsu, M., Tanaka, K., & Matsuda, N. (2010). p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. *Genes to Cells, 15*, 887–900.

Paisan-Ruiz, C., Jain, S., Evans, E. W., Gilks, W. P., Simon, J., van der Brug, M., Lopez de Munain, A., Aparicio, S., Gil, A. M., Khan, N., Johnson, J., Martinez, J. R., Nicholl, D., Carrera, I. M., Pena, A. S., de Silva, R., Lees, A., Marti-Masso, J. F., Perez-Tur, J., Wood, N. W., & Singleton, A. B. (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron, 44*, 595–600.

Paisan-Ruiz, C., Nath, P., Washecka, N., Gibbs, J. R., & Singleton, A. B. (2008). Comprehensive analysis of LRRK2 in publicly available Parkinson's disease cases and neurologically normal controls. *Human Mutation, 29*, 485–490.

Pan, T., Kondo, S., Le, W., & Jankovic, J. (2008a). The role of autophagy-lysosome pathway in neurodegeneration associated with Parkinson's disease. *Brain, 131*, 1969–1978.

Pan, T., Kondo, S., Zhu, W., Xie, W., Jankovic, J., & Le, W. (2008b). Neuroprotection of rapamycin in lactacystin-induced neurodegeneration via autophagy enhancement. *Neurobiology of Disease, 32*, 16–25.

Pandey, U. B., Nie, Z., Batlevi, Y., McCray, B. A., Ritson, G. P., Nedelsky, N. B., Schwartz, S. L., DiProspero, N. A., Knight, M. A., Schuldiner, O., Padmanabhan, R., Hild, M., Berry, D. L., Garza, D., Hubbert, C. C., Yao, T. P., Baehrecke, E. H., & Taylor, J. P. (2007). HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. *Nature, 447*, 859–863.

Pankiv, S., Clausen, T. H., Lamark, T., Brech, A., Bruun, J. A., Outzen, H., Overvatn, A., Bjorkoy, G., & Johansen, T. (2007). p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *The Journal of Biological Chemistry, 282*, 24131–24145.

Parisiadou, L., Xie, C., Cho, H. J., Lin, X., Gu, X. L., Long, C. X., Lobbestael, E., Baekelandt, V., Taymans, J. M., Sun, L., & Cai, H. (2009). Phosphorylation of ezrin/radixin/moesin proteins by LRRK2 promotes the rearrangement of actin cytoskeleton in neuronal morphogenesis. *Journal of Neuroscience, 29*, 13971–13980.

Paxinou, E., Chen, Q., Weisse, M., Giasson, B. I., Norris, E. H., Rueter, S. M., Trojanowski, J. Q., Lee, V. M., & Ischiropoulos, H. (2001). Induction of alpha-synuclein aggregation by intracellular nitrative insult. *Journal of Neuroscience, 21*, 8053–8061.

Petersen, A., Larsen, K. E., Behr, G. G., Romero, N., Przedborski, S., Brundin, P., & Sulzer, D. (2001). Expanded CAG repeats in exon 1 of the Huntington's disease gene stimulate dopamine-mediated striatal neuron autophagy and degeneration. *Human Molecular Genetics, 10*, 1243–1254.

Pickford, F., Masliah, E., Britschgi, M., Lucin, K., Narasimhan, R., Jaeger, P. A., Small, S., Spencer, B., Rockenstein, E., Levine, B., & Wyss-Coray, T. (2008). The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. *The Journal of Clinical Investigation, 118*, 2190–2199.

Plowey, E. D., Cherra, S. J., 3rd, Liu, Y. J., & Chu, C. T. (2008). Role of autophagy in G2019S-LRRK2-associated neurite shortening in differentiated SH-SY5Y cells. *Journal of Neurochemistry, 105*, 1048–1056.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., & Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science, 276*, 2045–2047.

Ramonet, D., Daher, J. P., Lin, B. M., Stafa, K., Kim, J., Banerjee, R., Westerlund, M., Pletnikova, O., Glauser, L., Yang, L., Liu, Y., Swing, D. A., Beal, M. F., Troncoso, J. C., McCaffery, J. M., Jenkins, N. A., Copeland, N. G., Galter, D., Thomas, B., Lee, M. K., Dawson, T. M., Dawson, V. L., & Moore, D. J. (2011). Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. *PLoS One, 6*, e18568.

Ramonet, D., Podhajska, A., Stafa, K., Sonnay, S., Trancikova, A., Tsika, E., Pletnikova, O., Troncoso, J. C., Glauser, L., & Moore, D. J. (2012). PARK9-associated ATP13A2 localizes to intracellular acidic vesicles and regulates cation homeostasis and neuronal integrity. *Human Molecular Genetics, 21*(8), 1725–1743.

Ravikumar, B., Vacher, C., Berger, Z., Davies, J. E., Luo, S., Oroz, L. G., Scaravilli, F., Easton, D. F., Duden, R., O'Kane, C. J., & Rubinsztein, D. C. (2004). Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nature Genetics, 36*, 585–595.

Ravikumar, B., Sarkar, S., Davies, J. E., Futter, M., Garcia-Arencibia, M., Green-Thompson, Z. W., Jimenez-Sanchez, M., Korolchuk, V. I., Lichtenberg, M., Luo, S., Massey, D. C., Menzies, F. M., Moreau, K., Narayanan, U., Renna, M., Siddiqi, F. H., Underwood, B. R., Winslow, A. R., & Rubinsztein, D. C. (2010). Regulation of mammalian autophagy in physiology and pathophysiology. *Physiological Reviews, 90*, 1383–1435.

Rochet, J. C., Outeiro, T. F., Conway, K. A., Ding, T. T., Volles, M. J., Lashuel, H. A., Bieganski, R. M., Lindquist, S. L., & Lansbury, P. T. (2004). Interactions among alpha-synuclein, dopamine, and biomembranes: Some clues for understanding neurodegeneration in Parkinson's disease. *Journal of Molecular Neuroscience, 23*, 23–34.

Sakaguchi-Nakashima, A., Meir, J. Y., Jin, Y., Matsumoto, K., & Hisamoto, N. (2007). LRK-1, a *C. elegans* PARK8-related kinase, regulates axonal-dendritic polarity of SV proteins. *Current Biology, 17*, 592–598.

Sakai, Y., Koller, A., Rangell, L., Keller, G., & Subramani, S. (1998). Peroxisome degradation by microautophagy in *Pichia pastoris*. Identification of specific steps and morphological intermediates. *The Journal of Cell Biology, 141*, 625–636.

Sanchez-Danes, A., Richaud-Patin, Y., Carballo-Carbajal, I., Jimenez-Delgado, S., Caig, C., Mora, S., Di Guglielmo, C., Ezquerra, M., Patel, B., Giralt, A., Canals, J. M., Memo, M., Alberch, J., Lopez-Barneo, J., Vila, M., Cuervo, A. M., Tolosa, E., Consiglio, A., & Raya, A. (2012). Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease. *EMBO Molecular Medicine., 4*, 380.

Santini, E., Heiman, M., Greengard, P., Valjent, E., & Fisone, G. (2009). Inhibition of mTOR signaling in Parkinson's disease prevents L-DOPA-induced dyskinesia. *Science Signaling, 2*, ra36. doi:10.1126/scisignal.2000308.

Sapp, E., Schwarz, C., Chase, K., Bhide, P. G., Young, A. B., Penney, J., Vonsattel, J. P., Aronin, N., & DiFiglia, M. (1997). Huntingtin localization in brains of normal and Huntington's disease patients. *Annals of Neurology, 42*, 604–612.

Sardi, S. P., Clarke, J., Kinnecom, C., Tamsett, T. J., Li, L., Stanek, L. M., et al. (2011). CNS expression of glucocerebrosidase corrects alpha-synuclein pathology and memory in a mouse model of Gaucher-related synucleinopathy. *Proceedings of the National Academy of Sciences of the United States of America, 108*(29), 12101–12106.

Satoh, J., & Kuroda, Y. (2001). A polymorphic variation of serine to tyrosine at codon 18 in the ubiquitin C-terminal hydrolase-L1 gene is associated with a reduced risk of sporadic Parkinson's disease in a Japanese population. *Journal of Neurological Sciences, 189*, 113–117.

Shin, N., Jeong, H., Kwon, J., Heo, H. Y., Kwon, J. J., Yun, H. J., Kim, C. H., Han, B. S., Tong, Y., Shen, J., Hatano, T., Hattori, N., Kim, K. S., Chang, S., & Seol, W. (2008). LRRK2 regulates synaptic vesicle endocytosis. *Experimental Cell Research, 314*, 2055–2065.

Singh, R., Xiang, Y., Wang, Y., Baikati, K., Cuervo, A. M., Luu, Y. K., Tang, Y., Pessin, J. E., Schwartz, G. J., & Czaja, M. J. (2009). Autophagy regulates adipose mass and differentiation in mice. *The Journal of Clinical Investigation, 119*, 3329–3339.

Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M. R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J., & Gwinn-Hardy, K. (2003). Alpha-synuclein locus triplication causes Parkinson's disease. *Science, 302*, 841.

Smith, W. W., Margolis, R. L., Li, X., Troncoso, J. C., Lee, M. K., Dawson, V. L., Dawson, T. M., Iwatsubo, T., & Ross, C. A. (2005). Alpha-synuclein phosphorylation enhances eosinophilic cytoplasmic inclusion formation in SH-SY5Y cells. *Journal of Neuroscience, 25*, 5544–5552.

Smith, W. W., Pei, Z., Jiang, H., Dawson, V. L., Dawson, T. M., & Ross, C. A. (2006). Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nature Neuroscience, 9*, 1231–1233.

Stefanis, L., Larsen, K. E., Rideout, H. J., Sulzer, D., & Greene, L. A. (2001). Expression of A53T mutant but not wild-type alpha-synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death. *Journal of Neuroscience, 21*, 9549–9560.

Sun, Y., Liou, B., Ran, H., Skelton, M. R., Williams, M. T., Vorhees, C. V., Kitatani, K., Hannun, Y. A., Witte, D. P., Xu, Y. H., & Grabowski, G. A. (2010). Neuronopathic Gaucher disease in the mouse: Viable combined selective saposin C deficiency and mutant glucocerebrosidase (V394L) mice with glucosylsphingosine and glucosylceramide accumulation and progressive neurological deficits. *Human Molecular Genetics, 19*, 1088–1097.

Taira, T., Saito, Y., Niki, T., Iguchi-Ariga, S. M., Takahashi, K., & Ariga, H. (2004). DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Reports, 5*, 213–218.

Tanaka, K., Matsuda, N., & Okatsu, K. (2010). Mechanisms underlying the cause of Parkinson's disease: The functions of Parkin/PINK1. *Rinshō Shinkeigaku, 50*, 867.

Thomas, K. J., McCoy, M. K., Blackinton, J., Beilina, A., van der Brug, M., Sandebring, A., Miller, D., Maric, D., Cedazo-Minguez, A., & Cookson, M. R. (2011). DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. *Human Molecular Genetics, 20*, 40–50.

Tong, Y., Yamaguchi, H., Giaime, E., Boyle, S., Kopan, R., Kelleher, R. J., III, & Shen, J. (2010). Loss of leucine-rich repeat kinase 2 causes impairment of protein degradation pathways, accumulation of alpha-synuclein, and apoptotic cell death in aged mice. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 9879–9884.

Tong, Y., Giaime, E., Yamaguchi, H., Ichimura, T., Liu, Y., Si, H., Cai, H., Bonventre, J. V., & Shen, J. (2012). Loss of leucine-rich repeat kinase 2 causes age-dependent bi-phasic alterations of the autophagy pathway. *Molecular Neurodegeneration, 7*, 2.

Usenovic, M., Knight, A. L., Ray, A., Wong, V., Brown, K. R., Caldwell, G. A., Caldwell, K. A., Stagljar, I., & Krainc, D. (2012a). Identification of novel ATP13A2 interactors and their role in alpha-synuclein misfolding and toxicity. *Human Molecular Genetics, 21*(17), 3785–3794.

Usenovic, M., Tresse, E., Mazzulli, J. R., Taylor, J. P., & Krainc, D. (2012b). Deficiency of ATP13A2 leads to lysosomal dysfunction, alpha-synuclein accumulation, and neurotoxicity. *Journal of Neuroscience, 32*, 4240.

Uversky, V. N., Yamin, G., Munishkina, L. A., Karymov, M. A., Millett, I. S., Doniach, S., Lyubchenko, Y. L., & Fink, A. L. (2005). Effects of nitration on the structure and aggregation of alpha-synuclein. *Brain Research. Molecular Brain Research, 134*, 84–102.

Vila, M., Bove, J., Dehay, B., Rodriguez-Muela, N., & Boya, P. (2011). Lysosomal membrane permeabilization in Parkinson disease. *Autophagy, 7*, 98–100.

Vitner, E. B., Dekel, H., Zigdon, H., Shachar, T., Farfel-Becker, T., Eilam, R., Karlsson, S., & Futerman, A. H. (2010). Altered expression and distribution of cathepsins in neuronopathic forms of Gaucher disease and in other sphingolipidoses. *Human Molecular Genetics, 19*, 3583–3590.

Vives-Bauza, C., Zhou, C., Huang, Y., Cui, M., de Vries, R.L., Kim, J., May, J., Tocilescu, M. A., Liu, W., Ko, H. S., Magrane, J., Moore, D. J., Dawson, V. L., Grailhe, R., Dawson, T. M., Li, C., Tieu, K., & Przedborski, S. (2009). PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proceedings of the National Academy of Sciences of the United States of America*.

Vives-Bauza, C., Zhou, C., Huang, Y., Cui, M., de Vries, R. L., Kim, J., et al. (2010). PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proceedings of the National Academy of Sciences of the United States of America, 107*(1), 378–383.

Vogiatzi, T., Xilouri, M., Vekrellis, K., & Stefanis, L. (2008). Wild type alpha-synuclein is degraded by chaperone-mediated autophagy and macroautophagy in neuronal cells. *The Journal of Biological Chemistry, 283*, 23542–23556.

Voisine, C., Pedersen, J. S., & Morimoto, R. I. (2010). Chaperone networks: Tipping the balance in protein folding diseases. *Neurobiology of Disease, 40*, 12–20.

Volles, M. J., & Lansbury, P. T., Jr. (2003). Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease. *Biochemistry, 42*, 7871–7878.

Wang, C. W., & Klionsky, D. J. (2003). The molecular mechanism of autophagy. *Molecular Medicine, 9*, 65–76.

Wang, L., Xie, C., Greggio, E., Parisiadou, L., Shim, H., Sun, L., Chandran, J., Lin, X., Lai, C., Yang, W. J., Moore, D. J., Dawson, T. M., Dawson, V. L., Chiosis, G., Cookson, M. R., & Cai, H. (2008). The chaperone activity of heat shock protein 90 is critical for maintaining the stability of leucine-rich repeat kinase 2. *Journal of Neuroscience, 28*, 3384–3391.

Webb, J. L., Ravikumar, B., Atkins, J., Skepper, J. N., & Rubinsztein, D. C. (2003). Alpha-synuclein is degraded by both autophagy and the proteasome. *The Journal of Biological Chemistry, 278*, 25009–25013.

West, A. B., Moore, D. J., Biskup, S., Bugayenko, A., Smith, W. W., Ross, C. A., Dawson, V. L., & Dawson, T. M. (2005). Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 16842–16847.

Westbroek, W., Gustafson, A. M., & Sidransky, E. (2011). Exploring the link between glucocerebrosidase mutations and parkinsonism. *Trends in Molecular Medicine, 17*, 485–493.

Whitworth, A. J., & Pallanck, L. J. (2009). The PINK1/Parkin pathway: A mitochondrial quality control system? *Journal of Bioenergetics and Biomembranes, 41*, 499.

Winslow, A. R., Chen, C. W., Corrochano, S., Acevedo-Arozena, A., Gordon, D. E., Peden, A. A., Lichtenberg, M., Menzies, F. M., Ravikumar, B., Imarisio, S., Brown, S., O'Kane, C. J., & Rubinsztein, D. C. (2010). Alpha-synuclein impairs macroautophagy: Implications for Parkinson's disease. *The Journal of Cell Biology, 190*, 1023–1037.

Wong, E., & Cuervo, A. M. (2010). Autophagy gone awry in neurodegenerative diseases. *Nature Neuroscience, 13*, 805–811.

Xilouri, M., Vogiatzi, T., Vekrellis, K., Park, D., & Stefanis, L. (2009). Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. *PLoS One, 4*, e5515.

Xilouri, M., Kyratzi, E., Pitychoutis, P. M., Papadopoulou-Daifoti, Z., Perier, C., Vila, M., Maniati, M., Ulusoy, A., Kirik, D., Park, D. S., Wada, K., & Stefanis, L. (2012). Selective

neuroprotective effects of the S18Y polymorphic variant of UCH-L1 in the dopaminergic system. *Human Molecular Genetics, 21*, 874–889.

Yang, Q., She, H., Gearing, M., Colla, E., Lee, M., Shacka, J. J., & Mao, Z. (2009). Regulation of neuronal survival factor MEF2D by chaperone-mediated autophagy. *Science, 323*, 124–127.

Zarranz, J. J., Alegre, J., Gomez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atares, B., Llorens, V., Gomez Tortosa, E., del Ser, T., Munoz, D. G., & de Yebenes, J. G. (2004). The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Annals of Neurology, 55*, 164–173.

Zhu, J. H., Guo, F., Shelburne, J., Watkins, S., & Chu, C. T. (2003). Localization of phosphorylated ERK/MAP kinases to mitochondria and autophagosomes in Lewy body diseases. *Brain Pathology, 13*, 473–481.

Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., Kachergus, J., Hulihan, M., Uitti, R. J., Calne, D. B., Stoessl, A. J., Pfeiffer, R. F., Patenge, N., Carbajal, I. C., Vieregge, P., Asmus, F., Muller-Myhsok, B., Dickson, D. W., Meitinger, T., Strom, T. M., Wszolek, Z. K., & Gasser, T. (2004). Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron, 44*, 601–607.

Ziviani, E., & Whitworth, A. J. (2010). How could Parkin-mediated ubiquitination of mitofusin promote mitophagy? *Autophagy, 6*, 660.

Ziviani, E., Tao, R. N., & Whitworth, A. J. (2010). Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 5018–5023.

Dopaminergic Neurons in Parkinson's Disease

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Abstract

The discovery of dopamine as a brain neurotransmitter by Arvid Carlsson and colleagues about 50 years ago contributed to better understand some of the brain diseases. Some of the drugs that are most widely used in neurologic and psychiatry illnesses, such as levodopa and antipsychotic drugs, act on dopaminergic mechanism. The discovery that the motor impairments of Parkinson's disease patients improved after restoring the physiological levels of striatal dopamine with levodopa attracted the attention of the neuroscience community for the role of this neurotransmitter in motor and brain functions. In the last decades, the knowledge has also been challenged by evidence that Parkinson's disease also affects cognitive and affective functions. Shortly after the introduction of levodopa as a therapy, a complex set of secondary phenomena such as dyskinesia was observed following repeated administration of the dopamine precursor. Information of dopaminergic cells and circuits has been enriched by findings obtained with several and highly sensitive technology in cellular biology, with sophisticated behavioral analyses of transgenic animals and functional neuroimaging. The present chapter attempts to review results reported in different clinical studies and animal models to provide a comprehensive picture of the role of dopamine in Parkinson's disease. Treatments have successfully been translated from preclinical to pharmacotherapeutic arsenal increasing clinical settings.

Keywords

Basal ganglia • Dopamine • Dopamine receptors • Dopaminergic drugs • Levodopa • Mesocortical system • Mesolimbic system • Nigrostriatal system • Nitric oxide • Parkinson's disease

List of Abbreviations

3-OMD	3-*O*-methyldopa
6-OHDA	6-hydroxydopamine
AIMs	Abnormal involuntary movements
AS	Alpha-synuclein
CNS	Central nervous system
COMT	Catechol-*O*-methyltransferase
DARPP-32	Dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa
DAT	Dopamine transporter
DLB	Dementia with Lewy body
DMV	Dorsal motor nucleus of vagus
DYN	Dynorphin
ENK	Enkephalin
FS	Fast spiking
GPCRs	G protein-coupled receptors
GPe	External segment of the globus pallidus
GPi	Internal segment of the globus pallidus

iLBP	Incident Lewy body pathology
IrtZ	Intermediate reticular zone
i.v.	Intravenous
LB	Lewy bodies
LC	Locus coeruleus
L-DOPA	L-3,4-dihydroxyphenylalanine
LN	Lewy neurites
LTS	Low-threshold spiking
MAO-B	Monoamine oxidase-B
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSN	Medium spiny neuron
NPY	Neuropeptide Y
PD	Parkinson's disease
PET	Positive emission tomography
PV	Parvalbumin
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
SOM	Somatostatin
SP	Substance P
SPECT	Single-photon emission computed tomography
STN	Subthalamic nucleus
TH	Tyrosine hydroxylase
VMAT2	Vesicular monoamine transporter 2
VTA	Ventral tegmental area

1 Introduction

At the beginning of the nineteenth century, when James Parkinson published the book entitled "*Essay on the Shaking Palsy*" (Parkinson 2002), life expectation was no longer than 45 years old. The "shaking palsy," later named Parkinson's disease (PD) by Charcot, affects mainly aged people. Nowadays, the prevalence of PD in industrialized countries is generally estimated at 0.3 % of the entire population and about 1 % in people over 60 years of age (Nussbaum and Ellis 2003). Age disease incidence ranges from 110 to 330 per 100,000 individuals over 50 years old, and its incidence increases with increasing age throughout the life span (Mayeux 2003). Now, in the beginning of the twenty-first century, life expectancy is near 80 years old, and the incidence rate of PD increases to 400–500 individuals per 100,000 annually (Mayeux 2003). Therefore, there are much more parkinsonian patients nowadays than two centuries ago, and with the increasing age of the general population, the prevalence of PD will rise constantly in the future.

PD is characterized clinically by bradykinesia, rest tremor, muscle rigidity, and postural reflex impairment. The discovery that the motor impairments of PD patients improved after restoring the physiological levels of striatal dopamine with levodopa attracted the attention of the neuroscience community for the role of this neurotransmitter in motor functions (Olanow and Tatton 1999). Since dopamine is the main modulator of the basal ganglia, their components have long been seen as part of the motor system (Graybiel et al. 1994). In the last decades, this view has been challenged by evidence that PD also affects cognitive and affective functions (Dubois and Pillon 1997; Chaudhuri et al. 2006; Da Cunha et al. 2009). In addition, dysfunction of dopamine neurotransmission has been implicated in many other psychiatric and neurological diseases, such as attention deficit hyperactivity disorder (ADHD), drug addiction, Tourette syndrome, schizophrenia, and obsessive-compulsive syndrome. Furthermore, a growing body of evidence has been accumulated supporting the view that some kinds of cognitive processes, such as instrumental learning, decision-making, and other executive functions, depend critically on the dopamine signaling (see Da Cunha et al. 2009).

The present chapter attempts to review results reported in different clinical studies and animal models to provide a comprehensive picture of the role of dopamine signaling in PD.

2 Dopamine Neurotransmission System: Pathways, Receptors, and Signaling

A detailed review of the history and neurobiology of the dopamine signaling is beyond the scope of this chapter and can be found in very interesting reviews by Hornykiewicz (2002), Iversen and Iversen (2007), and Fahn (2008). The neurotransmitter dopamine plays a crucial role in motor, motivational, and reward-related functions of the central nervous system (CNS). Our understanding of neuropsychiatric illnesses and the modern psychopharmacology was greatly influenced by the discovery of dopamine as a neurotransmitter in the brain by Arvid Carlsson and colleagues about 50 years ago. Some of the drugs that are most widely used in the treatment of neurologic and psychiatry illnesses, such as levodopa, methylphenidate, and antipsychotic drugs, act on dopaminergic mechanisms. Therefore, dopamine neurotransmitter system has been one of the major and fertile fields of behavioral neuroscience and its research culminated in the award of the Nobel Prize in 2000 to three scientists, Carlsson, Greengard, and Kandel, for their important contributions to this field.

Dopamine was the last catecholamine discovered, and Sir Henry Dale, in 1952, suggested dopamine as the current name of 3,4-dihydroxyphenethylamine, also known as 3-hydroxytyramine. Its biosynthesis begins with dietary amino acid tyrosine actively transported into the brain. Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis, is regulated by multiple mechanisms

(Molinoff and Axelrod 1971). Rapid activation of TH activity occurs via its phosphorylation by several protein kinases, including protein kinase A, Ca^{2+}/calmodulin-dependent protein kinase II, and protein kinase C. Possibly, its phosphorylation induces conformational change that results in a lower affinity of TH for catecholamines that trigger end-product inhibition of this enzyme. The end result is an increase of TH catalytic activity (Nestler et al. 2009). Several drugs can up- or downregulate TH expression, and extracellular stimuli may induce longer-term changes in TH activity through transcriptional regulation of its gene.

Dopamine is synthesized in the cytosol and transported into synaptic vesicles via the vesicular monoamine transporter 2 (VMAT2). From the synaptic vesicles, dopamine is released in a quantal manner when a nerve impulse traveling down the axon reaches the nerve terminal. Once in the synaptic cleft, dopamine can act on both postsynaptic and presynaptic dopamine receptors. Dopamine could be metabolized to homovanillic acid (HVA); 3,4-dihydroxyphenylacetic acid (DOPAC) via monoamine oxidase (MAO), an enzyme considered to be mostly intraneuronal; and 3-methoxytyramine (3-MT) via catechol-*O*-methyltransferase (COMT), an enzyme considered mostly extraneuronal. Entacapone and tolcapone are reversible COMT inhibitors which have been approved for clinical use in patients with PD as adjuvant to levodopa, significantly improving the clinical benefits of this therapy (Bonifácio et al. 2007).

Dopamine signal ends by spontaneous amine diffusion, local enzymatic degradation, and amine reuptake in the presynaptic neurons by dopamine transporter (DAT) (Cragg and Rice 2004; Nestler et al. 2009; Eriksen et al. 2010). This dopamine transporter seemingly play a key role in the dopamine transmission if we consider the behavioral consequences of their inhibition with blocking agents known to be powerful psychostimulants or drugs of abuse, as amphetamine or cocaine (see Nestler et al. 2009; Fig. 1).

Due to their diverse anatomy and disparate functions, different populations of dopamine neurons have been specifically implicated in a variety of neurological disorders. Biochemical characterization of dopamine in the brain revealed two families of dopamine receptors: D1-like receptors that also comprises D5 receptor and are able to stimulate adenylyl cyclase and increase cAMP production and D2-like receptors that comprises D3 and D4 receptors and are negatively coupled to adenylyl cyclase activity and inhibit cAMP production. In mammals, all of these receptors belong to the superfamily of GPCRs with seven transmembrane domains. D1 family is coupled to stimulatory G_s or the related G_{olf}, and the D2 family is coupled to inhibitory G_i/G_o (for review, see Herve and Girault 2005). D1 receptor activation stimulates the cAMP increase leading to the activation of protein kinase that have a broad array of cellular targets, including transcription factors, voltage-dependent ion channels, and glutamate receptors (Svenningsson et al. 2004).

Dopaminergic neurons and pathways were first described using classical histofluorescence techniques during the 1960s and 1970s (Carlsson et al. 1962). Knowledge of dopaminergic cells and circuits has been enriched by findings

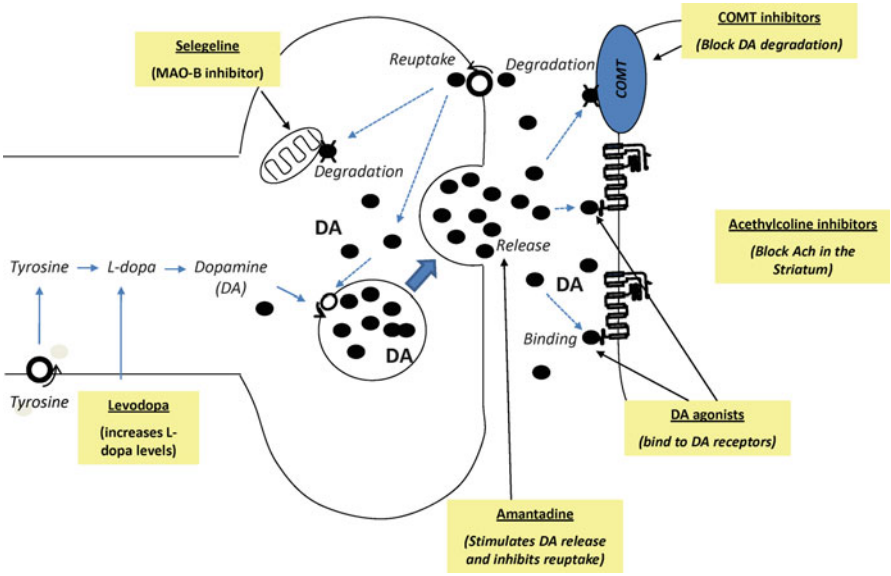

Fig. 1 *Dopamine synthesis, release, signaling, and reuptake and pharmacological approaches currently available for Parkinson's disease*. Dopamine synthesis originates from the amino acid tyrosine, which is converted to DOPA by the enzyme tyrosine hydroxylase (*TH*) (a rate-limiting enzyme). Subsequently, DOPA is decarboxylated, by the enzyme L-aromatic amino acid decarboxylase (DOPA decarboxylase), to form dopamine. L-dopa that is used in the Parkinson's disease treatment bypasses the rate-limiting enzymatic step and is decarboxylated by DOPA decarboxylase to form dopamine. The most significant mechanism by which the synaptic actions of dopamine is terminated is the reuptake into the nerve terminal via specific dopamine transporter (*DAT*) expressed on the membrane of presynaptic terminal. Dopamine is also catabolized by the enzymes monoamine oxidase (*MAO*) and catechol-*O*-methyltransferase (*COMT*). Possibly, MAO exists in mitochondria within dopaminergic nerve terminals, on extracellular space, and also in glia proximate to dopamine synapses. After its synthesis, dopamine acts on pre- and postsynaptic receptors from D1 or D2 dopamine receptor families, which, respectively, activates Gs protein and increases cAMP production or Gi protein and decreases cAMP production. Generally drugs including L-DOPA, carbidopa, amantadine, and COMT and MAO-B inhibitors revert temporarily the dopamine dysfunction caused by dopaminergic neurodegeneration in the basal ganglia of Parkinson's disease patients

obtained with several and highly-sensitive techniques, as immunohistochemistry (mainly to dopamine essential synthetic enzyme TH), cellular biology, transgenic mice or conditional mutants, functional anatomy, electrophysiological studies, sophisticated behavioral analyses, and functional neuroimaging. Altogether, these studies have also led to new concepts of healthy and pathological functions of dopaminergic circuits at the molecular and more integrated levels (Björklund and Dunnett 2007).

In the ventral mesencephalic tegmentum, dopaminergic cell bodies are very well organized in the following groups: the substantia nigra pars compacta (SNc) (A9),

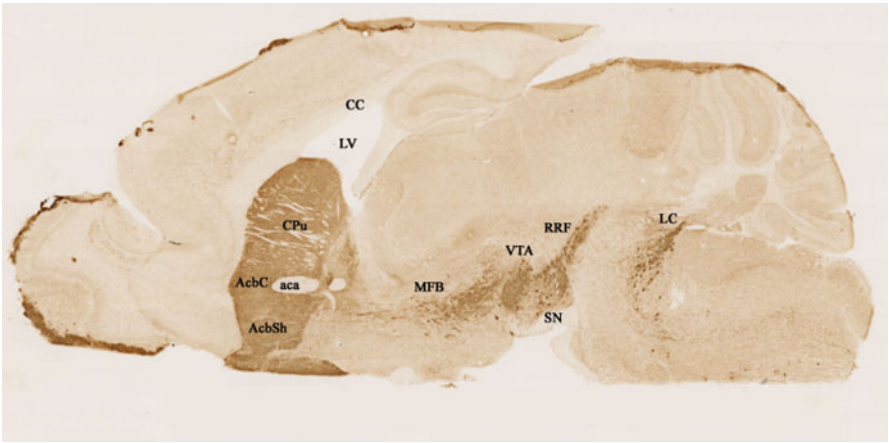

Fig. 2 Rat brain sagittal view (adult, *Wistar*) of the tyrosine hydroxylase immunocytochemistry showing the distribution of dopaminergic neurons. Abbreviations: *aca* anterior comm, anterior part, *AcbC* accumbens nucleus, core, *AcbSh* accumbens nucleus, shell, *cc* corpus callosum, *CPu* caudate putamen (striatum), *LC* locus coeruleus, *LV* lateral ventricle, *MFB* medial forebrain bundle, *RRF* retrorubral field, *SN* substantia nigra, *VTA* ventral tegmental area

the ventral tegmental area (VTA-A10), and the retrorubral area (A8) (Fig. 2). Dahlström and Fuxe (1964) defined the nomenclature of dopaminergic system in the rat midbrain: A8, A9 (substantia nigra), and A10 cell groups. The classification is nonetheless widely used.

Ascending dopaminergic pathways in the mammalian CNS can be simplified divided into three major systems. The two largest groups are located in the midbrain: the nigrostriatal system that originates in the substantia nigra (A9) and innervates predominantly the dorsal striatum and the mesocortical and mesolimbic systems that arise from the VTA and project to the cortical or several limbic areas (Bjorklund and Lindvall 1984; Krimer et al. 1997). The cell group of retrorubral area that forms a dorsal and caudal extension of the VTA contains cells that project to striatal, limbic, and cortical areas (Bentivoglio and Morelli 2005).

2.1 Nigrostriatal System

Since the first detailed studies of the SN by Cajal (1911), two main subdivisions were recognized. The SNc is characterized by densely packed neurons, and the substantia nigra *pars reticulata* (SNr) is marked by sparse dopaminergic cells, enmeshed in dopaminergic dendrites (Fig. 3). Most of the properties of dopamine neurotransmission in vivo are known from studies of the nigrostriatal dopamine pathway extending from the SNc to the dorsal striatum through the median

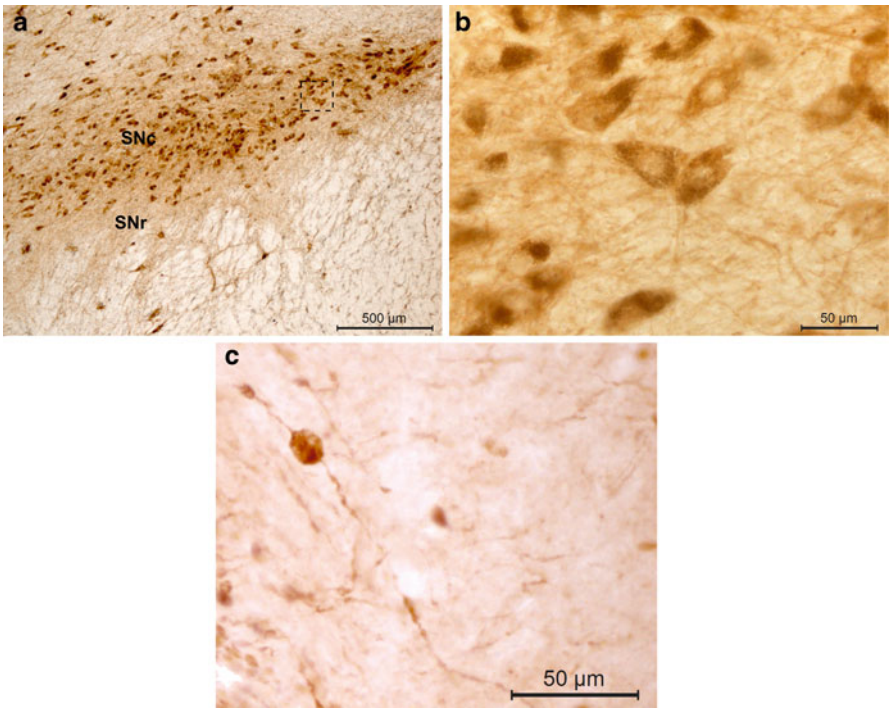

Fig. 3 Tyrosine hydroxylase immunocytochemistry of the regions of the human brain presenting positive reaction in the substantia nigra *compacta* (**a**, **b**; SNc), *reticulata* (**a** SNr,), and the striatum (**c**; caudate). Observe a high density of TH-positive neurons in the SNc, in contrast to sparse distribution in the SNr. TH- positive neurons are detected in the human and non human -primate striatum and and also in mice

forebrain bundle. Somato-dendritic release of dopamine in the SNc and axonal dopamine release in the dorsal striatum are both necessary for the expression of basal ganglia-mediated motor behaviors and various cognitive functions. In both rat and monkey, the striatal dopamine innervation is derived not only from SNc but also from cells located in the lateral VTA and retrorubral area. For this reason, the term "mesostriatal dopamine pathway" sometimes is used to include all components of the midbrain dopamine system projecting to the striatum (Björklund and Dunnett 2007).

The identification of cells of the SN as dopaminergic and of the dopaminergic innervation of the striatum through the nigrostriatal tract is inseparably intertwined with methodological achievements in experimental and chemical neuroanatomy in the 1960s and 1970s. These discoveries were intrinsically correlated to the histopathology of the midbrain dopaminergic system in PD in the 1960s (Bentivoglio and Morelli 2005).

2.2 Mesocortical and Mesolimbic Systems

Both of these systems connect the ventral tegmental dopamine cells (A8, A10) to prefrontal and cingulate cortex or to nucleus accumbens, amygdaloid complex, hippocampus, and piriform cortex. Ventral tegmental dopaminergic neurons play a critical role in motivation, reward-related behavior, attention, and multiple forms of memory (Nestler et al. 2009).

A third population of midbrain dopamine neurons resides in the retrorubral field and projects primarily to the dorsal striatum and the pontomedullary reticular formation; it is thought to play a role in orofacial movements. There are also connections between the retrorubral field and SN/VTA dopamine neurons. There are at least four groups of dopamine neurons in the hypothalamus that are intimately involved in neuroendocrine, hormonal, and arousal processes. Finally, there are populations of dopaminergic amacrine cells in the retina that contribute to neural adaptation to light and also some dopamine cells in the olfactory bulb (Nestler et al. 2009).

The striatum receives input from a number of regions, including the motor, sensory, and frontal cortex, integrates this information under the modulatory influence of dopamine, and uses this information to guide motor behavior. A cardinal feature of the striatum – the main integrator of cortical and thalamic information reaching the basal ganglia – is its dense dopamine innervation, which arises from the midbrain nigrostriatal pathway. Nigrostriatal dopaminergic neurons form highly branched networks within their striatal targets. The full extent of this arborization was elegantly demonstrated by Matsuda and colleagues (2009). A single dopaminergic neuron innervated up to 5.7 % of the neostriatum, while adjacent input dopaminergic neurons showed high degrees of overlapping with targets. It was calculated that a single postsynaptic medium spiny neuron (MSN) could be influenced by up to 194 dopaminergic inputs. As a result of this redundancy, clinical signs of PD only develop after up to 70 % of SNc neurons are lost. Each striatal MSN receives input from several thousand different cortical neurons on its spines. The input from each synapse is weak and many inputs are needed to discharge the neuron (Rothwell 2011).

Anatomically, striatal cells fall into two main classes: (a) spiny projection neurons and (b) aspiny interneurons. Spiny projection neurons, also known as medium spiny neurons (MSNs), represent the vast majority of striatal neurons. MSNs receive glutamatergic inputs from cortex and thalamus that terminate predominantly on spines (Kemp and Powell 1971). In addition, they are a main target of midbrain dopaminergic neuron axons that form synapses on MSN dendrites and spine necks (Smith et al. 1994).

One of the major targets of this innervation is the principal neuron of the striatum: GABAergic spiny projection neurons. Spiny projection neurons constitute as much as 90 % of the striatal neuron population. They can be divided into two approximately equally sized populations based on axonal projections. One population – **direct pathway spiny projection neurons** – projects axons to the nuclei at the interface between the basal ganglia and the rest of the brain,

whereas the other population, **indirect pathway spiny projection neurons**, projects only indirectly to the interface nuclei. The dopamine D1 receptor is expressed selectively by direct pathway spiny projection neurons. Also, **direct pathway** spiny projection neurons express high levels of substance P (SP) and dynorphin (DYN). The dopamine D2 receptor is expressed by **indirect pathway** spiny projection neurons, which also express enkephalin (ENK). These two G protein-coupled receptors (GPCRs) have distinctive intracellular signaling cascades and targets, leading to fundamentally different cellular responses to extracellular dopamine (see below).

There are four well-characterized classes of striatal interneuron: cholinergic interneuron, parvalbumin-expressing GABAergic interneuron, calretinin-expressing GABAergic interneuron, and neuropeptide Y (NPY)/nitric oxide-expressing GABAergic interneuron (Tepper et al. 2008). Together, these interneurons constitute 5–10 % of all striatal neurons. One of them, the cholinergic interneuron, co expresses dopamine D2 and D5 receptors (Bergson et al. 1995) and modulates both spiny projection neurons populations through muscarinic receptors (Yan and Surmeier 1996). Two other prominent interneurons, the somatostatin (SOM)/NPY-expressing GABAergic interneuron and the parvalbumin (PV) GABAergic interneuron, express dopamine D5 receptors (Centonze et al. 2003). The PV interneuron is strongly innervated by globus pallidus neurons that express dopamine D2 receptors (Bevan et al. 1998), creating a microcircuit that is influenced by both dopamine D1 and D2 receptors.

According to the classic model of basal ganglia function (see Fig. 4; DeLong 1990), movements occur during pauses in the tonic inhibitory activity of the basal ganglia output interface, generated by activity in the **direct pathway**. This model had its origins in neurophysiologic studies showing that corticostriatal activation of the direct striatonigral pathway results in pauses of the tonic activity of GABAergic neurons in the SNr (Chevalier et al. 1985). The opposing role of the **indirect pathway** in suppressing movements was originally proposed on the basis of studies in animal models of PD. Mink (2003) reviewed the classic model, proposing that the activity of direct and indirect pathways is coordinated to select particular motor programs and to inhibit competing motor programs. This model predicts that during ongoing behavior, there will be increased activity in neuronal ensembles that are part of direct and indirect pathway circuits, rather than one or the other. The execution of a movement sequence would then generate a complex pattern of activity in specific neuronal ensembles. Recent work using optogenetic or genetic approaches supports the general tenets of the model (Hikida et al. 2010; Kravitz et al. 2010). Although this model has proven to be of considerable clinical value, it fails to account for the great diversity and complexity of the decision-making process in action selection (Cisek and Kalaska 2010).

Dopamine acted by stimulating the MSNs of the direct and inhibiting those of the indirect pathway (Albin et al. 1989; Gerfen et al. 1990; Gerfen and Surmeier 2011). These opposing effects were suggested to depend on the expression of dopamine D1 receptors in the MSNs of the direct pathway and of dopamine D2 receptors in the MSNs of the indirect pathway. These receptors have distinctive

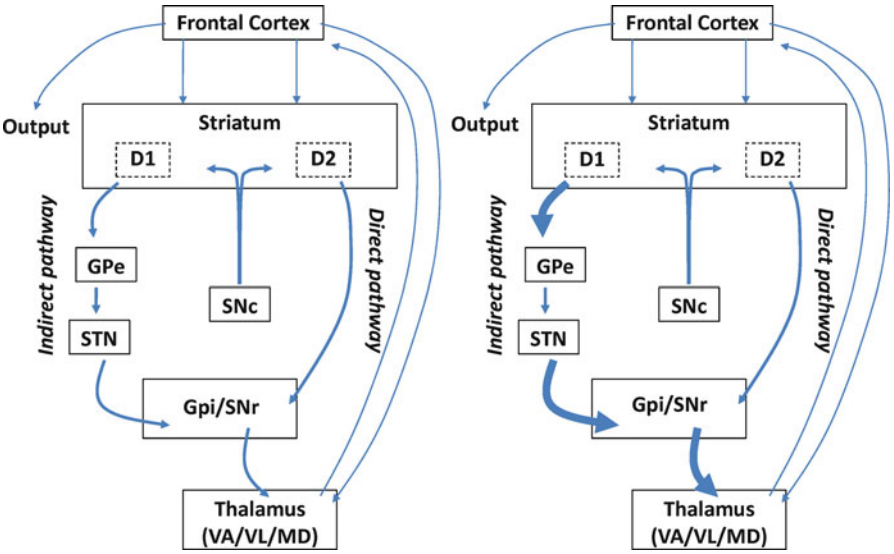

Fig. 4 *Overview of schematic representation of the main basal ganglia-thalamocortical circuits* (*left*). Normal status: the two output routes ("indirect" and "direct") are in balance at the level of the output structures (the GPi and the SNr) (*right*). Neurodegenerative changes in the central nervous system in Parkinson's disease (*PD*): depletion of dopamine in the striatum leads to imbalance in the two output routes and suppression of thalamocortical activity. The thickness of the *arrows i*ndicates the level of activity in the pathways. For some time, the symptoms of PD have been described as a loss of balance between the direct and indirect striatal output pathways. The indirect pathway is attenuated primarily by D2 receptor stimulation, whereas the direct pathway is activated via D1 receptor stimulation. With normal tonic/phasic balance, cortical activation of the indirect pathway (attenuated by tonic D2 stimulation) is in balance with other presumably motor cortical inputs influencing the direct pathway (augmented by phasic D1 stimulation). However, in PD, this balance is disrupted, with the loss of DA resulting in hyperactivity (with akinesia) in the indirect pathway and depression (with loss of voluntary movement) within the direct pathway. The model predicts that neuronal firing in the STN and GPi is increased in the parkinsonian state, leading to excessive inhibition of brain stem and thalamocortical neurons with the development of parkinsonian motor features. In contrast, the model proposes that dyskinesia is related to the decreased firing in the STN and GPi, with reduced inhibition of thalamic and cortical motor regions. Abbreviations: *D* dopamine, *GPe* external segment of globus pallidus, *GPi* internal segment of globus pallidus, *MD* mediodorsal nucleus, *SNr* reticular part of the substantia nigra, *SNc pars compacta* of the substantia nigra, *STN* subthalamic nucleus, *VA/VL* ventral anterior/ventral lateral thalamic nuclei (Modified from Hauser 2009)

intracellular signaling cascades and targets, leading to fundamentally different cellular responses to extracellular dopamine (Gerfen and Surmeier 2011). Also, direct pathway expresses high levels of substance P and dynorphin, whereas indirect pathway expresses enkephalin. Direct and indirect pathways are representing on Fig. 4.

Dopamine stimulation of D1 receptors acts via cAMP and protein kinase A to phosphorylate dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa

(DARPP-32), an important mediator of cAMP signaling and striatal excitability. The DARPP-32 signaling pathway has a central role in mediating signal transduction within MSNs in the striatum, and the function of this phosphoprotein depends on its relative state of phosphorylation at two main regulatory sites, threonine 34 (T34) and threonine T75 (T75). When DARPP-32 is phosphorylated at T34 by protein kinase A, it becomes a potent inhibitor of protein phosphatase 1, which in turn regulates the phosphorylation state of several classes of effector proteins, including transcription factors, ionotropic receptors, and ion channels (Greengard et al. 1999; Grace 2002). It has been shown that DARPP-32 is also present in other non-D1-containing neurons as well, including the enkephalin-containing striatal neurons (Langley et al. 1997). In this case, in contrast, stimulation of dopamine D2 receptors causes a dephosphorylation of DARPP-32 via calcineurin activation by calcium influx (Grace 2002).

Dopamine release appears to occur via two functionally distinct components. One is known as phasic component of dopamine release and is believed to underlie most of the behavioral indices of this transmitter. Phasic dopamine release occurs in a high-amplitude, brief pulsatile manner by means of action potentials and then is rapidly removed from the synaptic cleft via reuptake (Grace 1991, 2002). The other component is known as tonic dopamine release that occurs in very low concentration but is sufficient to activate extrasynaptic receptors, including dopamine terminal autoreceptors. D2 and D3 receptors act as inhibitory presynaptic autoreceptors and as postsynaptic receptors. In addition, D2 receptors have two splice variants, $D2_{short}$ and $D2_{long}$ (Nestler et al. 2009). Autoreceptors can exist on most portions of dopamine cells including the soma, dendrites, and nerve terminals. Stimulation of dopamine autoreceptors slows or inhibits dopamine synthesis or release. Thus, autoreceptors are supposed to exert a tonic downregulation of dopamine neuron activity, maintaining their firing within a stable range of activity (Harden and Grace 1995).

3 Dopamine-Related Disease: Parkinson's Disease

Several important diseases of the CNS are associated with dysfunctions of the dopamine system, ranging from PD to schizophrenia and drug addiction. In this review, we shall focus only the Parkinson's disease and some consequences for human beings. The discovery of mechanism of action of several drugs that act on dopamine synapses really improved the knowledge about the dopaminergic system and its related diseases.

PD is characterized by complex motor symptomatology, including tremor at rest, bradykinesia, rigidity, postural instability, stooped posture, and freezing that occur by hypofunctional dopamine states when nigrostriatal pathway is degenerated. Dopamine became clinically relevant when dopamine depletion in the caudate nucleus of patients with PD was discovered, whereas intravenous (i.v.) administration of L-dihydroxyphenylalanine (L-DOPA), the amino acid precursor of dopamine,

dramatically and rapidly alleviated parkinsonian symptoms (Hornykiewicz 2010). Studies with animal models of PD provided the first clear indication of the dichotomy in the regulation of the direct and indirect pathways, suggesting that their excitability shifts in opposite directions following the loss of dopamine, creating an imbalance in the regulation of the motor thalamus favoring suppression of movement (Albin et al. 1989). Specifically, direct pathway was suggested to spike less in the PD state, whereas indirect pathway was thought to spike more (Gerfen and Surmeier 2011).

Besides motor symptoms, dopaminergic-related non-motor symptoms, as depression, apathy, and sleep disorders, are part of the clinical spectrum of PD. There is increasing evidence that such symptoms are treatable, at least in part, with various dopaminergic agents (Chaudhuri and Schapira 2009).

One current drawback of dopaminergic therapy is that it cannot be targeted to a specific subpopulation of dopamine neurons. A consequence of this is significant dopamine-related side effects, such as hallucinations or compulsive gambling during treatment for PD or extrapyramidal and sexual side effects caused by antipsychotics. In addition, from the standpoint of pathogenesis, it is clear that dopaminergic neurotransmission is not the sole dysfunction in this neurological disease.

4 Dopamine Signaling in Parkinson's Disease

4.1 The Neuropathology of Parkinson's Disease: A Brief Historical Overview

The association of PD with the mesencephalic dopaminergic zone was hypothesized by Edouard Brissaud in 1893, based on an autopsy case of unilateral Parkinsonism associated with a tumor confined in the SN (see Parent and Parent 2010). The hypothesis was validated by Konstantin Tretiakoff in 1919, in his doctoral thesis, reporting the marked loss of neurons in the SNc and the presence of intracytoplasmic inclusions which he called "*corps de Lewy*" (reference to Friedrich Lewy's description of these pathological findings) in the surviving neurons of PD patients (Lees et al. 2008), after confirmed by Hassler in 1938, which found a constant cell loss in the SNc of 10 PD patients (Hassler 1938). Just on the beginning of 1960, Hornykiewicz described the presence of dopamine in SN and that it is reduced in the midbrain and in the striatum of parkinsonian patients, suggesting the possible existence of a link between SN and the corpus striatum (Hornykiewicz 1963).

Using new fluorescence techniques, Dahlström and Fuxe (1964) described firstly, in rat, the occurrence of catecholaminergic cell in the midbrain and lower brain stem, and Andén et al. (1964) noticed that lesions of SNc caused a substantial loss of the catecholaminergic fluorescence in the striatum, helping the acceptance of the existence of a nigrostriatal fiber system originating from dopaminergic midbrain neurons. The utilization of immunohistochemistry labeling of enzymes

participating in the metabolism was very useful to study the catecholaminergic neurons of midbrain, and Hökfelt et al. (1973) were the first to identify dopaminergic neurons in midbrain using antibodies to aromatic acid decarboxylase, followed by the immunohistochemical revelation of the rate-limiting enzyme of dopamine synthesis, the TH, done by Ljungdahl et al. (1975), confirming the dopaminergic nature of nigrostriatal pathway and starting a new era for basal ganglia research.

Jakes and co-workers described two proteins of synuclein family, the alpha- and beta-synuclein, in human brain (Jakes et al. 1994). After some descriptions of familial cases of PD associated with mutation on alpha-synuclein (AS) (Polymeropoulos et al. 1997; Goedert 1997), Spillantini et al. (1997) reported, for the first time, the strong immunolabeling of Lewy bodies (LB) and neurites (LN) with AS in PD and dementia with Lewy body (DLB), followed by several publications which reinforced the relevance of AS on PD pathogenesis.

4.2 The Topographic Braak Staging of Parkinson's Disease

After the recent observations on relationship of AS and PD, Del Tredici et al. (2002) evaluated the presence of AS in brain stem of subjects without history of neurologic or psychiatric disease. They described that all patients with AS inclusions in dorsal motor nucleus of vagus (DMV) and in intermediate reticular zone (IRtZ) presented also AS-immunoreactivity in other brain stem nuclei, as the locus coeruleus (LC) and the SN, suggesting that the pathological progression of PD could begin in the medulla and spread to the whole brain. The absence of clinical signs of PD in these individuals, even with AS pathology, can be explained by the existence of a presymptomatic phase before the onset of motor dysfunction.

The study of Braak et al. (2003) changed the classic concepts of an essentially dopaminergic dysfunction in PD. Using the same methods of Del Tredici et al. (2002) in 3 groups of (1) 41 individuals with clinical diagnosis of PD, (2) 69 individuals without neurological or psychiatric disease but with AS brain pathology, and (3) 58 individuals without neurological or psychiatric disease nor AS pathology, they showed that LB and LN marked with AS were present in nigral and extranigral areas, with a particular vulnerability and independent of neurotransmitter produced by the neurons, which became involved at different times in the progression of the disease, in a pattern of topographical caudorostral progression, as follows:

- Stage 1: The presence of LN and LB was detected in two major nuclei: DMV and the IRtZ. At this stage LN was observed first than LB; however, in advanced cases, the LN outnumbers the LB in these areas. This pattern was seen at all stages of disease progression. Some neuromelanin-laden nerve cells in these nuclei do not show LB/LN. The anterior olfactory structures (olfactory bulb/stalk and the anterior olfactory nucleus) are also affected by AS inclusions. This stage of pathology spares the pontine melanized neurons, as the LC.

- Stage 2: The more accentuated presence of LB/LN in DMV and IRtZ and the loss of neurons are seen at this stage. Furthermore, the pathological LB/LN appears in pontine areas of caudal raphe nuclei, the gigantocellular reticular nucleus and the coeruleus-subcoeruleus complex, but the latter nucleus has unaffected neurons. The midbrain nuclei remain uninvolved.
- Stage 3: There is worsening of lesions on affected nuclei in medulla and pons, and the LB/LN is seen in midbrain structures and nuclei from the basal forebrain. In the midbrain, the presence of LB/LN in SN is observed initially in melanized projection neurons of posterolateral and posteromedial subnuclei, without macroscopically detectable depigmentation. The compact portion of pedunculopontine tegmental nucleus is affected only by LN in stage 3. The magnocellular nuclei of the basal forebrain (medial septal nucleus, interstitial nucleus of diagonal band, basal nucleus of Meynert) develop only a network of LN. There is no pathological alteration in temporal mesocortex or neocortex, but LNs are seen in anterior olfactory nucleus and second sector of Ammon's horn (CA2) (for a wide review about extranigral brain stem nuclei affected by AS pathology, see Grinberg et al. 2010).
- Stage 4: The midbrain shows accentuated involvement of neuromelanin-laden neurons of SNc, more significant in posterior region of pars compacta; the depigmentation is macroscopically seen at this stage and is observed in the extracellular melanin granules in SNc. Other mesencephalic regions are affected, such as the paranigral and parabrachial nucleus, and the compact portion of pedunculopontine tegmental nucleus (PPN) is more affected by LB and LN. The basal forebrain becomes severely involved, and areas as stria terminalis, central nucleus of amygdala, and ventral claustrum show LB. The anterior olfactory nucleus is much damaged. This stage is characterized by AS accumulation in the anteromedial temporal mesocortex, with outer layers developing networks of LN and LB within inner layers.
- Stage 5: The last two stages indicate diffuse AS immunolabeling, including the neocortex. The SN is almost without melanin, and LB develops in melanized nerve cells as DMV and reticular formation. The network of LN in CA2 extends into adjacent sectors, comprising large portions of hippocampus. From the anteromedial temporal mesocortex (the periallocortical transentorhinal region and the proneocortical entorhinal area), the pathological progression moves toward the sensory association areas, anterior cingulate cortex, and prefrontal area, with LB in infragranular layers and LN in supragranular layers. The primary neocortical areas are spared.
- Stage 6: The final stage encloses the entire neocortex, but the pathological changes on premotor area, primary motor field, and primary sensory area are mild.

The authors of this study suggested that this staging model permits the distinction of clinically manifested cases, even as non-motor initial PD patients, as REM sleep disorders, psychiatric diseases, intestinal constipation, olfactory dysfunctions, and dysautonomia. The Braak hypothesis shows a correlation among pathological and clinical phenotypes. Jellinger (2003) observed that some individuals from

Braak's study had stages 3–4 PD pathology, but no motor symptoms were diagnosed, in which he called incident Lewy body pathology (iLBP). Furthermore, as noticed by Parkkinen et al. (2003) and Ishizawa et al. 2003, other diseases as DLB and AD showed LB with AS-immunoreactivity in the brain, reducing the specificity of Braak scheme for PD. After the publication of those works, the Braak staging model had gained acceptance rapidly. However, there are no conclusive proofs confirming a caudal-to-rostral temporal progression in PD pathology. Additional studies are required to elucidate these questions.

4.3 The Human Midbrain Dopaminergic System and Parkinson's Disease

Classically, the bradykinesia, rigidity, and tremor in parkinsonian syndromes are attributed to mesencephalic dysfunctions, and diseases as strokes and tumors localized in SN can cause a rigid-akinetic phenotype (Garcia de Yebenes et al. 1982; Akyol et al. 2006; Orta Daniel and Ulises 2008). PD is modernly considered as a multisystem neurodegenerative illness, affecting dopaminergic and non-dopaminergic neurons (serotoninergic, glutamatergic, cholinergic, nitrergic) as seen on Braak hypothesis. However, the major motor symptoms (bradykinesia and rigidity) and the motor complications (dyskinesia, wearing off phenomenon) are associated to dopaminergic transmission, justifying the special attention given to the nigrostriatal degeneration. There are other dopamine-rich areas in CNS, as the hypothalamus and retina, but its involvement in PD is controversial (for review see Archibald et al. 2009).

Based on the immunoreactivity to calbindin, the midbrain dopaminergic system can be divided into two tiers: the dorsal and the ventral tier. The dorsal tier encompasses the retrorubral groups and the VTA, its neurons are calbindin-positive, and they have horizontal orientation and low expression of DAT and D2 receptors. The ventral tier is composed by SNc, with calbindin-negative and ventrally oriented neurons, showing high amounts of DAT and D2 receptors. This cytochemical phenotypic classification has a practical application: the ventral tier is particularly vulnerable to the neurodegeneration of PD but with selective sparing of the dorsal tier (Haber and Gdowski 2004). However, the neuromelanin-laden neuron loss in SNc is not necessarily uniform (Ma et al. 1996).

Fearnley and Lees (1991) studied the micro-architecture of SN and the rates of neuron loss in normal aging and parkinsonian syndromes, and there was a linear reduction of pigmented neurons in advancing aging, more affected in dorsal tier. In opposite pattern, the PD individuals had 45 % of neuron loss in the first decade of disease, being greatest in the lateral ventral tier (average loss 91 %), followed by the medial ventral tier (71 %) and the dorsal tier (56 %). These results show a specific pattern of nerve cell loss in SNc on PD (Fearnley and Lees 1991). Ma et al. (1996) performed a morphometric analysis of SNc in PD individuals, and they described a marked reduction in pigmented neuron area, perimeter, diameter, and number in PD cases.

Damier et al. (1999a, b) developed a new method to analyze the SNc, based in immunolabeling to calbindin, identifying calbindin-rich regions ("matrix") and calbindin-poor islands ("nigrosomes"), and these pocket zone patterns were recognized in all brain as five nigrosomes. The nigrosome 1, localized in the ventral third and the lateral two-thirds of the calbindin-rich neuropil, corresponds to the ventral tier (Damier et al. 1999a). In another study (Damier et al. 1999b), the same authors used this SN analysis in PD subjects, and the neuronal depletion was maximum (98 %) in nigrosome 1, corroborating the findings of Fearnley and Lees (1991). A spatiotemporal progression of cell loss on PD was suggested, beginning in nigrosome 1, after spreading to other nigrosomes and to the matrix.

Another method used for analysis of dopamine depletion in parkinsonian syndromes is the radionuclide imaging with positive emission tomography (PET) and single-photon emission computed tomography (SPECT), applied as biomarkers for early disease and study of disease progression, mostly the presynaptic dopamine function, of the nigrostriatal pathway. There are four major ways to examine the dopaminergic terminals function: (1) the ^{18}F-dopa in SPECT and ^{11}C-dopa in PET, measuring the DOPA-decarboxylase activity; (2) molecules as ^{11}C or ^{18}F (for PET), or ^{123}I or ^{99m}Tc (for SPECT, as ^{99m}Tc-TRODAT-1) quantify the availability of DATs; (3) 11C-dihydrotetrabenazine or its 18F-labeled analogue measures the VMAT2 density; and (4) the ability of dopaminergic terminals to release dopamine after pharmacological stimuli (e.g., amphetamine) can be evaluated by changes in dopamine D2 receptor availability with ^{11}C-raclopride PET or ^{123}I-iodobenzamide SPECT (Brooks 2007; Stoessl et al. 2011).

It is known that ^{18}F-dopa uptake has correlation with nigral dopaminergic cell counts and dopamine concentrations in striatum of humans and nonhuman primates (Pate et al. 1993; Snow et al. 1993). In unilateral Hoehn & Yahr stage 1 PD patients, the dorsal posterior putamen ^{18}F-dopa uptake is first reduced, even contralateral to the asymptomatic limb, corresponding to the evidences that ventrolateral nigral dopaminergic projections to the dorsal putamen are more affected in the onset of clinical phase of PD (Kish et al. 1988).

The subsequent PD progression does not reduce the ^{18}F-dopa uptake in the first 2 years (Whone et al. 2003), but after that the functional loss occurs more rapidly in PD than aged controls (Brooks 2003), and the initial posterior-anterior gradient of striatal involvement is substituted by similar rates of decline in the whole striatum. Furthermore, the PET/SPECT measures show inverse correlation with severity of rigidity and bradykinesia in PD but poorly with tremor, suggesting the parkinsonian tremor may be associated with extranigral mechanisms (Vingerhoets et al. 1997; Benamer et al. 2000). Another finding is the increase of ^{18}F-dopa uptake into the internal globus pallidus in initial PD as a possible compensatory action, but this phenomenon vanishes with disease progression, simultaneously with onset of fluctuating responses to levodopa (Whone et al. 2003).

The motor complications on PD are also associated with radioimaging changes. PD patients with fluctuating responses to levodopa present 20 % reduction in ^{18}F-dopa uptake than those with sustained therapeutic responses (de la Fuente-Fernandez et al. 2000), and the ^{11}C-raclopride binding shows a mean 10 % fall in

posterior putamen of advanced PD subjects compared with early-stage patients (Torstenson et al. 1997). Nevertheless, PET studies with dyskinetic individuals show similar levels of striatal dopamine D1 and D2 receptor availability (Turjanski et al. 1997). The dementia in PD, a frequent non-motor complication of advanced stages, shows a reduction in ^{18}F-dopa uptake in cingulate and medial prefrontal areas, suggesting the involvement of the mesocortical dopaminergic pathway on cognitive decline in PD (Ito et al. 2002).

In conclusion, the major importance of midbrain dopaminergic neuronal degeneration on symptomatic progression of PD is unquestionable. However, it must be emphasized that there are several other non-dopaminergic neurons involved in its pathogenesis, as shown by Braak hypothesis, and the AS aggregation and accumulation can be the common link between these vulnerable nerve cells.

4.4 Dopamine-Related Treatments of Parkinson's Disease

The treatment of PD is very complex due to the progressive nature of the disease and the array of motor and non-motor features combined with early and late side effects associated with therapeutic interventions. While no treatments have yet been shown conclusively to slow the progression of the disease, a growing number of symptomatic pharmacologic therapies are available, as well as several forms of surgery and numerous non-pharmacological approaches.

Among dopaminergic drugs, L-DOPA standard and controlled-release formulations are marketed as fixed associations with a DOPA-decarboxylase inhibitor carbidopa or benzeraside. There are several dopamine agonists, with different receptor binding, pharmacokinetic profiles, and routes of administration and two types of indirect dopamine transmission enhancers, monoamine oxidase-B (MAO-B) and COMT inhibitors. Among non-dopaminergic medications, there are several anticholinergics, although amantadine is the only drug widely available for anti-glutamatergic effects. Functional neurosurgery for PD attempts to restore functional balance in basal ganglia relays. There are currently three targets: the ventral intermediate nucleus of the thalamus, the internal segment of the globus pallidus, and the subthalamic nucleus. Either CNS lesions (thalamotomy, pallidotomy, or subthalamic nucleus lesions) or implants of chronic stimulating electrodes at these sites (deep brain stimulation) can be used. Reconstructive surgery involves the implantation of human fetal mesencephalic cells (fetal transplant) or other dopamine-producing cells into the striatum. Rehabilitation for PD (speech, occupational, and physical therapies) has been applied empirically based on its accepted use in other chronic disorders.

It is important to consider that while drug treatments available for PD increased in the last 30 years, the first dopaminergic drug L-DOPA remains the more effective. Actually, the more recent drugs refined the treatment but they do not alter the clinical aspect and the evolution of the disease, and everyone can only temporary control and ameliorate the motor symptoms. The increasing evidences that cognitive dysfunctions, depression, constipation, pain, and nocturnal incapacity can play

Table 1 US Food and Drug Administration (FDA) approved medications for Parkinson's disease

Medication	Adverse effects	Indications and comments
Anticholinergics	Dry mouth, dry eyes, constipation, hypotension, cognitive impairment, urinary retention	Useful for symptomatic control of Parkinson's disease (benefits are mild to moderate); associated with more adverse effects than other drugs
Benztropine trihexyphenidyl		
Carbidopa/ levodopa	Nausea, somnolence, dyskinesia, hypotension, hallucinations	Levodopa is the most effective medication and remains the primary treatment for symptomatic Parkinson's disease; no added benefit for motor complications with sustained-release versus immediate-release preparations
Immediate- and sustained-release carbidopa/levodopa		
COMT inhibitors	Diarrhea; exacerbates levodopa adverse effects; bright orange urine	Useful for managing motor fluctuations ("wearing off" effect) in patients taking levodopa; levodopa dose may need to be reduced
Entacapone		
Tolcapone	Diarrhea; exacerbates levodopa adverse effects if dyskinesia appears; rare liver failure (liver function monitoring needed)	
Dopamine agonists	Nausea, headache, dizziness	Useful for early and advanced disease
Bromocriptine Pergolide	Somnolence; hallucinations; nausea; edema; fibrosis of cardiac valves, lung, and retroperitoneum; retroperitoneal and pulmonary fibrosis	Useful for the initial treatment of parkinsonism and as adjunct therapy in patients taking levodopa
Pramipexole Ropinirole	Nausea, sleep attacks, edema, hallucinations, hypotension	Useful for early disease and in patients with Parkinson's disease and motor fluctuations
MAO-B inhibitors	Nausea, insomnia, drug interactions with other MAO inhibitors/tyramine	Useful for symptomatic control of Parkinson's disease (benefits are mild to moderate) and as adjuvant therapy for patients with Parkinson's disease and motor fluctuations
Selegiline		
Rasagaline	Weight loss, hypotension, dry mouth, drug interactions with other MAO inhibitors/tyramine	
NMDA receptor inhibitor	Nausea, hypotension, hallucinations, confusion, edema	Useful for treating akinesia, rigidity, tremor, dyskinesia
Amantadine		

Abbreviations: *FDA* US Food and Drug Administration, *COMT* catechol-O-methyltransferase, *MAO-B* monoamine oxidase-B, *NMDA N*-methyl-d-aspartate. Based on information from Goetz et al. (2005)

important role in the quality of life of patients and the benefits of any new treatment should be evaluated in their impact in the well-bearing, mental health, and others deficiencies of the patients. A summarized review of the pharmacological approaches currently available for PD is illustrated in Table 1.

All the dopaminergic therapies are based in the pathway of catecholamine synthesis in the body, as presented in Fig. 1. Since most parkinsonian motor

symptoms occur as a consequence of dopamine depletion in the basal ganglia, dopamine replacement strategies represent the main therapeutic approach used to counteract PD motor impairment.

4.4.1 3,4-Dihydroxyphenylalanine (L-DOPA)

Since its introduction in the routine treatment of PD at the beginning of the 1970s (Cotzias 1971), L-DOPA (L-3,4-dihydroxyphenylalanine), a dopamine metabolic precursor, still remains as the most effective treatment for the treatment of the classical motor parkinsonian signs. L-DOPA, the naturally occurring isomer of the amino acid 3,4-dihydroxyphenylalanine, was first isolated in 1913 from legumes (seedlings of *Vicia faba*) by Marcus Guggenheim (Hauser 2009). Already 2 years earlier, Casimir Funk had synthesized D,L-DOPA in the laboratory (Hauser 2009). Funk and Guggenheim considered the amino acid as a possible parent compound of adrenaline. The discovery by Peter Holtz (1959) of an enzyme, DOPA decarboxylase, in mammalian tissue (kidney) extracts that converted L-DOPA to the corresponding – biologically active – amine, that is, dopamine, represented a turning point in catecholamine research (Hauser 2009). After the discovery of dopamine occurrence in the body, many biochemical studies were performed in an attempt to provide experimental support for the proposed biosynthesis of the catecholamines, mainly dopamine, from the amino acid L-DOPA (Hornykiewicz 2002).

The introduction of high dosage of L-DOPA by George Cotzias revolutionized the treatment of PD (Singh et al. 2007). As a prodrug of dopamine, L-DOPA crosses the blood-brain barrier, and it is decarboxylated to dopamine in the nigrostriatal pathways by brain enzymes Nowadays, L-DOPA is always given with carbidopa or benserazide, peripheral DOPA-decarboxylase inhibitors, which prevents peripheral metabolism of L-DOPA and allows a higher percentage of a dose to cross the blood-brain barrier. Primarily, they block L-DOPA metabolism in the periphery, thereby reducing the rate of the first-pass metabolism, and slowing the plasma clearance of L-DOPA; however, the enzyme inhibitors do not cross the blood-brain barrier.

The effects of the dopamine replacement therapy are predictable (as are the side effects), and none of the more recently introduced synthetic dopamine agonists has surpassed the clinical benefit derived from L-DOPA. L-DOPA has a long-duration response in early disease that enables adequate symptomatic control with dosage schedules (Table 1). This is a consequence of the ability of the nigrostriatal system to convert L-DOPA to dopamine, store it in presynaptic vesicles, and release it in response to physiological stimuli. However, as the disease progresses, the conversion of L-DOPA to dopamine in dopaminergic neurons becomes limited.

4.4.2 L-DOPA and Dyskinesia

Although the precise mechanisms of dyskinesia have remained elusive, three main risk factors for this side effect have been conclusively identified in clinical studies, namely, a young age at disease onset, disease severity (reflecting the extent of putaminal dopamine denervation), and high doses of L-DOPA (Jenner 2008; Stocchi 2009; see a recent review of Huot et al. 2013). These risk factors are

reproduced in both nonhuman primate and rodent species by applying neurotoxic lesions to the nigrostriatal dopamine pathway, followed by daily administration of L-DOPA at a sufficient dosage.

Although initially effective, dopaminergic therapies are eventually complicated by motor fluctuations, including off-time (periods of return of PD symptoms when medication effect wears off) and dyskinesia (drug-induced involuntary movements including chorea and dystonia) in most patients (Jenner 2008; Stocchi 2009). The mechanisms by which dyskinesia develop are not completely understood, but pulsatile stimulation of dopamine receptors by short-acting agents as L-DOPA and the degree of striatal denervation have been implicated (Obeso et al. 2000; Stocchi 2009). Dyskinesia may occur at the time of maximal clinical benefit and peak concentration of L-DOPA (peak-dose dyskinesia) or may appear at the beginning and/or at the end of the L-DOPA effect (diphasic dyskinesia, off-dystonia, off-dyskinesia). The progressive reduction in the duration of the clinical effect is called "wearing off." This change in the response to L-DOPA is more related to the disease progression and loss of buffering capacity of the presynaptic terminals to keep constant the levels of dopamine in the striatum (Chase 1998). When wearing off begins, it required modification of dosage and/or dose frequency of L-DOPA or the introduction of additional therapies. Interestingly, at this stage of the disease, so long as the plasma L-DOPA concentration is kept constant, the clinical response will persist (Mouradian et al. 1990; Schapira et al. 2009) and "wearing off" does not occur if the drug is given by continuous infusion (Nutt et al. 1989; Schapira et al. 2009). Motor complications can be an important source of disability for some patients who cycle between "on" periods, which are complicated by dyskinesia and "off" periods in which they suffer severe motor disability (Schapira et al. 2009).

Nonhuman primates have been used to produce models of L-DOPA-induced dyskinesia since the late 1980s (Crossman 1987). Following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesion and subsequent treatment with L-DOPA, nonhuman primates (and macaques in particular) exhibit choreiform and dystonic movements that look very similar to those seen in dyskinetic PD patients. These models have been extensively used to define the basic pharmacological features of L-DOPA-induced dyskinesia (Jenner 2003; Cenci et al. 2009). Rats with 6-hydroxydopamine (6-OHDA) lesions have been the most widely used animal model of PD since the 1970s, but the idea that these animals can exhibit L-DOPA-induced dyskinesia has become accepted only recently (Henry et al. 1998; Cenci et al. 2009). For many years, drug-induced rotation remained the only available method to study motor effects of dopaminergic therapies in this animal model. When given to unilaterally 6-OHDA-lesioned rats, L-DOPA or dopamine receptor agonists induce the animals to turn in the direction contralateral to the lesion, a behavior traditionally regarded as a correlate of the antiparkinsonian activity of these drugs. Later on, it was realized that contralateral rotation shows sensitization during chronic dopaminergic treatment (Henry et al. 1998) – a feature reminiscent of the priming for dyskinesia described in nonhuman primates and supposed to occur in PD patients. About 10 years ago came the first reports that 6-OHDA-lesioned rats treated with L-DOPA exhibit movements with dystonic and

hyperkinetic features engaging the forelimb contralateral to the lesion, as well as axial and orofacial muscles (Lundblad et al. 2002; Winkler et al. 2002). These movements interfere with the rat's physiological motor activities, and they are reduced by treatments that have antidyskinetic efficacy in nonhuman primates and PD patients. Rating scales for rodent abnormal involuntary movements (AIMs) were developed to reflect the topographic distribution, frequency, duration, and amplitude of dyskinetic behaviors induced by L-DOPA (Dekundy et al. 2007; Cenci et al. 2009). Producing and validating an animal model is a fundamental task for investigators involved in translational PD research.

Nevertheless, it must be emphasized that L-DOPA remains the "gold standard" medication in the treatment of PD. The use of other dopaminergic drugs at the beginning of the treatment helps to delay the onset of the motor complications in patients with PD. However, at some time when the disability of the patient increases, L-DOPA needs to be introduced (Olanow et al. 2009b). L-DOPA use is appropriate when all other reasonable strategies fail to produce adequate control of symptoms or when patient's symptoms pose an imminent danger to them (e.g., loss of postural reflexes with high risk for falling).

Recently, there was a growing discussion about a possible neurotoxic effect of L-DOPA that could increase the disease progression, and many patients and doctors became afraid to use the drug and delayed its use at the most (Kurlan 2005). However, a critical evaluation of older and most recent studies fails to describe a deleterious effect of L-DOPA in the evolution of the degenerative process (Olanow et al. 2004). In contrast our work showed that chronic L-DOPA treatment to partial 6-OHDA-lesioned rats induces partial recovery of the dopaminergic system suggesting their trophic effect which was confirmed by the increase of the trophic factor pleiotrophin (Murer et al. 1998; Ferrario et al. 2004).

Therefore, in the initial stages of the disease, L-DOPA therapy is the most effective for improving motor symptoms in PD. However, long-term treatment is accompanied by fluctuations in motor performance, dyskinesia, and neuropsychiatric complications. Furthermore, as PD progresses, patients develop features that do not respond well to L-DOPA therapy, such as freezing episodes, autonomic dysfunction, falling, and dementia. The increasingly diverse possibilities in the therapy of PD, and the many side effects and complications of therapy, require the formulation of reliable standards for patient care that are based on current scientific knowledge.

4.4.3 Catechol-O-Methyltransferase Inhibitors (COMT)

L-DOPA is usually combined with a DOPA-decarboxylase inhibitor (carbidopa or benserazide) to improve absorption and reduce peripheral metabolism (Schapira et al. 2009). However, the majority of L-DOPA is still metabolized in the gut and liver by COMT to form 3-*O*-methyldopa (3-OMD). 3-OMD accumulates and may interfere with the brain penetration of L-DOPA. COMT inhibitors reduce the metabolism of L-DOPA, extending its plasma half-life by 30 % and prolonging the clinical effect of each L-DOPA dose. This effect was suspected to be of enough magnitude to produce more stable L-DOPA plasma levels if a COMT inhibitor was

used with each dose of L-DOPA. The hypothesis was that this combined therapy could delay the appearance of motor complications because of producing more continuous dopaminergic stimulation. However, clinical studies were unable to show this positive effect, and in opposition, the combined therapy led to earlier appearance of dyskinesia (Klivenyi and Vecsei 2010). This unexpected effect may be explained by the potentializing effect of the COMT inhibitors in the L-DOPA effect, since its dosage, or effect, is associated with earlier beginning of motor complications.

Entacapone and tolcapone are the two selective COMT inhibitors currently in use for the treatment of PD (see Table 1), while newer molecules are now in clinical evaluation. Both COMT inhibitors only have observable clinical effects when used in association to L-DOPA. The most significant clinical effect is the reduction in the daily off-time, by small prolongation in the time of L-DOPA effect (Olanow et al. 2004; Jankovic and Stacy 2007). The COMT inhibitors also potentialize the effect of L-DOPA, and they can be used to achieve a stronger symptomatic effect (Gallagher and Schrag 2008). Tolcapone is a central and peripheral COMT inhibitor and is apparently more efficacious than entacapone that only inhibits peripheral metabolism, but there are some concerns about its safety and in particular to its potential hepatotoxicity (Assal et al. 1998). Entacapone is much more widely used and appears to be less hepatotoxic (Gershanik et al. 2003; Schrag 2005). The main adverse effects are related to its potentializing dopaminergic effects and include dyskinesia, orthostatic hypotension, nausea, vomiting, and confusion. Other important side effects are the occurrence of diarrhea and urinary discoloration. The control of hepatic enzymes is always indicated during the use of tolcapone and eventually during the use of entacapone. The association of entacapone in a single tablet with L-DOPA and carbidopa is now available. Another effect of the COMT inhibitor is the reduction in the systemic levels of 3-OMD that was supposed to interfere with L-DOPA metabolism in the central nervous system. However, any clinical benefit could be proved to be due to this property (Muller and Kuhn 2006). Another systemic effect of the COMT inhibitors is the reduction in the blood levels of homocysteine in treated PD patients (Muller and Kuhn 2006; Muller and Muhlack 2009). This could be a hypothetical benefit in the long term, since homocysteine could mediate the progression of neuronal degeneration and increase the risk for development of dementia, vascular disease, and polyneuropathy in levodopa-treated PD patients (Muller and Muhlack 2009).

Recently, it was established that a continuous duodenal L-DOPA infusion may be an efficient strategy to obtain continuous dopaminergic stimulation and to decrease the complications of the therapy (Clarke et al. 2009). Other ways to obtain a stable plasma level of L-DOPA were under research and include the development of new controlled-release tablets and cutaneous patches of the drug.

4.4.4 Monoamine Oxidase Isoenzyme Type B (MAO-B) Inhibitor

Substantial experimental evidences indicate that the oxidative stress is an important pathogenic mechanism in PD (Yacoubian and Standaert 2009). There are many possible mechanisms associated to the generation of free radicals in PD. The own

dopamine metabolism produces free radicals in excess. Blocking the first step of dopamine metabolism by inactivating MAO-B reduces the production of free radicals. Selegiline and rasagiline are selective MAO-B inhibitors currently in clinical use (see Table 1). MAO-B inhibition may increase the concentration of dopamine in the synaptic cleft due to the inhibition in the dopamine degradations and also produce an antiparkinsonian effect (Wu et al. 2000). Unlike selegiline, rasagiline is not metabolized to amphetamine and has no sympathomimetic activity. Rasagiline has also shown neuroprotective effects in in vitro and in vivo models of PD (Jenner 2004).

Selective MAO inhibitors become nonselective at doses greater than those recommended. In this condition, selegiline and rasagiline may increase the risk of a hypertensive crisis if used in combination with tyramine-containing foods (e.g., fermented cheese, aged meats) or amine-containing medications (e.g., over-the-counter cough/cold medicines). Serotoninergic crisis also has been associated with the use of MAO-B inhibitors in association with antidepressive drugs, since they can increase brain serotonin levels. The motor symptomatic effect of the MAO-B inhibitors is only modest, and these drugs are most commonly used to treat patients at the earlier stage of the disease. However, the MAO-B inhibitors may also be used at the later stages to increase the daily on-time. Despite recent clinical studies with rasagiline suggested that the drug may also have a neuroprotective effect (Olanow et al. 2009a), this is still a matter of controversy (see Ahlskog and Uitti 2010).

4.4.5 Dopamine Receptor Agonists

Dopamine agonists directly stimulate dopamine receptors. Of the nine dopamine agonists marketed for the treatment of PD, five were ergot derivatives (bromocriptine, cabergoline, dihydroergocryptine, lisuride, and pergolide) and four are non-ergot derivatives (apomorphine, piribedil, pramipexole, and ropinirole) (see Table 1). Pergolide was retired of the market of most countries due to tendency to induce cardiac valvar fibrosis; however, the use of ergot-derived dopamine agonists (pergolide and cabergoline) in patients with PD was associated with increased risk of cardiac valvar regurgitation (Steiger et al. 2009). The acute side effects of dopamine agonists are similar to those observed with L-DOPA and include nausea, vomiting, and postural hypotension. It is generally accepted that the shared D2-like receptor agonistic activity produces the antiparkinsonian effect. This D2 effect also explains peripheral (gastrointestinal nausea and vomiting), cardiovascular (orthostatic hypotension), and neuropsychiatric (somnolence, psychosis, and hallucinations) side effects. Even at lower doses, patients experience orthostatic hypotension, constipation, dyskinesia, confusion, and excessive somnolence with these agents (Wong et al. 2003). These adverse effects tend to occur with the initiation of treatment and to abate as tolerance develops usually over the ensuing days to weeks. Other problems related to the use of dopamine agonists include weight gain (possibly related to overeating) and edema (especially in the lower extremities). More recently, attention has been focused on the association of dopamine agonist treatment with the development of pathologic gambling, hypersexuality, and compulsive eating and shopping. This has attracted

considerable scientific interest because of the known relationship between dopamine and reward (Obeso et al. 2000).

Almost all currently used dopamine agonists are able to protect dopamine neurons from death in many in vitro and in vivo experiments. This neuroprotective effect may be the result of different mechanisms: antioxidation, scavenging of free radicals, suppression of lipid peroxidation, and inhibition of apoptosis. Dopamine agonists have a significant clinical effect in the motor parkinsonian signs, but most of them were not comparable to that observed with L-DOPA. The use of dopamine agonists at the beginning of the treatment of PD to spare L-DOPA is indicated specially for younger patients. The monotherapy with this class of drugs is associated with lower incidence of dyskinesia and wearing off but with a lower antiparkinsonian effect than L-DOPA (Hitzeman and Rafii 2009). One explanation for their lower risk to induce motor complications may be due to the long half-life of these drugs in relation to the L-DOPA. One exception for this is apomorphine that has a pharmacokinetic curve very similar to the L-DOPA and is the only dopamine agonist which a similar symptomatic effect to it. The main limitation for the clinical use of this drug is the administration route that must be subcutaneous. In some countries apomorphine is infused subcutaneously and continuously by a pump mechanism in patients with PD. The clinical effect is significant and persistent (Clarke et al. 2009). This is now recognized as a potential continuous dopaminergic stimulation strategy and reduces functional impairment and motor complications.

New delivery ways for dopamine agonist molecule are in development. Subcutaneous patches of rotigotine seem to be efficient and may produce more continuous dopaminergic stimulation (Chen et al. 2009). Long-release tablets of non-ergot dopamine agonists are now in evaluation and may be useful to increase adherence of the patient to the therapeutics.

4.4.6 Is There Nitric Oxide Inhibition Utility in the Dopamine Replacement Therapy?

Notwithstanding recent advances in the management of PD, L-DOPA-induced dyskinesia continues to be a clinical and therapeutic challenge (Cenci and Lindgren 2007; Jenner 2008; Santini et al. 2008). However, many other neurotransmitters may play a central role in the neurobiology and management of L-DOPA-induced dyskinesia. Nitric oxide is a free radical that can also act as an atypical neurotransmitter and influence dopamine-mediated neurotransmission. Nitric oxide also regulates synaptic and neural plasticity being enrolled in the regulation of motor activity (Pierucci et al. 2011; Del Bel et al. 2005, 2011). There is evidence whereby communication between nitrergic and dopaminergic systems plays an essential role in the control of the nigrostriatal pathway. The dual localization of immunoreactivity for nitric oxide synthase (NOS) and tyrosine hydroxylase, enzymes responsible for the synthesis of nitric oxide and dopamine, respectively, proposes a close anatomical link between the two neurotransmitters (Mitkovski et al. 2012).

Recently, our group (Padovan-Neto et al. 2009, 2011; Novaretti et al. 2010; Del Bel et al. 2011) and other laboratories (Takuma et al. 2012; Yuste et al. 2011)

described that nitric oxide synthase inhibitors are able to decrease the motor changes related to prolonged administration of L-DOPA in rodent models of PD. Similar results were described, in latest meeting communication, for nonhuman primates by Iravani et al. (2008) and Yuste et al. (2011). Postmortem studies in human brain of patients with Parkinson's disease have greatly contributed to figure out the disease. In spite of this, few human brain studies have focused on the relationship between the development of dyskinesia and biochemical changes in the human brain. It limits the possible understanding of the nitric oxide system in the treatment of Parkinson's disease. But Parkinsonian patients presented in serum a marked rise of the production of the nitric oxide second messenger cyclic guanosine monophosphate (cGMP) levels (Chalimoniuk et al. 2004) induced by L-DOPA. Similar result was described in the cerebellum (Gumulka et al. 1976). Comparable result was described in animals (Itokawa et al. 2006). In mice, cGMP is regulated by L-DOPA (Chalimoniuk and Langfort 2007). In addition, Sanchez and cols. (2002) showed that nitric oxide stimulates L-DOPA release in the striatum in a time- and concentration-dependent manner.

A role for nitrergic neurotransmission in the pathophysiology of dyskinesia and as a potential drug target for new therapeutic has been proposed also by others (Iravani and Jenner 2011; Pierucci et al. 2011; Huot et al. 2013). NOS inhibitor treatment was able to prevent abnormal involuntary movements without significantly changing normal motor (Padovan-Neto et al. 2009). Different from the cataleptic effects of these drugs, there was no tolerance to the antidyskinetic effects of NOS inhibitors (Novaretti et al. 2010). The antidyskinetic effects of 7-nitroindazole, a preferential neuronal nitric oxide synthase (nNOS) inhibitor, seem to involve reduction in gene and protein expression in denervated striatal neurons (Padovan-Neto et al. 2011). L-DOPA increased nNOS mRNA levels in the contra- and ipsilateral side to the dopamine lesion of the frontal cortex but did not produce any further increases of nNOS protein in the striatum compared to the 6-OHDA-induced increase (Padovan-Neto et al. 2011).

Despite promising studies, efforts in synthesizing highly selective nNOS inhibitors have been difficult and have limited the development of such drugs (Hobbs et al. 1999; Vallance and Leiper 2002). To date, clinical studies of NOS inhibitors have been limited by the small number of potent and selective drugs that are safe for human administration (Vallance 2003; Petros et al. 1994: Rees 1995; Ashina et al. 1999). The recent discovery that multiple subunits of NOS exist, with apparent unique anatomic distribution and functional and pharmacological diversity (for review see Garthwaite 2008), holds considerable therapeutic potential. One novel therapeutic approach could be to manipulate the levels of endogenous NOS inhibitors. NOS inhibitors are also likely synthesized within the body. L-NMMA (NG-monomethylarginine) and asymmetric dimethylarginine are synthesized by methylation of arginine residues in proteins (Leiper and Vallance 1999). Once the proteins are broken down, the free methylarginines are released into the cell cytosol and act as competitive inhibitors of all NOS isoforms. They are metabolized to citrulline by the action of dimethylarginine dimethylaminohydrolase (Leiper and Vallance 1999; MacAllister et al. 1996). Inhibition of this enzyme leads to

the accumulation of methylarginines and thereby blocks nitric oxide production. Antagonists might be developed to target-specific pathways within the basal ganglia.

A novel class of drugs named membrane-associated guanylate kinase inhibitors has recently been proposed in treatment of excitotoxicity (Lau and Tymianski 2010; Doucet et al. 2012). These peptides are competitive antagonists designed to bind postsynaptic scaffolding proteins (as, e.g., PSD-95, postsynaptic density 95 kDa), aimed at dissociating the spatial relationship between nNOS and NMDA (*N*-methyl-d-aspartate) receptors (NMDA/PSD-95/nNOS receptor complex) (Zhou et al. 2010; Doucet et al. 2012). The association of nNOS with NMDARs, via PSD-95 (Christopherson et al. 1999), plays an important role in a range of normal neuronal functions including synaptic plasticity (Garthwaite et al. 1988; Steinert et al. 2010) as well as pathophysiological disorders of the brain (Aarts et al. 2002; Sun et al. 2008; Zhou et al. 2010; Doucet et al. 2012). Since (a) NMDARs couple to nNOS and (b) NMDAR antagonists (amantadine) and nNOS inhibitors possess antidyskinetic properties, the disruption of the NMDAR/PSD-95/nNOS complex may possibly represent an alternative approach to specifically block nitrergic signaling coupled to NMDA receptor activity. It may prevent the problems associated general reduction of NMDA or nNOS function. To date, PDZ-based interactions represent a drug target that still remains largely unused (Dev 2004). They are however currently undergoing phase II clinical trials (Lau and Tymianski 2010).

The reduction in L-DOPA-induced dyskinesia in rodents and primates treated with NOS inhibitors suggests the involvement of nitric oxide in this altered behavior. These preclinical findings suggest that nitric oxide is a promising therapeutic target for the reduction of L-DOPA-induced dyskinesia.

5 Conclusion

The discoveries of dopamine as a neurotransmitter in the brain, its depletion in patients with PD, and its replacement with L-DOPA therapy were major revolutionary events in the rise to effective therapy for patients with PD. However, although drugs that act on the dopaminergic system provide effective symptomatic control for the classic parkinsonian motor features, the patients' quality of life continues to deteriorate as a consequence of gait and balance difficulties, autonomic dysfunction, and cognitive impairment. L-DOPA remains at present the most powerful symptomatic drug for the treatment of this condition. However, motor complications of chronic L-DOPA treatment have emerged as a major limitation of this therapy. Nonetheless, the importance of dopamine neurotransmitter system in a broad array of human disorders ranging from PD to schizophrenia has driven an intensive array of investigations oriented toward increasing our understanding of this complex system in normal conditions as well as disease states. Knowledge of dopaminergic cells and circuits has been enriched by findings obtained with several and highly sensitive techniques, as immunohistochemistry, cellular biology, transgenic mice or conditional mutants, functional anatomy,

electrophysiological studies, sophisticated behavioral analyses, and functional neuroimaging. Altogether, these studies have also led to new concepts of healthy and pathological functions of dopaminergic circuits at the molecular and system levels.

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References

Aarts, M., Liu, Y., Liu, L., Besshoh, S., Arundine, M., et al. (2002). Treatment of ischemic brain damage by perturbing NMDA receptor- PSD-95 protein interactions. *Science, 298*, 846–850.

Ahlskog, J. E., & Uitti, R. J. (2010). Rasagiline, Parkinson neuroprotection, and delayed-start trials: Still no satisfaction? *Neurology, 74*, 1143–1148.

Akyol, A., Akyildiz, U. O., & Tataroglu, C. (2006). Vascular Parkinsonism: a case of lacunar infarction localized to mesencephalic substantia nigra. *Parkinsonism & Related Disorders, 12*, 459–461.

Albin, R. L., Young, A. B., & Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences, 12*, 366–375.

Andén, N. E., Carlsson, A., Dahlström, A., Fuxe, K., Hillarp, N., et al. (1964). Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life Sciences, 3*, 523–530.

Archibald, N. K., Clarke, M. P., Mosimann, U. P., & Burn, D. J. (2009). The retina in Parkinson's disease. *Brain, 132*, 1128–1145.

Ashina, M., Lassen, L. H., Bendtsen, L., Jensen, R., & Olesen, J. (1999). Effect of inhibition of nitric oxide synthase on chronic tension-type headache: A randomised crossover trial. *Lancet, 353*, 287–289.

Assal, F., Spahr, L., Hadengue, A., Rubbia-Brandt, L., & Burkhard, P. R. (1998). Tolcapone and fulminant hepatitis. *Lancet, 352*, 958.

Benamer, H. T., Patterson, J., Wyper, D. J., Hadley, D. M., Macphee, G. J., et al. (2000). Correlation of Parkinson's disease severity and duration with 123I-FP-CIT SPECT striatal uptake. *Movement Disorders, 15*, 692–698.

Bentivoglio, M., & Morelli, M. (2005). The organisation and circuits of mesencephalic dopaminergic neurons and the distribution of dopamine receptors in the brain. In S. B. Dunnett, M. Bentivoglio, A. Björklund, & T. Hokfelt (Eds.), *Handbook of chemical neuroanatomy* (Dopamine, Vol. 21, pp. 1–107). Amsterdam, Boston: Elsevier.

Bergson, C., Mrzljak, L., Smiley, J. F., Pappy, M., Levenson, R., et al. (1995). Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *Journal of Neuroscience, 15*, 7821–7836.

Bevan, M. D., Booth, P. A., Eaton, S. A., & Bolam, J. P. (1998). Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. *Journal of Neuroscience, 18*, 9438–9452.

Björklund, A., & Dunnett, S. B. (2007). Dopamine neuron systems in the brain: an update. *Trends in Neurosciences, 30*, 194–202.

Björklund, A., & Lindvall, O. (1984). Dopamine-containing systems in the CNS. In A. Björklund & T. Hökfelt (Eds.), *Handbook of chemical neuroanatomy* (Classical transmitters in the CNS, Vol. 2, pp. 55–122). Amsterdam: Elsevier.

Bonifácio, M. J., Palma, P. N., Almeida, L., & Soares-da-Silva, P. (2007). Catechol-*O*-methyltransferase and its inhibitors in Parkinson's disease. *CNS Drug Reviews, 13*, 352–379.

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., et al. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging, 24*, 197–211.

Brooks, D. J. (2003). Imaging end points for monitoring neuroprotection in Parkinson's disease. *Annals of Neurology, 53*, 110–118.

Brooks, D. J. (2007). Functional neuroimaging in movement disorders. In J. Jankovic & E. Tolosa (Eds.), *Parkinson's disease & movement disorders* (5th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.

Carlsson, A., Falck, B., & Hillarp, N. A. (1962). Cellular localization of brain monoamines. *Acta Physiologica Scandinavica, 56*, 1–28.

Cenci, M. A., & Lindgren, H. S. (2007). Advances in understanding L-DOPA-induced dyskinesia. *Current Opinion in Neurobiology, 7*, 665–671.

Cenci, M. A., Ohlin, K. E., & Rylander, D. (2009). Plastic effects of L-DOPA treatment in the basal ganglia and their relevance to the development of dyskinesia. *Parkinsonism & Related Disorders, 15*, 59–63.

Centonze, D., Grande, C., Usiello, A., Gubellini, P., Erbs, E., et al. (2003). Receptor subtypes involved in the presynaptic and postsynaptic actions of dopamine on striatal interneurons. *Journal of Neuroscience, 23*, 6245–6254.

Chalimoniuk, M., & Langfort, J. (2007). The effect of subchronic, intermittent L-DOPA treatment on neuronal nitric oxide synthase and soluble guanylyl cyclase expression and activity in the striatum and midbrain of normal and MPTP-treated mice. *Neurochemistry International, 50*, 821–833.

Chalimoniuk, M., Stepień, A., & Strosznajder, J. B. (2004). Pergolide mesylate, a dopaminergic receptor agonist, applied with L-DOPA enhances serum antioxidant enzyme activity in Parkinson disease. *Clinical Neuropharmacology, 27*, 223–229.

Chase, T. N. (1998). The significance of continuous dopaminergic stimulation in the treatment of Parkinson's disease. *Drugs, 55*, 1–9.

Chaudhuri, R., & Schapira, A. (2009). Non-motor symptoms of Parkinson's disease: Dopaminergic pathophysiology and treatment. *Lancet Neurology, 8*, 464–474.

Chaudhuri, K. R., Healy, D. G., & Schapira, A. H. (2006). Non-motor symptoms of Parkinson's disease: Diagnosis and management. *Lancet Neurology, 5*, 235–245.

Chen, J. J., Swope, D. M., Dashtipour, K., & Lyons, K. E. (2009). Transdermal rotigotine: A clinically innovative dopamine-receptor agonist for the management of Parkinson's disease. *Pharmacotherapy, 29*, 1452–1467.

Chevalier, G., Vacher, S., Deniau, J. M., & Desban, M. (1985). Disinhibition as a basic process in the expression of striatal functions. I. The striato-nigral influence on tecto-spinal/tecto-diencephalic neurons. *Brain Research, 334*, 215–226.

Christopherson, K. S., Hillier, B. J., Lim, W. A., & Bredt, D. S. (1999). PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. *Journal of Biological Chemistry, 274*, 27467–27473.

Cisek, P., & Kalaska, J. F. (2010). Neural mechanisms for interacting with a world full of action choices. *Annual Review of Neuroscience, 33*, 269–298.

Clarke, C. E., Worth, P., Grosset, D., & Stewart, D. (2009). Systematic review of apomorphine infusion, levodopa infusion and deep brain stimulation in advanced Parkinson's disease. *Parkinsonism & Related Disorders, 15*, 728–741.

Cotzias, G. C. (1971). Levodopa in the treatment of Parkinsonism. *JAMA: The Journal of the American Medical Association, 218*, 1903–1908.

Cragg, S. J., & Rice, M. E. (2004). DAncing past the DAT at a DA synapse. *Trends in Neurosciences, 27*, 270–277.

Crossman, A. R. (1987). Primate models of dyskinesia: The experimental approach to the study of basal ganglia-related involuntary movement disorders. *Neuroscience, 21*, 1–40.

Da Cunha, C., Wietzikoski, E. C., Bortolanza, M., Dombrowski, P., Santos, L. M., et al. (2009). Non-motor function of the midbrain dopaminergic neurons. In G. Di Giovanni, V. Di Matteo, & E. Esposito (Eds.), *Birth, life and death of dopaminergic neurons in the substantia nigra*. New York: Springer-Verlag/Wien.

Dahlström, A., & Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiologica Scandinavica, 232*, 1–55.

Damier, P., Hirsch, E. C., Agid, Y., & Graybiel, A. M. (1999a). The substantia nigra of the human brain. I. Nigrosomes and the nigral matrix, a compartmental organization based on calbindin D(28K) immunohistochemistry. *Brain, 122*, 1421–1436.

Damier, P., Hirsch, E. C., Agid, Y., & Graybiel, A. M. (1999b). The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain, 122*, 1437–1448.

De la Fuente-Fernandez, R., Pal, P. K., Vingerhoets, F. J., Kishore, A., Schulzer, M., et al. (2000). Evidence for impaired presynaptic dopamine function in parkinsonian patients with motor fluctuations. *Journal of Neural Transmission, 107*, 49–57.

Dekundy, A., Lundblad, M., Danysz, W., & Cenci, M. A. (2007). Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: Further validation of the rat dyskinesia model. *Behavioural Brain Research, 179*, 76–89.

Del Bel, E. A., Guimaraes, F. S., Bermudez-Echeverry, M., Gomes, M. Z., Schiaveto-de-Souza, A., et al. (2005). Role of nitric oxide on motor behavior. *Cellular and Molecular Neurobiology, 25*, 371–392.

Del Bel, E., Padovan-Neto, F. E., Raisman-Vozari, R., & Lazzarini, M. (2011). Role of nitric oxide in motor control: Implications for Parkinson's disease pathophysiology and treatment. *Current Pharmaceutical Design, 17*, 471–488.

Del Tredici, K., Rub, U., De Vos, R. A., Bohl, J. R., & Braak, H. (2002). Where does parkinson disease pathology begin in the brain? *Journal of Neuropathology and Experimental Neurology, 61*, 413–426.

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends in Neurosciences, 13*, 281–285.

Dev, K. K. (2004). Making protein interactions druggable: Targeting PDZ domains. *Nature Reviews. Drug Discovery, 3*, 1047–1056.

Doucet, M. V., Harkin, A., & Dev, K. K. (2012). The PSD-95/nNOS complex: New drugs for depression? *Pharmacology and Therapeutics, 133*, 218–229.

Dubois, B., & Pillon, B. (1997). Cognitive deficits in Parkinson's disease. *Journal of Neurology, 244*, 2–8.

Eriksen, J., Jørgensen, T. N., & Gether, U. (2010). Regulation of dopamine transporter function by protein-protein interactions: new discoveries and methodological challenges. *Journal of Neurochemistry, 113*, 27–41.

Fahn, S. (2008). The history of dopamine and levodopa in the treatment of Parkinson's disease. *Movement Disorders, 23*, 497–508.

Fearnley, J. M., & Lees, A. J. (1991). Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain, 114*, 2283–2301.

Ferrario, J. E., Taravini, I. R. E., Mourlevat, S., Stefano, A. V., Delfino, M. A., et al. (2004). Differential gene expresión induced by chronic levodopa treatment in the striatum of rats with lesions of the nigrostriatal system. *Journal of Neurochemistry, 90*, 1348–1358.

Gallagher, D. A., & Schrag, A. (2008). Impact of newer pharmacological treatments on quality of life in patients with Parkinson's disease. *CNS Drugs, 22*, 563–586.

Garcia de Y'ebenes, J., Gervas, J. J., Iglesias, J., Mena, M. A., Martin del Rio, R., et al. (1982). Biochemical findings in a case of parkinsonism secondary to brain tumor. *Annals of Neurology, 11*, 313–316.

Garthwaite, J. (2008). Concepts of neural nitric oxide-mediated transmission. *European Journal of Neuroscience, 27*, 2783–2802.

Garthwaite, J., Charles, S. L., & Chess-Williams, R. (1988). Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature, 336*, 385–388.

Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma, F.J. Jr, Sibley, D.R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science, 250*(4986), 1429–1432.

Gerfen, C. R., & Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annual Review of Neuroscience, 34*, 441–446.

Gershanik, O., Emre, M., Bernhard, G., & Sauer, D. (2003). Efficacy and safety of levodopa with entacapone in Parkinson's disease patients suboptimally controlled with levodopa alone, in daily clinical practice: An international, multicentre, open-label study. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27*, 963–971.

Goedert, M. (1997). Familial Parkinson's disease. The awakening of alpha-synuclein. *Nature, 388*, 232–233.

Goetz, C. G., Poewe, W., Rascol, O., & Sampaio, C. (2005). Evidence-based medical review update: Pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. *Movement Disorders, 20*, 523–539.

Grace, A. A. (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. *Neuroscience, 41*, 1–24.

Grace, A. A. (2002). Dopamine. In K. L. Davis, D. Charney, J. T. Coyle, & C. Nemeroff *Neuropsychopharmacology: The fifth generation of progress editors*. Philadelphia, PA: Lippincott, Williams, & Wilkins.

Graybiel, A. M., Aosaki, T., Flaherty, A. W., & Kimura, M. (1994). The basal ganglia and adaptive motor control. *Science, 265*, 1826–1831.

Greengard, P., Allen, P. B., & Nairn, A. C. (1999). Beyond the dopamine receptor: The DARPP-32/protein phosphatase-1 cascade. *Neuron, 23*, 435–447.

Grinberg, L. T., Rueb, U., Alho, A. T., & Heinsen, H. (2010). Brainstem pathology and non-motor symptoms in PD. *Journal of Neurological Sciences, 289*, 81–88.

Gumulka, S. W., Dinnendahl, V., Schönhöfer, P. S., & Stock, K. (1976). Dopaminergic stimulants and cyclic nucleotides in mouse brain. Effects of dopaminergic antagonists, olinolytics, and GABA agonists. *Naunyn-Schmiedeberg's Archives of Pharmacology, 295*, 21–26.

Haber, S. N., & Gdowski, M. J. (2004). The basal ganglia. In G. Paxinos & J. K. Mai (Eds.), *The human nervous system* (2nd ed.). San Diego, CA: Elsevier Academic Press.

Harden, D. G., & Grace, A. A. (1995). Activation of dopamine cell firing by repeated L-DOPA administration to dopamine-depleted rats: Its potential role in mediating the therapeutic response to LDOPA treatment. *Journal of Neuroscience, 15*, 6157–6166.

Hassler, R. (1938). Zur die Pathologie der Paralysis Agitans and des postenzephalitischen Parkinsonismus. *Journal für Psychologie und Neurologie, 48*, 387–476.

Hauser, R. A. (2009). Levodopa: Past, present, and future. *European Neurology, 62*, 1–8.

Henry, B., Crossman, A. R., & Brotchie, J. M. (1998). Characterization of enhanced behavioral responses to L-DOPA following repeated administration in the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Experimental Neurology, 151*, 334–342.

Herve, D., & Girault, J. A. (2005). Signal transduction of dopamine receptors. In S.B. Dunnett, M. Bentivoglio, A. Björklund & Hökfelt T (Eds.), *Handbook of chemical neuroanatomy*. Dopamine, Vol. 21, pp. 109–151.

Hikida, T., Kimura, K., Wada, N., Funabiki, K., & Nakanishi, S. (2010). Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron, 66*, 896–907.

Hitzeman, N., & Rafii, F. (2009). Dopamine agonists for early Parkinson disease. *American Family Physician, 80*, 28–30.

Hobbs, A. J., Higgs, A., & Moncada, S. (1999). Inhibition of nitric oxide synthase as a potential therapeutic target. *Annual Review of Pharmacology and Toxicology, 39*, 191–220.

Hokfelt, T., Fuxe, K., & Goldstein, M. (1973). Immunohistochemical studies on monoamine-containing cell systems. *Brain Research, 62*, 461–469.

Holtz, P. (1959). Role of L-DOPA decarboxylase in the biosynthesis of catecholamines in nervous tissue and the adrenal medulla. *Pharmacological Reviews, 11*, 317–329.

Hornykiewicz, O. (1963). Die topische Lokalisation und das Verhalten von Noradrenalin und Dopamin (3-Hydroxytyramin) in der Substantia nigra des normalen und Parkinsonkranken Menschen. *Wien Klin Wschr, 75*, 309–312.

Hornykiewicz, O. (2002). Dopamine miracle: From brain homogenate to dopamine replacement. *Movement Disorders, 17*, 501–508.

Hornykiewicz, O. (2010). A brief history of levodopa. *Journal of Neurology, 257*, 249–252.

Huot, P., Johnston, T. H., Koprich, J. B., Fox, S. H., & Brotchie, J. M. (2013). The pharmacology of L-DOPA-induced dyskinesia in Parkinson's disease. *Pharmacological Reviews, 65*, 171–222.

Iravani, M. M., & Jenner, P. (2011). Mechanisms underlying the onset and expression of levodopa-induced dyskinesia and their pharmacological manipulation. *Journal of Neural Transmission, 118*, 1661–1690.

Iravani, M. M., Stockwell, K. A., Tayarani-Binazir, K., Jackson, M. J., Smith, L. A., et al. (2008). Inhibition of neuronal nitric oxide synthase as a novel target for suppression of levodopa-induced dyskinesia in primates. Neuroscience Meeting Planner Society for Neuroscience. Abstract 139.15/M6.

Ishizawa, T., Mattila, P., Davies, P., Wang, D., & Dickson, D. W. (2003). Colocalization of tau and alpha-synuclein epitopes in Lewy bodies. *Journal of Neuropathology and Experimental Neurology, 62*, 389–397.

Ito, K., Nagano-Saito, A., Kato, T., et al. (2002). Striatal and extrastriatal dysfunction in Parkinson's disease with dementia: A 6-[^{18}F]fluoro-L-dopa PET study. *Brain, 125*, 1358–1365.

Itokawa, K., Ohkuma, A., Araki, N., Tamura, N., & Shimazu, K. (2006). Effect of L-DOPA on nitric oxide production in striatum of freely mobile mice. *Neuroscience Letters, 402*, 142–144.

Iversen, S. D., & Iversen, L. L. (2007). Dopamine: 50 years in perspective. *Trends in Neurosciences, 30*, 188–193.

Jakes, R., Spillantini, M. G., & Goedert, M. (1994). Identification of two distinct synucleins from human brain. *FEBS Letters, 345*, 27–32.

Jankovic, J., & Stacy, M. (2007). Medical management of levodopa-associated motor complications in patients with Parkinson's disease. *CNS Drugs, 21*, 677–692.

Jellinger, K. A. (2003). Alpha-synuclein pathology in Parkinson's and Alzheimer's disease brain: Incidence and topographic distribution–a pilot study. *Acta Neuropathologica, 106*, 191–201.

Jenner, P. (2003). The MPTP-treated primate as a model of motor complications in PD: Primate model of motor complications. *Neurology, 61*, 4–11.

Jenner, P. (2004). Preclinical evidence for neuroprotection with monoamine oxidase-B inhibitors in Parkinson's disease. *Neurology, 63*, 13–22.

Jenner, P. (2008). Molecular mechanisms of L-DOPA-induced dyskinesia. *Nature Reviews Neuroscience, 9*, 665–677.

Kemp, J. M., & Powell, T. P. (1971). The connexions of the striatum and globus pallidus: Synthesis and speculation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 262*, 441–457.

Kish, S. J., Shannak, K., & Hornykiewicz, O. (1988). Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. *The New England Journal of Medicine, 318*, 876–880.

Klivenyi, P., & Vecsei, L. (2010). Novel therapeutic strategies in Parkinson's disease. *European Journal of Clinical Pharmacology, 66*, 119–125.

Kravitz, A. V., Freeze, B. S., Parker, P. R., Kay, K., Thwin, M. T., et al. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature, 466*, 622–626.

Krimer, L. S., Jakab, R. L., & Goldman-Rakic, P. S. (1997). Quantitative three dimensional analysis of the catecholaminergic innervation of identified neurons in the macaque prefrontal cortex. *Journal of Neuroscience, 17*, 7450–7461.

Kurlan, R. (2005). "Levodopa phobia": A new iatrogenic cause of disability in Parkinson disease. *Neurology, 64*, 923–924.

Langley, K. C., Bergson, C., Greengard, P., & Ouimet, C. C. (1997). Co-localization of the D1 dopamine receptor in a subset of DARPP-32-containing neurons in rat caudate-putamen. *Neuroscience, 78*, 977–983.

Lau, A., & Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflügers Archiv, 460*, 525–542.

Lees, A. J., Selikhova, M., Andrade, L. A., & Duyckaerts, C. (2008). The black stuff and Konstantin Nikolaevich Tretiakoff. *Movement Disorders, 23*, 777–783.

Leiper, J., & Vallance, P. (1999). Biological significance of endogenous methylarginines that inhibit nitric oxide synthases. *Cardiovascular Research, 43*, 542–548.

Ljungdahl, A., Hokfelt, T., Goldstein, M., & Park, D. (1975). Retrograde peroxidase tracing of neurons combined with transmitter histochemistry. *Brain Research, 84*, 313–319.

Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N., et al. (2002). Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *European Journal of Neuroscience, 15*, 120–132.

Ma, S. Y., Rinne, J. O., Collan, Y., Roytta, M., & Rinne, U. K. (1996). A quantitative morphometrical study of neuron degeneration in the substantia nigra in Parkinson's disease. *Journal of Neurological Sciences, 140*, 40–45.

MacAllister, R. J., Parry, H., Kimoto, M., Ogawa, T., Russell, R. J., Hodson, H., Whitley, G. S., & Vallance, P. (1996). Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase. *British Journal of Pharmacology, 119*, 1533–1540.

Matsuda, W., Furuta, T., Nakamura, K., Hioki, H., Fujiyama, F., et al. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *Journal of Neuroscience, 29*, 444–453.

Mayeux, R. (2003). Epidemiology of neurodegeneration. *Annual Review of Neuroscience, 26*, 81–104.

Mink, J. W. (2003). The basal ganglia and involuntary movements: Impaired inhibition of competing motor patterns. *Archives of Neurology, 60*, 1365–1368.

Mitkovski, M., Padovan-Neto, F. E., Raisman-Vozari, R., Ginestet, L., da-Silva, C. A., et al. (2012). Investigations into potential extrasynaptic communication between the dopaminergic and nitrergic systems. *Frontiers in Physiology, 3*, 372.

Molinoff, P. B., & Axelrod, J. (1971). Biochemistry of catecholamines. *Annual Review of Biochemistry, 40*, 465–500.

Mouradian, M. M., Heuser, I. J., Baronti, F., & Chase, T. N. (1990). Modification of central dopaminergic mechanisms by continuous levodopa therapy for advanced Parkinson's disease. *Annals of Neurology, 27*, 18–23.

Muller, T., & Kuhn, W. (2006). Tolcapone decreases plasma levels of S-adenosyl-L-homocysteine and homocysteine in treated Parkinson's disease patients. *European Journal of Clinical Pharmacology, 62*, 447–450.

Muller, T., & Muhlack, S. (2009). Peripheral COMT inhibition prevents levodopa associated homocysteine increase. *Journal of Neural Transmission, 116*, 1253–1256.

Murer, M. G., Dziewczapolski, G., Menalled, L., Garcia, M., Agid, Y., Gershanik, O. S., et al. (1998). Chronic levodopa is not toxic for remaining dopaminergic neurons, but instead promotes their recovery, in rats with moderate nigrostriatal lesions. *Annals of Neurology, 43*, 561–575.

Nestler, E. J., Hyman, S. E., & Malenka, R. C. (2009). *Molecular neuropharmacology: A foundation for clinical neuroscience*. New York: McGraw-Hill Medical.

Novaretti, N., Padovan-Neto, F. E., Tumas, V., da-Silva, C. A., & Del Bel, E. A. (2010). Lack of tolerance for the anti-dyskinetic effects of 7-nitroindazole, a neuronal nitric oxide synthase inhibitor, in rats. *Brazilian Journal of Medical and Biological Research, 43*(11), 1047–1053.

Nussbaum, R. L., & Ellis, C. E. (2003). Alzheimer's disease and Parkinson's disease. *The New England Journal of Medicine, 348*, 1356–1364.

Nutt, J. G. (1987). On-off phenomenon: Relation to levodopa pharmacokinetics and pharmacodynamics. *Annals of Neurology, 22*, 535–540.

Nutt, J. G., Gancher, S. T., & Woodward, W. R. (1989). Motor fluctuations in Parkinson's disease. *Annals of Neurology, 25*, 633–634.

Obeso, J. A., Rodriguez-Oroz, M. C., Chana, P., Lera, G., Rodriguez, M., et al. (2000). The evolution and origin of motor complications in Parkinson's disease. *Neurology 55*, S13–20; discussion S1–3.

Olanow, C. W., & Tatton, W. G. (1999). Etiology and pathogenesis of Parkinson's disease. *Annual Review of Neuroscience, 22*, 123–144.

Olanow, C. W., Agid, Y., Mizuno, Y., Albanese, A., Bonuccelli, U., et al. (2004). Levodopa in the treatment of Parkinson's disease: Current controversies. *Movement Disorders, 19*, 997–1005.

Olanow, C. W., Rascol, O., Hauser, R., Feigin, P. D., Jankovic, J., ADAGIO Study Investigators, et al. (2009a). A double-blind, delayed-start trial of rasagiline in Parkinson's disease. *The New England Journal of Medicine, 361*, 1268–1278.

Olanow, C. W., Stern, M. B., & Sethi, K. (2009b). The scientific and clinical basis for the treatment of Parkinson disease. *Neurology, 72*, S1–S136.

Orta Daniel, S. J., & Ulises, R. O. (2008). Stroke of the substance nigra and parkinsonism as first manifestation of systemic lupus erythematosus. *Parkinsonism & Related Disorders, 14*, 367–369.

Padovan-Neto, F. E., Echeverry, M. B., Tumas, V., & Del-Bel, E. A. (2009). Nitric oxide synthase inhibition attenuates L-DOPA-induced dyskinesias in a rodent model of Parkinson's disease. *Neuroscience, 159*, 927–935.

Padovan-Neto, F. E., Echeverry, M. B. D., Chiavegatto, S., & Del Bel, E. (2011). Nitric oxide synthase inhibitor improves de novo and long-term L-DOPA-induced dyskinesia in hemiparkinsonian rats. *Frontiers in Systems Neuroscience, 5*, 40.

Parent, M., & Parent, A. (2010). Substantia nigra and Parkinson's disease: A brief history of their long and intimate relationship. *Canadian Journal of Neurological Sciences, 37*, 313–319.

Parkinson, J. (2002). An essay on the shaking palsy. 1817. *The Journal of Neuropsychiatry and Clinical Neurosciences, 14*, 223–236.

Parkkinen, L., Soininen, H., & Alafuzoff, I. (2003). Regional distribution of alpha-synuclein pathology in unimpaired aging and Alzheimer disease. *Journal of Neuropathology and Experimental Neurology, 62*, 363–367.

Pate, B. D., Kawamata, T., Yamada, T., McGeer, E. G., Hewitt, K. A., et al. (1993). Correlation of striatal fluorodopa uptake in the MPTP monkey with dopaminergic indices. *Annals of Neurology, 34*, 331–338.

Petros, A., Lamb, G., Leone, A., Moncada, S., Bennett, D., et al. (1994). Effects of a nitric oxide synthase inhibitor in humans with septic shock. *Cardiovascular Research, 28*(1), 34–39.

Pierucci, M., Galati, S., Valentino, M., Di Matteo, V., Benigno, A., et al. (2011). Nitric oxide modulation of the basal ganglia circuitry: Therapeutic implication for Parkinson's disease and other motor disorders. *CNS & Neurological Disorders Drug Targets, 10*(7), 777–791.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., et al. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science, 276*, 2045–2047.

Rees, D. D. (1995). Role of nitric oxide in the vascular dysfunction of septic shock. *Biochemical Society Transactions, 23*(4), 1025–1029.

Rothwell, J. C. (2011). The motor functions of the basal ganglia. *Journal of Integrative Neuroscience, 10*, 303–315.

Sanchez, J. J., Abreu, P., & Gonzalez, M. C. (2002). Sodium nitroprusside stimulates L-DOPA release from striatal tissue through nitric oxide and cGMP. *European Journal of Pharmacology, 438*(1–2), 79–83.

Santini, E., Valjent, E., & Fisone, G. (2008). Parkinson's disease: Levodopa-induced dyskinesia and signal transduction. *FEBS Journal, 275*(7), 1392–1399.

Schapira, A. H., Emre, M., Jenner, P., & Poewe, W. (2009). Levodopa in the treatment of Parkinson's disease. *European Journal of Neurology, 16*, 982–989.

Schrag, A. (2005). Entacapone in the treatment of Parkinson's disease. *Lancet Neurology, 4*, 366–370.

Singh, N., Pillay, V., & Choonara, Y. E. (2007). Advances in the treatment of Parkinson's disease. *Progress in Neurobiology, 81*, 29–44.

Smith, Y., Bennett, B. D., Bolam, J. P., Parent, A., & Sadikot, A. F. (1994). Synaptic relationships between dopaminergic afferents and cortical or thalamic input in the sensorimotor territory of the striatum in monkey. *The Journal of Comparative Neurology, 344*, 1–19.

Snow, B. J., Tooyama, I., McGeer, E. G., et al. (1993). Human positron emission tomographic [^{18}F]fluorodopa studies correlate with dopamine cell counts and levels. *Annals of Neurology, 34*, 324–330.

Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., et al. (1997). Alpha-synuclein in Lewy bodies. *Nature, 388*, 839–840.

Steiger, M., Jost, W., Grandas, F., & Van Camp, G. (2009). Risk of valvular heart disease associated with the use of dopamine agonists in Parkinson's disease: A systematic review. *Journal of Neural Transmission, 116*, 179–191.

Steinert, J. R., Chernova, T., & Forsythe, I. D. (2010). Nitric oxide signaling in brain function, dysfunction, and dementia. *The Neuroscientist, 16*(4), 435–452.

Stocchi, F. (2009). The therapeutic concept of continuous dopaminergic stimulation (CDS) in the treatment of Parkinson's disease. *Parkinsonism & Related Disorders, 15*(Suppl 3), S68–S71.

Stoessl, A. J., Martin, W. W., McKeown, M. J., & Sossi, V. (2011). Advances in imaging in Parkinson's disease. *Lancet Neurology, 10*, 987–1001.

Sun, H. S., Doucette, T. A., Liu, Y., Fang, Y., Teves, L., et al. (2008). Effectiveness of PSD95 inhibitors in permanent and transient focal ischemia in the rat. *Stroke, 39*, 2544–2553.

Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., Nairn, A. C., et al. (2004). DARPP-32: An integrator of neurotransmission. *Annual Review of Pharmacology and Toxicology, 44*, 269–296.

Takuma, K., Tanaka, T., Takahashi, T., Hiramatsu, N., Ota, Y., Ago, Y., Matsuda, T. (2012). Neuronal nitric oxide synthase inhibition attenuates the development of L-DOPA-induced dyskinesia in hemi-Parkinsonian rats. *European Journal of Pharmacology, 683*, 166–173.

Tepper, J. M., Wilson, C. J., & Koós, T. (2008). Feedforward and feedback inhibition in neostriatal GABAergic spiny neurons. *Brain Research Reviews, 58*, 272–281.

Torstenson, R., Hartvig, P., Langstrom, B., Westerberg, G., & Tedroff, J. (1997). Differential effects of levodopa on dopaminergic function in early and advanced Parkinson's disease. *Annals of Neurology, 41*, 334–340.

Turjanski, N., Lees, A. J., & Brooks, D. J. (1997). In vivo studies on striatal dopamine D1 and D2 site binding in L-dopa-treated Parkinson's disease patients with and without dyskinesias. *Neurology, 49*, 717–723.

Vallance, P. (2003). Nitric oxide: Therapeutic opportunities. *Fundamental and Clinical Pharmacology, 17*(1), 1–10.

Vallance, P., & Leiper, J. (2002). Blocking NO synthesis: How, where and why? *Nature Reviews. Drug Discovery, 1*, 939–950.

Vingerhoets, F. J., Schulzer, M., Calne, D. B., & Snow, B. J. (1997). Which clinical sign of Parkinson's disease best reflects the nigrostriatal lesion? *Annals of Neurology, 41*, 58–64.

Whone, A. L., Moore, R. Y., Piccini, P. P., & Brooks, D. J. (2003). Plasticity of the nigropallidal pathway in Parkinson's disease. *Annals of Neurology, 53*, 206–213.

Winkler, C., Kirik, D., Bjorklund, A., & Cenci, M. A. (2002). L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: Relation to motor and cellular parameters of nigrostriatal function. *Neurobiology of Disease, 10*, 165–186.

Wong, K. S., Lu, C. S., Shan, D. E., Yang, C. C., Tsoi, T. H., & Mok, V. (2003). Efficacy, safety, and tolerability of pramipexole in untreated and levodopa-treated patients with Parkinson's disease. *Journal of Neurological Sciences, 216*, 81–87.

Wu, R. M., Chen, R. C., & Chiueh, C. C. (2000). Effect of MAO-B inhibitors on MPP^+ toxicity in Vivo. *Annals of the New York Academy of Sciences, 899*, 255–261.

Yacoubian, T. A., & Standaert, D. G. (2009). Targets for neuroprotection in Parkinson's disease. *Biochimica et Biophysica Acta, 1792*, 676–687.

Yan, Z., & Surmeier, D. J. (1996). Muscarinic (m2/m4) receptors reduce N- and P-type Ca^{2+} currents in rat neostriatal cholinergic interneurons through a fast, membrane-delimited, G-protein pathway. *Journal of Neuroscience, 16*, 2592–2604.

Yuste, J. E., Bermúdez, M., Bernal, F. R., Barcia, C., Martin, J., et al. (2011). NOS inhibitors improve L-DOPA-induced dyskinesias in experimental models of Parkinsonism. *Movement Disorders, 26*(Suppl 2), S257–S258.

Zhou, L., Li, F., Xu, H. B., Luo, C. X., Wu, H. Y., et al. (2010). Treatment of cerebral ischemia by disrupting ischemia-induced interaction of nNOS with PSD-95. *Nature Medicine, 16*, 1439–1443.

Iron Neurotoxicity in Parkinson's Disease

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Abstract

Iron plays essential roles in the development of early cognitive functions and in the maintenance of neuronal functions in the mature brain, so neurons have in place expeditious mechanisms to ensure a readily available supply of iron. However, iron is an intrinsic producer of reactive oxygen species (ROS), and increased levels of iron promote neurotoxicity because of hydroxyl radical formation, glutathione consumption, protein aggregation, lipid peroxidation, and nucleic acid modification. In this chapter, the components of iron homeostasis and the mechanisms by which iron homeostasis is lost in Parkinson's disease (PD) are discussed. In particular, it will be discussed the relevance of endogenous toxins such as aminochrome as mediators of mitochondrial dysfunction and of hepcidin as a mediator of inflammatory stimuli. A model is proposed that describes a positive feedback loop between changes in iron, glutathione, and ROS levels that ends in cell death.

Keywords

DMT1 • FPN1 • Glutathione • Hepcidin • Iron • Iron accumulation • Iron chelation • IRP1 • Mitochondrial complex I • Mitochondrial dysfunction • MPTP • NTBI • Parkinson's disease • Reactive oxygen species

List of Abbreviations

AC	Aminochrome
CDC14A	Dual specificity protein phosphatase
CNS	Central nervous system
CP	Ceruloplasmin
CSF	Cerebrospinal fluid
DAT	Dopamine transporter
Dcytb	Duodenal cytochrome b ferrireductase
DMT1	SLC11A2, divalent metal transporter 1
EMSA	Electrophoretic mobility shift assay
FPN1	SCL40A, ferroportin 1
GPAT	Glutamyl phosphoribosyltransferase
GSH	Glutathione
GSSG	Oxidized glutathione
HIF	Hypoxia-inducible factor
IFN-γ	Interferon gamma
iNOS	Inducible nitric oxide synthase
IRE	Iron regulatory element
IRP	Iron regulatory protein
L-DOPA	L-3,4-dihydroxyphenylalanine
LIMK1	LIM domain kinase 1
LIP	Labile iron pool
LPS	Lipopolysaccharide
MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRCKα	Myotonic dystrophy kinase-related Cdc42-binding kinase α

NFκB	Nuclear factor kappa B
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NTBI	Non-transferrin-bound iron
PAP7	PKA-associated protein 7
PD	Parkinson's disease
PINK-1	PTEN-induced putative kinase 1
ROS	Reactive oxygen species
SDR2	Stromal cell-derived receptor 2
SN	Substantia nigra
Tf	Transferrin
TfR1	Transferrin receptor 1
TH	Tyrosine hydroxylase
TNF	Tumor necrosis factor
TNFR	TNF receptor
TRPC6	Transient receptor potential canonical 6
XO	Xanthine oxidase
Zip 14	Zrt-, Irt-like protein 14

1 Introduction

Early observations in postmortem PD tissue showed decreased glutathione (GSH) and increased oxygen-mediated damage as detected by 4-hydroxynonenal protein adducts (Perry et al. 1982; Youdim et al. 1989; Jenner 1991). These findings established the evidence for the participation of oxidative damage in nigral neuronal death occurring in PD. At present, the causes for this deregulation remained obscure.

An important development in the study of the molecular mechanisms underlying the disease arose from the serendipitous discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as a Parkinsonian agent (Langston et al. 1983). This initial finding was followed by the finding that the primary effect of MPTP, inhibition of mitochondria electron transport chain complex I, was a customary characteristic of Parkinsonian tissue (Schapira et al. 1990). Thus, it became fairly well established that mitochondrial dysfunction and the consequences therein are most probably germane to the development of the disease.

The finding of genetic mutations that induce familiar Parkinsonism has provided important insights into the cellular and molecular processes that go awry in PD (for a recent review see (Martin et al. 2011)). PINK1, parkin, DJ-1, and α-synuclein are proteins that interact with the mitochondrion and modulate its function. The fact that their loss of function results in familiar PD supports the notion that mitochondrial dysfunction is a central event to PD pathogenesis (Schapira and Gegg 2011).

There is abundant evidence showing iron accumulation in dopaminergic neurons of the SN pars compacta (Jellinger 1999; Bartzokis et al. 2000; Sayre et al. 2000; Perry et al. 2003; Zecca et al. 2004; Berg and Youdim 2006; Wilson 2006; Weinreb et al. 2011).

The observation that pharmacological agents with iron chelation capacity prevent neuronal death induced by Parkinsonian toxins (Zhu et al. 2007) highlights the pivotal role of iron in PD neuronal death. It is therefore important to understand the relationships between mitochondrial complex I dysfunction, oxidative stress, and iron accumulation and how the conjunction of these factors results in neuronal death. The pivotal role of iron in the events leading to nigral neuronal death is the subject of this chapter.

2 Iron Homeostasis and Dyshomeostasis: The Role of Iron Transporters on Iron Accumulation

2.1 The Iron Responsive Element: Iron Regulatory Protein (IRE/IRP) System. A Brief Description

In vertebrates, cellular iron levels are posttranscriptionally controlled by the activity of iron regulatory proteins (IRP1 and IRP2), cytoplasmic proteins that bind to structural elements called iron-responsive elements (IREs). IREs are found in the untranslated region of the mRNAs of the major proteins that regulate cellular iron homeostasis: the transferrin receptor, the iron transporter DMT1, involved in iron import, the iron-storage protein ferritin and the exo-transporter ferroportin 1, involved in decreasing the levels of cytosolic redox-active iron (for a recent review see (Wang and Pantopoulos 2011)).

The activities of both IRP1 and IRP2 respond to changes in cellular Fe status, although through different mechanisms. In iron-replete conditions, IRP1 has a 4S-4Fe cubane structure that renders the protein active as a cytoplasmic aconitase but inactive for IRE binding. Low levels of intracellular Fe induce disassembling of the 4S-4Fe cluster, which causes IRP1 to bind and stabilize TfR and DMT1 mRNA. Besides being regulated by iron levels, IRP1 is also regulated by effectors such as nitric oxide (Bouton 1999), hydrogen peroxide (H_2O_2) (Martins et al. 1995), hypoxia (Hanson et al. 1999), and phosphorylation (Schalinske and Eisenstein 1996). In contrast to IRP1, IRP2 activity is downregulated through iron-induced oxidative damage followed by ubiquitination and proteasome degradation (Guo et al. 1995). The genetic ablation of IRP2 revealed that IRP2−/− mice are born normal but develop in adulthood a movement disorder characterized by ataxia, bradykinesia, and tremor (LaVaute et al. 2001). In contrast, IRP1−/− mice are apparently normal during adulthood, with only slight misregulation of iron metabolism in the kidney and brown fat (Meyron-Holtz et al. 2004). Thus, IRP2 seems to dominate the physiological regulation of iron metabolism, whereas IRP1 seems to predominate in pathophysiological conditions.

A genome-wide search for IRE sequences resulted in 35 new target genes for IRPs (Campillos et al. 2010; Sanchez et al. 2011). Thus, the scope of the IRE/IRP system has lately gone from a restricted iron homeostasis system to a more global system, still undetermined, possibly linking iron homeostasis to a myriad of other cell functions (Fig. 1).

Newly found genes that contain an IRE motif include Hif-2α, which regulates the expression of proteins involved in erythropoiesis, angiogenesis, programmed

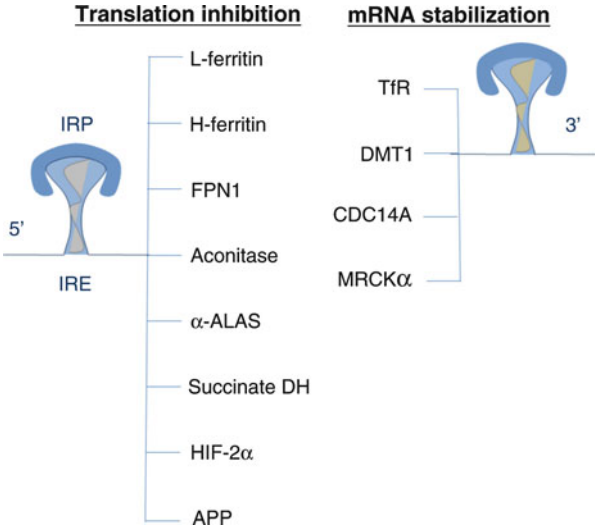

Fig. 1 Genes that contain an IRE motif in the untranslated regions of their mRNA

cell death, cancer, and ischemia (Patel and Simon 2008; Davis et al. 2011). Other prominent members are Cdc14, a tyrosine phosphatase involved in exit from mitosis and initiation of DNA replication. Cdc14 has been shown to dephosphorylate tumor suppressor protein p53, possibly regulating its function and MRCKα, a serine/threonine kinase regulated by Cdc42, a member of the Rho subfamily of small GTPases that regulate cytoskeletal organization. MRCKα also regulates myosin assembly by phosphorylation of the myosin-binding subunit of myosin light chain and phosphorylates LIMK1 and LIMK2, kinases that regulate actin assembly (Cmejla et al. 2010).

2.2 Cellular Components of Neuronal Iron Homeostasis

The components of neuronal iron homeostasis are shown in Fig. 2.

The scheme includes transferrin and transferrin receptor (TfR), inflow (DMT1; SLC11A2) and efflux (ferroportin 1, FPN1) iron transporters, the iron-storage protein ferritin, the ferrireductase Dcytb that reduces extracellular Fe^{3+} to Fe^{2+} prior to transport by DMT1, and the ferroxidase ceruloplasmin (CP) responsible for the oxidation of Fe^{2+} after transport by FPN1 and prior to the binding by apoTf. Transferrin-bound iron uptake starts with the binding of transferrin to surface receptors, followed by internalization into the endosomal system, release of iron mediated by the acidification of the endosome, reduction possibly mediated by Steap2, and transport into the cytoplasm by endosomal DMT1. Once in the cytoplasm, Fe^{2+} becomes part of the labile or reactive iron pool where it distributes to mitochondria, neuromelanin, and ferritin or engages in electron exchange reactions (Kakhlon and Cabantchik 2002; Kruszewski 2003). All the components described in Fig. 1 have been detected in the brain (Moos et al. 2007; Rouault et al. 2009; Haeger et al. 2010).

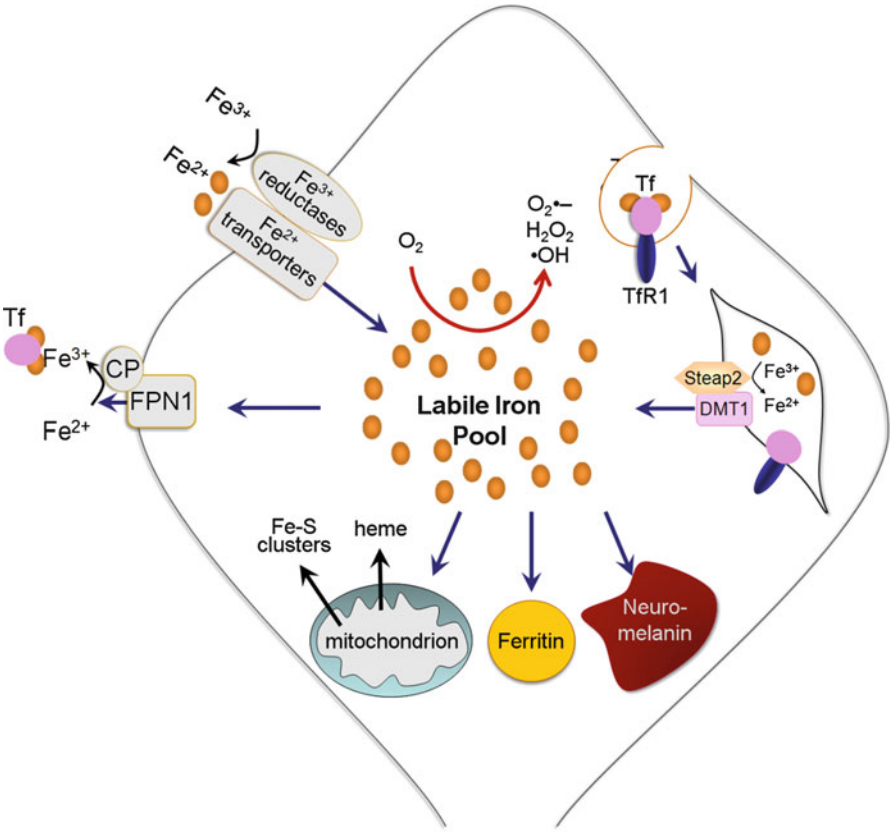

Fig. 2 Components of neuronal iron homeostasis. The molecular components comprise the transferrin–transferrin receptor complex; the ferrireductases Dcytb and/or SDR2, responsible for the reduction of Fe^{3+} prior to transport by DMT1; uptake (DMT1, TRPC6, VGCC, Zip14) and efflux (ferroportin 1, FPN1) iron transporters; the iron-storage protein ferritin; the ferrireductase Steap2, probably responsible for the reduction of Fe^{3+} prior to transport by DMT1 in endosomes in the brain; and the ferroxidase CP, responsible for the oxidation of Fe^{2+} after transport by FPN1 and prior to Fe^{3+} binding to apoTf. Once in the cytoplasm, iron forms part of the labile iron pool from where it distributes to mitochondria, ferritin, and neuromelanin, or it is exo-transported by FPN1. In the cellular environment, LIP iron is a net ROS producer. For simplicity, the molecular components of mitochondrial iron homeostasis were omitted (From Núñez et al. (2012) with permission from the publisher)

2.2.1 The Iron-Uptake Transporters

Iron concentration in cerebrospinal fluid (CSF) ranges between 0.2 and 1.1 μM, whereas transferrin concentration lies around 0.24 μM (Moos and Morgan 1998; Symons and Gutteridge 1998). Thus, CSF iron often exceeds the binding capacity of transferrin, and non-transferrin-bound iron (NTBI) uptake is expected to occur in neurons that express DMT1 or other iron-uptake transporters.

DMT1 (SLC11A2) is the paradigmatic transporter that brings iron inside cells (Gunshin et al. 1997). In the brain, DMT1 is expressed in hippocampal pyramidal

and granule cells, cerebella granule cells, pyramidal cells of the piriform cortex, SN and the ventral portion of the anterior olfactory nucleus, striatum, cerebellum, hippocampus and thalamus, as well as in vascular cells throughout the brain and ependymal cells in the third ventricle (Gunshin et al. 1997; Williams et al. 2000; Burdo et al. 2001). The pervasive presence of DMT1 in neurons suggests that DMT1 is necessary for their regular function (Hidalgo and Núñez 2007; Wright and Baccarelli 2007; Haeger et al. 2010; Pelizzoni et al. 2011).

The mammalian DMT1 gene undergoes alternative splicing generating four isoforms, all with similar activities for Fe^{2+} transport. The 1A and 1B mRNA DMT1 variants originate from alternative splicing at the 5′ end (exons 1A and 1B), while the +IRE or –IRE variants originate from splicing on the 3′ end (exons 16/16A and 17) (Hubert and Hentze 2002; Ludwiczek et al. 2007). The 1B isoforms are expressed in the brain (Haeger et al. 2010), whereas the 1A isoforms are almost exclusively expressed in the intestine (Hubert and Hentze 2002).

Given its crucial role on cell iron uptake, knowledge on the regulation of DMT1 expression is vital to understand pathological conditions in which iron accumulation is observed. It is generally accepted that the +IRE isoforms of DMT1 are regulated by the IRE/IRP system, which posttranscriptionally regulates the expression of iron homeostasis proteins such as transferrin receptor 1 (TfR1), DMT1, FPN1, and ferritin, in response to the concentration of reactive iron in the cytoplasm (Garrick and Garrick 2009). Knowledge on the regulation of DMT1(−IRE) mRNA levels through its 3′-UTR is scarce. Recently, its regulation by the levels of miRNA Let-7d was reported in developing erythroid cells (Andolfo et al. 2010). The authors demonstrated that when the levels of miRNA Let-7d increase, the expression of DMT1(−IRE) decreases, probably by inducing its degradation through binding to imperfect complementary sites within the 3′-UTR of its mRNA.

Understanding on the transcriptional regulation of DMT1 expression is emerging. The inflammatory cytokine NFκB and nuclear factor Y regulate DMT1(1B) expression in embryonic carcinoma cells (Paradkar and Roth 2006b), whereas hypoxia upregulates expression of the DMT1(1A) isoform, presumably through activation of HIF-1 (Lis et al. 2005; Wang et al. 2010a). HIF-1 also promotes the expression of the 1B isoform by binding to a putative hypoxia-response element sequence in DMT1 exon1B gene (Qian et al. 2011). Recently, upregulation of DMT1 mRNA by the endogenous neurotoxin aminochrome was reported (Aguirre et al. 2012). Micromolar concentrations of aminochrome induced increased DMT1(+IRE) expression coupled to decreased FPN1 expression, in a process blocked by the antioxidant N-acetyl cysteine. Thus, it is possible that an increase in the oxidative tone triggers, through an undefined mechanism, increased expression of DMT1.

2.2.2 The Iron Exo-transporter FPN1

FPN1 (SCL40A) is the only member of the SLC40 family of transporters and the first reported protein that mediates the exit of iron from cells (McKie et al. 2000). The protein is expressed mainly in enterocytes and macrophages, but its presence has also been described in neurons and astrocytes (Burdo et al. 2001).

In enterocytes, FPN1 is responsible for iron efflux during the process of intestinal iron absorption, while in Kupffer cells FPN1 mediates iron export for reutilization by the bone marrow (Devalia et al. 2002).

Knowledge on the regulation of FPN1 expression is incipient. Most probably, FPN1 expression is regulated at both transcriptional and translational levels (McKie et al. 2000; Knutson et al. 2003). There are two closely related isoforms of FPN, one (FPN1) that has an IRE motif in the 5′-UTR and another (FPN1B) that does not have it (Zhang et al. 2009). The 5′-UTR motif gives FPN1 a ferritin-like response to variations in cell iron, increasing its expression under elevated cell iron conditions (McKie et al. 2000; Yang et al. 2002; Aguirre et al. 2005). For the contrary, the 1A isoform, mainly expressed in enterocytes and erythroid precursor cells, responds to an iron challenge decreasing its expression (Zhang et al. 2009).

The expression of FPN1 in mouse brain is ubiquitous. It is present in oligodendrocytes, microglia, astrocytes, and neurons (Song et al. 2010). Space-temporal expression of FPN1 in neurons is variable (Moos and Rosengren 2006). In young brain high immunoreactivity is found in the neurons of the hippocampus and striatum (both in cell bodies and in projection fibers), a mild expression in the SN pars compacta and the superior colliculus and low expression in the SN pars reticulata (Boserup et al. 2011). In the adult brain, FPN1 immunoreactivity is lower in the projections of the striatum, but no differences have been found in neuronal cell bodies (Moos and Rosengren 2006).

2.2.3 TfR1

The TfR1 mediates the incorporation of iron into cells by binding the plasma iron-transport protein transferrin and undergoing recycling clathrin-mediated endocytosis (Harding et al. 1983). The presence of TfR in neurons, microglia, and astrocytes and its response to iron deficiency have been described (Han et al. 2003; Beard et al. 2005). Early studies by Faucheux et al. revealed that the density of ^{125}I-labeled transferrin binding sites were highest in the central gray substance, intermediate in the catecholaminergic cells of the superior colliculus and ventral tegmental area, and almost nonexistent in the SN (Faucheux et al. 1993).

As noted above, TfR1 is posttranscriptionally regulated by the IRE/IRP system. When cellular iron is low, IRPs bind to five IRE motifs in the 3′-UTR of TfR1 mRNA increasing its mRNA stability. TfR1 expression is transcriptionally regulated by hypoxia through HIF-1 (Tacchini et al. 1999). TfR1 expression is also regulated by inflammatory stimuli: LPS/IFN-γ treatment to mouse macrophages upregulates NF-κB, which in turn transiently activates HIF-1-dependent TfR1expression and iron uptake (Tacchini et al. 2008).

2.2.4 Ferritin

Ferritin, a multimeric protein formed by 24 subunits of H and L monomers, is the only well-characterized iron-storage protein in living organisms (Liu and Theil 2005). The 24 subunits in ferritin form a hollow cavity that can store as much as 4,500 Fe^{3+} atoms in the form of crystallized diferric oxo-hydroxyl complexes (Harrison and Arosio 1996). Ferritin plays a fundamental role in controlling the

size of the cytoplasmic redox-active iron pool (Salgado et al. 2010). Although ferritin is expected to reduce this pool by storing iron, there is a dynamic exchange of iron between its ferritin-bound form and a cytoplasmic form amenable to transport by FPN1 (De Domenico et al. 2006). In addition, ferritin contributes to the pool of redox-active iron every time it turns over (Mehlhase et al. 2005). H-ferritin, the subunit responsible for Fe^{2+} oxidation, is relatively poorly expressed in melanized dopaminergic neurons of the SN pars compacta, as compared to neurons in other parts of the brain, but is strongly expressed in oligodendrocytes (Snyder and Connor 2009).

2.2.5 The Ferrireductases and Ferroxidases

Fe^{2+} is the preferred species for transport, so ferrireductases must reduce Fe^{3+} prior to its transport into the cell. In contrast, Fe^{2+} transported from inside the cell to the extracellular milieu must be oxidized by ferroxidases to Fe^{3+} prior to transferrin binding. Three membrane ferroxidases have been described: Dcytb, its homologous cytochrome b561, and SDR2 (Vargas et al. 2003). Dcytb, initially described as the ferroxidase responsible for reduction of nonheme iron in the duodenal lumen (McKie et al. 2001), is the only member of the iron transport machinery that lacks an IRE in its mRNA. Recently, the upregulation of Dcytb by Hif-2α was reported (Latunde-Dada et al. 2011). As mentioned before, DMT1 and FPN1 exhibit transcriptional regulation by Hif-1. Thus, the control of iron acquisition through the upregulation of Dcytb and DMT1 by Hif-1, coupled to the downregulation of FPN1 by Hif-2α, results in a concerted response of increased iron accumulation under hypoxic conditions. The presence of Dcytb and SDR2 was recently reported in astrocytes, where they may have a limited role in iron accumulation by these cells (Tulpule et al. 2010), but there is no report on their presence in neurons. Maybe the high concentration of ascorbate in the CSF, which should keep NTBI in the 2+ state, makes unnecessary a membrane-bound iron reduction system in brain cells.

The Steap family of ferrireductases comprises the members Steap1, Steap2, Steap3, and Steap4 (Ohgami et al. 2006). Steap3 was described in endosomes of erythroid precursor cells, catalyzing the reduction of Fe^{3+} to Fe^{2+} prior to transport by DMT1 (Ohgami et al. 2005). In situ hybridization studies show that Steap2 is expressed in the brain (Ohgami et al. 2006). Whether Steap2 or other member of the Steap family has a role in iron uptake by neurons remains speculative.

Two multi-copper ferroxidases, ceruloplasmin (CP) and hephaestin (Heph), are involved in iron export from the cell. CP seems to be required for iron release from most tissues in the body. Although the liver is the predominant source of serum ceruloplasmin, ceruloplasmin gene expression has also been demonstrated in many tissues including spleen, lung, testis, and brain (Hellman and Gitlin 2002). The scope of action of the membrane-bound CP homolog Heph is restricted to the small intestine, where it is required for efficient iron absorption (Vulpe et al. 1999).

2.2.6 Neuromelanin

Neuromelanin is a polymer formed by oxidized metabolites of dopamine, containing a peptide component of about 15 % (Zecca et al. 2002). Neuromelanin

avidly binds iron in its +3 form, presenting high- and low-affinity binding sites (Double et al. 2003). Given the low expression of ferritin, neuromelanin is the main iron-storage moiety in SN pars compacta neurons. It is believed that the high-affinity sites are protective as they sequester iron in a redox-inactive form, whereas iron in the low-affinity sites is redox active (Gerlach et al. 2008). Thus, when iron concentration increases above the high-affinity binding capacity of neuromelanin, the neurons become prone to oxidative damage.

3 Iron Accumulation and Glutathione Depletion in PD

Iron is an intrinsic ROS producer. When one or more of its six ligand binding sites are not tightly bound, iron engages in one-electron exchange reactions, producing free radicals (Graf et al. 1984) through the Haber-Weiss and Fenton reactions. The thermodynamic balance of these reactions indicates that in the reductive environment of the cell, iron, in the presence of oxygen, catalyzes the consumption of GSH and the production of the hydroxyl radical ($^{\bullet}OH$) (Núñez et al. 2012).

In dopaminergic cells, another source of free radicals derives from the nonenzymatic oxidation of dopamine mediated by redox-active iron, resulting in semiquinones and H_2O_2 production (Zoccarato et al. 2005). Thus, redox-active iron, both through the Fenton reaction or via dopamine oxidation, is a dangerous prooxidant agent.

3.1 Iron Accumulation in PD

Overwhelming evidence indicates that iron accumulation is a common feature of a number of neurodegenerative disorders of the central nervous system that include Huntington's disease, Alzheimer's disease, Friedreich's ataxia, amyotrophic lateral sclerosis, and Parkinson's disease (Jellinger 1999; Bartzokis et al. 2000; Sayre et al. 2000; Perry et al. 2003; Zecca et al. 2004; Berg and Youdim 2006; Wilson 2006; Weinreb et al. 2011). Indeed, the autocatalytic production of hydroxyl radicals because of iron accumulation has been proposed as the unifying mechanism of cell death in neurodegenerative diseases (Kell 2010).

Iron accumulation has been demonstrated in the dopaminergic neurons of the SN pars compacta (Youdim et al. 1989; Hirsch et al. 1991; Gorell et al. 1995; Vymazal et al. 1999). Interestingly, MPTP, a drug that causes experimental Parkinson's disease, upregulates DMT1(+IRE) protein expression in mice ventral mesencephalon, where it increases neuronal death presumably through abnormal increases in cellular iron content (Salazar et al. 2008; Jiang et al. 2010). Additionally, DMT1(−IRE) mediates L-DOPA neurotoxicity in primary cortical neurons (Du et al. 2009).

The contribution of iron dyshomeostasis to the progression of events leading to neuronal death is likely, since iron accumulation has been detected in brain tissue from patients who have died, after the final steps of the pathology. Since neuronal

death caused by MPTP or 6-hydroxydopamine intoxication is blocked by pharmacologic or genetic chelation of iron (Kaur et al. 2003; Shachar et al. 2004; Youdim et al. 2004; Youdim and Buccafusco 2005; Zheng et al. 2010) or by dysfunction of the iron transporter DMT1 (Salazar et al. 2008), it is possible that iron dyshomeostasis takes place in the late stages of the disease as part of a vicious cycle resulting in uncontrolled oxidative damage and cell death. A recent study in mesencephalic dopaminergic neurons shows that low (0.25–0.5 μM) concentrations of 1-methyl-4-phenylpyridinium (MPP+), the active metabolite of MPTP, induce neuritic tree collapse without loss of cell viability (Gómez et al. 2010). This collapse was effectively prevented by decreasing iron supply or by the addition of antioxidants. Thus, it seems plausible that increased intracellular iron and ROS are involved in the early steps of dopaminergic neuron dysfunction, prior to cell death. Later, a vicious cycle of further iron accumulation, complex I dysfunction, and ROS increase may result in uncontrolled oxidative damage and cell death.

Iron toxicity is not restricted to dopaminergic neurons. Neurotoxic concentrations of NMDA induce the NO-Dexras1-PAP7 signaling cascade in glutamatergic PC12 cells. Upon activation, PAP7 binds to intracellular DMT1 and relocates it to the plasma membrane, increasing intracellular iron and the production of hydroxyl radicals. Thus, the DMT1-iron-uptake-hydroxyl radical signaling pathway appears to mediate NMDA neurotoxicity (Cheah et al. 2006).

3.2 Glutathione (GSH) Metabolism in PD

The tripeptide GSH (γ-L-glutamyl-L-cysteinylglycine) is the most abundant and the main antioxidant agent in the central nervous system, where it reaches mM concentrations in the cytoplasm (Meister and Anderson 1983; Dringen et al. 2000). In its redox cycling, glutathione is present either in its reduced (GSH) form or its oxidized disulfide (GSSG) form, the ratio GSH:GSSG being faithful reflection of the redox state of the cell (Schafer and Buettner 2001).

Early postmortem studies revealed decreased levels of GSH in degenerating SN of PD patients (Perry et al. 1982; Sofic et al. 1988; Sian et al. 1994), the observation implicating that GSH depletion may play a major role in the neurodegenerative process. The question arises whether GSH depletion is an early event during the progression of the disease or a reflection of increased oxidative stress resulting, for example, from mitochondrial complex I inhibition and/or iron accumulation.

Chronic submaximal inhibition of GSH synthesis in N27 dopaminergic cells results in about 50 % inhibition of mitochondrial electron transport chain complex I without ensuing cell death (Chinta and Andersen 2006). Thus, increased oxidative stress generated by complex I inhibition should result in decreased GSH levels and further inhibition of complex I. Conversely, a decrease in GSH levels, provoked by unknown causes, could result in inhibition of complex I activity.

Iron induces the consumption of GSH. After exposure to increasing concentrations of iron, SH-SY5Y dopaminergic cells undergo sustained iron accumulation and a biphasic change in intracellular GSH levels, increasing at low (1–5 μM) iron

concentrations and decreasing thereafter. Indeed, cell exposure to high iron concentrations (20–80 μM) markedly decreases the GSH:GSSG molar ratio and the GSH half-cell reduction potential, with the associated loss of cell viability (Núñez et al. 2004).

It is therefore possible that a decrease in GSH levels is a consequence of the increased oxidative load produced by the increased intracellular iron. Nevertheless, increased iron and decreased GSH may be intertwined in a positive feedback loop, since in dopaminergic neurons the pharmacological reduction of GSH levels results in increased levels of TfR and an increased labile iron pool (Kaur et al. 2009). Thus, the question remains as to which of the three processes initiates the oxidative spiral, but a reasonable assumption is that if one of them develops, the others will follow.

4 Mitochondrial Dysfunction in Parkinson's Disease

Mitochondrial dysfunction is another event involved in Parkinson pathogenesis. Evidence of mitochondrial dysfunction in PD began in the 1980s, when, after an intravenous injection of illicit drugs, four people developed a marked Parkinsonism. Further analysis of the substances injected revealed the existence of MPTP, a potent inhibitor of complex I of the mitochondrial respiratory chain (Langston et al. 1983). Since its discovery, mice and monkeys intoxicated with MPTP have been used as experimental models of PD.

MPTP crosses the blood–brain barrier and it is oxidized by astrocytes to the active metabolite MPP+. Subsequently, MPP+ is selectively taken up by dopaminergic neurons through the dopamine transporter DAT (Gainetdinov et al. 1997). Inside neurons MPP+ inhibits mitochondrial complex I (Mizuno et al. 1987). Among the effects of this inhibition are decreased levels of ATP (Scotcher et al. 1990) and increased production of free radicals and oxidative stress (Wong et al. 1999).

Decreased activity of mitochondrial complex I, found in postmortem tissue of PD patients (Schapira et al. 1990; Tretter et al. 2004; Banerjee et al. 2009; Hattingen et al. 2009), is probably a founding event in neuronal death. The association between complex I inhibition and PD is further supported by the observation that rats intoxicated with rotenone, a selective inhibitor of complex I, develop a syndrome similar to PD, characterized by neuronal degeneration and the formation of inclusion bodies rich in alpha-synuclein (Betarbet et al. 2000). Likewise, inhibition of glutaredoxin 2, an enzyme involved in mitochondrial Fe–S synthesis, produces an alteration in iron metabolism in a model of Parkinson's disease (Lee et al. 2009). Additionally, the fact that mutations in mitochondrial proteins PINK-1, a putative serine/threonine kinase involved in mitochondrial response to oxidative stress (Valente et al. 2004), and DJ-1, an antioxidant protein that relocalizes to mitochondria under conditions of oxidative stress (Blackinton et al. 2005), result in a genetic form of PD lends further support for an important role of mitochondria in PD neurodegeneration.

ROS seem to have a negative effect on complex I activity. Experiments with isolated synaptosomal mitochondria revealed that low concentrations of H_2O_2 decrease complex I activity by 10 %. This relatively minor effect of H_2O_2 was additive to partial inhibition of complex I induced by low concentrations (5 nM–1 μM) of rotenone (Chinopoulos and Adam-Vizi 2001). Similarly, submitochondrial particles exposed to $O_2^{\bullet-}$, H_2O_2, or $^{\bullet}OH$ present decreased activity of NADH dehydrogenase, a marker of complex I activity (Zhang et al. 1990). Thus, an initial inhibition of complex I could generate a positive loop between ROS generation and further complex I inhibition.

5 Fe–S Cluster Synthesis and Its Relevance to PD

5.1 Fe–S Cluster Biogenesis

Iron–sulfur (Fe–S) clusters are small inorganic cofactors formed by tetrahedral coordination of iron atoms with sulfur groups. The Fe–S clusters most commonly found in eukaryotes are 2Fe–2S and 4Fe–4S. Fe–S clusters are cofactors for proteins that are involved in many cellular processes, including electron transport, enzymatic catalysis and regulation, and DNA synthesis (Lill and Muhlenhoff 2008). The proteins that contain Fe–S clusters in eukaryotes are present in mitochondria, endoplasmic reticulum, cytoplasm, and nucleus (Lill et al. 2006; Sheftel et al. 2010).

The mitochondrion plays a central role in the generation and biology of Fe–S clusters since it holds the assembly machinery responsible for their synthesis (Lill et al. 2006). Fe–S cluster synthesis also occurs in the cytoplasm, albeit at a minor scale (Ye and Rouault 2010). In eukaryotes, the Fe–S cluster assembly machinery of mitochondria comprises the cysteine desulfurase, Nfs1, which provides sulfur and Isu1, a protein that serves as a molecular scaffold for the assembly of the Fe–S cluster. Synthesis of the transiently bound Fe–S cluster on the Isu scaffold proteins requires reduced (ferrous) iron and the input of electrons, presumably to generate sulfide (S^{2-}) from cysteine (Ye and Rouault 2010). Finally, synthesized Fe–S clusters either are transferred to mitochondrial apoproteins or are exported into the cytoplasm by the Fe–S cluster export machinery. This machinery involves the ABCB7 transporter, the antioxidant GSH, and the sulfhydryl oxidase Erv1, which is also involved in protein import (Lill et al. 2006; Sheftel et al. 2010). Iron is transported inside the mitochondria by the transporter mitoferrin (Shaw et al. 2006).

5.2 Biological Functions of Iron–Sulfur Clusters

Mitochondria contain numerous Fe–S proteins essential for its function. Complex I has eight Fe–S clusters, succinate dehydrogenase (complex II) has three Fe–S clusters, and ubiquinone cytochrome c oxidoreductase (complex III) has one 2Fe–2S cluster. Additionally, mitochondrial aconitase contains a 4Fe–4S cluster in its catalysis center (Beinert et al. 1983). Proteins involved in purine metabolism in the

cytoplasm like xanthine oxidase and phosphoribosylpyrophosphate amidotransferase (GPAT) contain two 2Fe–2S and one 4Fe–4S cluster, respectively (Unciuleac et al. 2004; Martelli et al. 2007). As mentioned above, disassembly of the 4Fe–4S cluster in IRP1 alters the active site accessibility and determines IRP1 binding to target mRNAs (Wallander et al. 2006; Cairo and Recalcati 2007). In the nucleus, proteins involved in DNA repair and replication contain Fe–S clusters (Sheftel et al. 2010). For a compendium of Fe–S clusters, see the Prosthetic Groups and Metal Ions in Protein Active Sites (PROMISE) http://metallo.scripps.edu/promise/MAIN.html.

5.3 IRP1: A Link Between Fe–S Clusters and Iron Homeostasis

Recent data indicate that inhibition of complex I by rotenone results in decreased synthesis of Fe–S clusters, as shown by the decreased activity of the Fe–S cluster-containing enzymes cytoplasmic aconitase, mitochondrial aconitase, xanthine oxidase (XO), and GPAT, as well as the activation of cytoplasmic IRP1 (Mena et al. 2011) (Fig. 3).

It is considered that decreased activity of complex I results, via decreased Fe–S cluster synthesis and the consequent activation of IRP1, in a false "low iron" signal that activates the iron-uptake system. In consequence, diminished Fe–S cluster synthesis could play a fundamental role in the accumulation of iron observed in Parkinson's disease. Future research is needed to evaluate its participation in neurodegenerative diseases in which iron accumulation is observed.

6 Aminochrome: An Inhibitor of Complex I of the Mitochondrial Electron Transport Chain

The causes for complex I dysfunction in sporadic PD are a matter of active research. Exogenous toxins can be of relevance, given the existing correlation between PD and exposure to toxins such as MPTP, rotenone, paraquat, fenazaquin, annonacin, and Guadeloupe PSP-PDC (reviewed in Schapira (2010)). Endogenous toxins could also cause mitochondrial dysfunction, as is the case for nitric oxide (Clementi et al. 1998; Broom et al. 2011), 4-hydroxynonenal (Humphries et al. 1998; Castellani et al. 2002), and advanced glycosylated end products (Munch et al. 2000; Remor et al. 2011).

An endogenous source of free radicals proper to dopaminergic neurons derives from the nonenzymatic oxidation of dopaminoquinone to dopamine o-quinone that at physiological pH spontaneously cycles to o-quinone *aminochrome* (Arriagada et al. 2004; Zoccarato et al. 2005). A one-electron gain by aminochrome results in the highly reactive leukoaminochrome o-semiquinone radical that reacts with oxygen to generate $O_2^{\bullet -}$ (Segura-Aguilar et al. 1998). Moreover, aminochrome seems to mediate the transfer of electrons from respiration-active complex I into oxygen, thus increasing the production of $O_2^{\bullet -}$ and H_2O_2 (Zoccarato et al. 2005). The increase in reactive oxygen species has direct

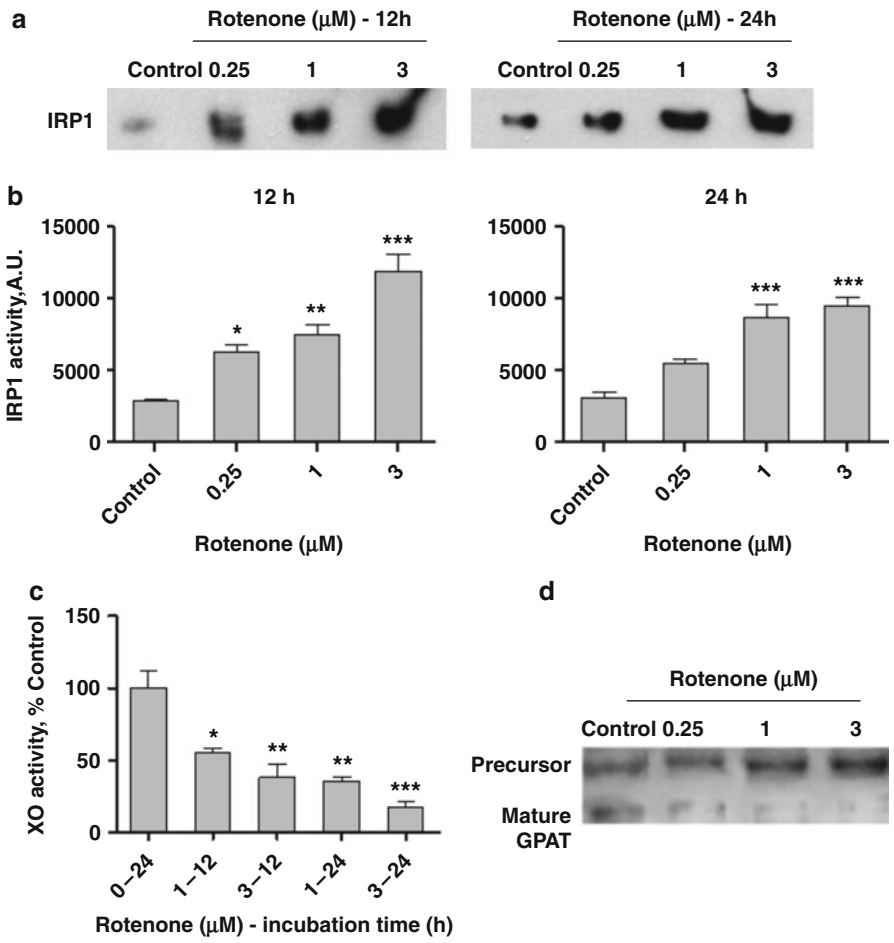

Fig. 3 Complex I inhibition affects IRP activity, XO activity, and Fe–S cluster content of GPAT. (**a**) EMSA for IRP activity. Cells were treated for 24 h with 0.25, 1, or 3 μM rotenone. Subsequently, the cytosolic extracts were probed with an IRE sequence tagged with biotin. (**b**) Densitometric quantification of data in A. Data represent mean ± SEM; n = 3. (**c**) XO activity of cells treated for 12 or 24 h with 0.25, 1, or 3 μM rotenone. Data represent mean ± SEM; n = 3. (**d**) Cells were treated for 24 h with 0.25, 1, or 3 μM rotenone. Subsequently, the holo and apo forms of GPAT were detected by Western blot. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with the control condition (Modified from Mena et al. (2011) with permission from the publisher)

consequences on the mitochondrial electron transport chain since both H_2O_2 and the $^{\bullet}OH$ inhibit complex I activity and decrease ATP production (Zhang et al. 1990; Bautista et al. 2000; Sims et al. 2000; Chinopoulos and Adam-Vizi 2001).

Recent work from our laboratory has established that aminochrome inhibits complex I and ATP production in SH-SY5Y neuroblastoma cells differentiated into a dopaminergic phenotype (Fig. 4) (Aguirre et al. 2012).

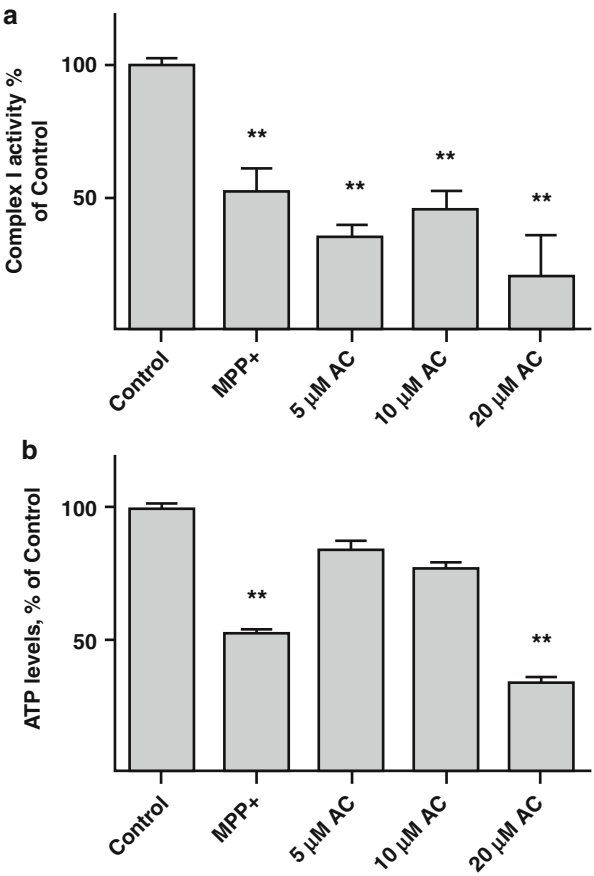

Fig. 4 Aminochrome inhibits complex I activity. SH-SY5Y cells differentiated to a dopaminergic phenotype were treated with 5–20 μM aminochrome or 100 μM MPP+ for 20 h prior to determination of complex I activity as described (Mena et al. 2011). (**a**) Complex I activity (nmoles/min/mg) normalized to the untreated (Control) condition. (**b**) Determination of ATP levels in cells treated as above. Results represent mean ± SEM of three independent experiments. ** $P < 0.01$ compared with the control condition (Modified from Aguirre et al. (2012) with permission from the publisher)

The inhibition is apparently direct on the mitochondria and not secondary to other process since it was replicated in isolated mitochondria. Additionally, overnight treatment with low micromolar concentrations of aminochrome induced increased expression of the iron import transporter DMT1 and a decrease in the export transporter FPN1. Both changes resulted in increased iron uptake. These results strongly suggest that aminochrome promotes complex I inhibition and increased iron accumulation that ends up in cell death. Therefore, aminochrome should be considered an endogenous toxin that, if uncontrolled, can induce deregulation of iron homeostasis.

7 Inflammation, Hepcidin, and PD

In addition to iron accumulation, another event strongly associated with neuronal death in PD and other neurodegenerative disorders is the presence of inflammatory processes characterized by the occurrence of reactive microglia and massive production of pro-inflammatory cytokines. Both phenomena have been studied as

independent events leading to the progression of the disease, but, as discussed above, the expression of iron homeostasis proteins such as ferritin, DMT1, and Dcytb is regulated by inflammatory cytokines. Moreover, the recent identification of hepcidin in the central nervous system reinforces the idea that in PD a chronic inflammation state and deregulation of iron homeostasis are intimately related.

7.1 Hepcidin, the Master Regulator of Iron Homeostasis

Hepcidin is a 25-amino acids cationic peptide of secreted into bloodstream by the liver. Initially, hepcidin was described as a peptide with antimicrobial activity (Krause et al. 2000); however, further studies revealed that it also acts as a major regulator of circulating iron levels (Nicolas et al. 2001; Pigeon et al. 2001). Two processes contribute to blood iron levels: the recycling of senescent red blood cells (RBC) and intestinal iron absorption. The recycling by spleen macrophages of heme iron from senescent RBC is the main contributor of iron to the circulation, providing about 95 % of daily turnover. The recycling of RBC iron comprises the phagocytosis of senescent RBC, the release of iron contained in the heme moiety of hemoglobin by heme oxygenase-1, and the subsequent iron release into the blood mediated by FPN1 (De Domenico et al. 2008; Kovtunovych et al. 2010).

The physiological function of hepcidin is to reduce the levels of circulating iron by downregulation of the iron exo-transporter FPN1 in macrophages. The binding of hepcidin to FPN1 present in the plasma membrane of splenic macrophages induces the endocytosis of the complex and the subsequent lysosomal degradation of FPN1 (Nemeth et al. 2004). The decreased levels of FPN1 lead to the accumulation of iron in macrophages and the decrease of circulating iron.

Hepcidin synthesis is regulated by multiple stimuli that have an effect in the regulation of circulating iron levels. Erythropoietin, a hormone that stimulates red blood cell production, and hypoxia downregulate the synthesis of hepcidin, increasing circulating levels of iron as required for the production of new RBCs (Peyssonnaux et al. 2007; Pinto et al. 2008). On the contrary, increased plasma iron levels and inflammatory cytokines stimulate hepcidin synthesis, which results in reduced levels of plasma iron and its accumulation in cells (Lee et al. 2005; Wrighting and Andrews 2006; Gnana-Prakasam et al. 2008; Frazier et al. 2011).

7.2 Hepcidin Expression in the CNS

Hepcidin shows a wide distribution in the CNS, most notably in the midbrain, with a clear presence in the superior colliculus, the geniculate nucleus, some fiber bundles of the SN pars reticulata, the SN pars compacta, and the striatum (Zechel et al. 2006) (Wang et al. 2010b). Hepcidin is expressed mainly in glial cells, as well as in endothelial cells of the choroid plexus (Zechel et al. 2006; Marques et al. 2009). Hepcidin expression changes with age: increased mRNA levels of hepcidin in cortex, striatum, and hippocampus have been observed with aging (Wang et al. 2010b).

As stated above, hepcidin synthesis is induced by inflammatory stimuli. Bacterial lipopolysaccharide (LPS) induces liver hepcidin expression. LPS also increases hepcidin expression in the brain. After an intraventricular injection of LPS, a transient transcription of the gene for hepcidin ensues in the choroid plexus, which correlates with increased levels of pro-hepcidin in the cerebrospinal fluid (Marques et al. 2009). The highest hepcidin expression was observed at 3 h, returning to baseline levels 24 h after the injection. Interestingly, LPS treatment induced a tenfold increase in hepcidin expression in the SN (Wang et al. 2008), which correlates with a marked increase in iron levels observed in this region in PD. Recently, it was reported that in ischemic brain there is an increase in hepcidin expression in cerebral cortex, hippocampus, and corpus striatum, in parallel with increased levels of free iron and ferritin (Ding et al. 2011). These data suggest that hepcidin could be an important contributor to iron accumulation in the brain.

7.3 FPN1–Hepcidin Interactions in the CNS

As described above, the iron transporter FPN1 is a receptor for hepcidin. An interesting fact is that the spatial distribution of FPN1 and hepcidin is similar. Although the effects of hepcidin on FPN1 levels differ according to cell type (Chaston et al. 2008), the injection of hepcidin in mice lateral cerebral ventricle causes a decrease in the levels of FPN1 in the cerebral cortex, hippocampus, and striatum (Wang et al. 2010b). These results suggest that in the brain, hepcidin generates the same response as that observed in macrophages, that is, iron retention inside the cells. This conclusion is strengthened by the fact that high doses of hepcidin produce an increase in the iron-storage protein ferritin, thus indicating increased cellular iron concentration in these brain areas. Unexpected for a high cell iron situation, treatment with hepcidin induces the decrease of both FPN1 protein and mRNA and an increase in total DMT1 in the hippocampus and cortex of rats (Li et al. 2011), a situation that should drive further iron accumulation. Hippocampal neurons in culture treated with hepcidin also show a decrease in the expression of FPN1, which is reflected in a reduction of the iron released from these cells (Wang et al. 2010b). In astrocytes, one study recently reported that treatment with hepcidin significantly reduces iron uptake (both Tf-Fe and NTBI) and iron release, accompanied by decreased expressions of TfR1, DMT1, and FPN1 (Du et al. 2011).

7.4 Hepcidin: A Nexus Between Inflammation and Iron Accumulation in PD

Many cases of PD are accompanied by general inflammation of the brain, with a dramatic proliferation of reactive amoeboid macrophages and microglia in the SN (McGeer et al. 1988). In the striatum, macrophage proliferation is accompanied by high expression of pro-inflammatory cytokines such as TNF-α, IL-1β, IFN-γ, and

IL-6 by glial cells (Mogi et al. 1994; Hirsch et al. 1998; Muller et al. 1998). The increase of many of these cytokines has also been observed in cerebrospinal fluid and the basal ganglia of patients with PD (Nagatsu 2002). The aforementioned characteristics are also observed in several animal models of PD, such as those injected with 6-hydroxydopamine, MPTP, rotenone, or LPS (Herrera et al. 2000; Barnum and Tansey 2010).

The induction of hepcidin synthesis by cytokines suggests that in PD high brain hepcidin levels may mediate iron accumulation. For example, an increase in hepcidin secretion in PD triggered by inflammatory stimuli could be responsible for the decrease of FPN1 observed in this neurodegenerative condition and the subsequent accumulation of iron in neurons and glia (Lv et al. 2011).

7.5 Hepcidin-Independent Relationships Between Iron Accumulation and the Inflammatory Response

Inflammatory cytokines modulate the activity of IRP1 and IRP2 through nitric oxide-dependent and nitric oxide-independent mechanisms, thus influencing iron homeostasis (Mulero and Brock 1999). Stimulation of macrophages with IFN-γ, TNF-α, and LPS produces an increase in IRE-binding activity of IRP1 and IRP2, reducing FPN1 and increasing DMT1 mRNA expression. Interestingly, pretreatment with the anti-inflammatory cytokine IL-10 reverts this response (Wardrop and Richardson 2000; Yang et al. 2002; Ludwiczek et al. 2003; Wang et al. 2005). In addition, DMT1 expression is transcriptionally enhanced by the transcription factor NFκB (Paradkar and Roth 2006a), whose activation is downstream of many cytokine receptors such as TNF receptor (TNFR) and IL-1 receptor (IL-1R). Activation of NFκB by inflammatory stimuli may play a significant role in iron accumulation by dopaminergic neurons of the SN, which express high levels of TNFR (Boka et al. 1994). Interestingly, an increase in the nuclear immunoreactivity of NFκB has been observed in PD brains or in animal models for this disease (Hunot et al. 1997), so it is possible that activation of NFκB contributes to iron accumulation in PD.

Finally, the iron transporter Zip14 could be important in the regulation of brain iron homeostasis. Highly expressed in the brain (Girijashanker et al. 2008) and upregulated by inflammatory mediators (Liuzzi et al. 2005; Lichten et al. 2009), Zip 14 could additionally contribute to iron accumulation in dopaminergic neurons of PD brains.

8 A Positive Feedback Loop in the Death of Neurons

It is proposed that inhibition of mitochondrial complex I by endogenous and/or exogenous toxins and inflammatory processes produced by trauma or other causes result in a vicious cycle of increased oxidative stress, increased iron accumulation, and decreased GSH content (Fig. 5).

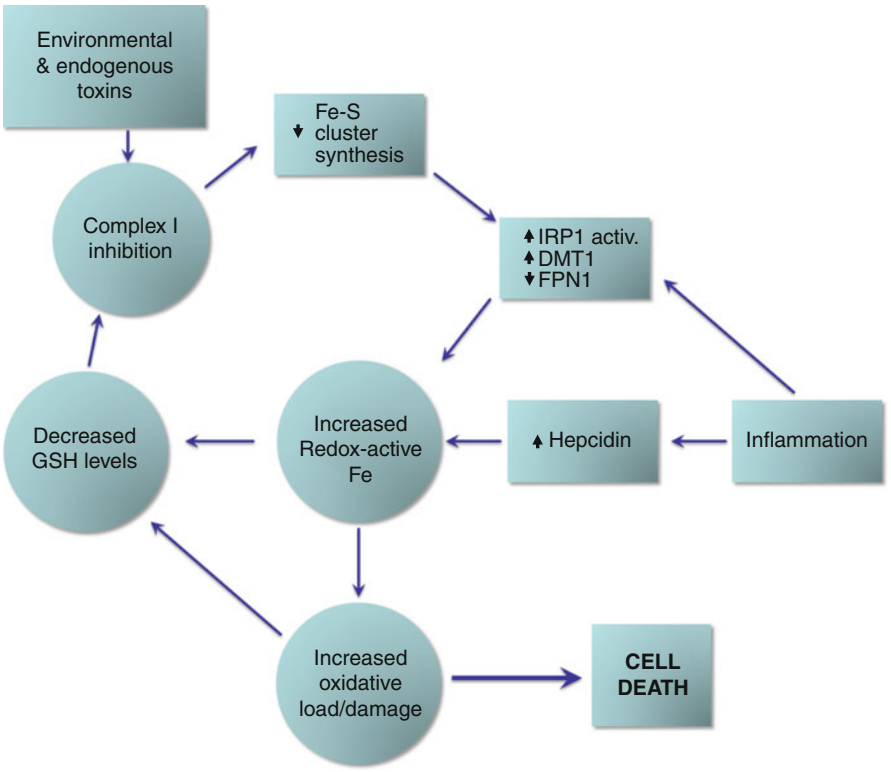

Fig. 5 A positive feedback loop resulting in uncontrolled oxidative load. Complex I inhibition by endogenous or exogenous toxins results in decreased Fe–S cluster synthesis (see text). Decreased Fe–S cluster synthesis leads to the activation of IRP1 that needs a 4Fe–4S cluster to acquire its inactive state. Increased IRP1 activity results in increased DMT1 and decreased FPN1 synthesis, which produce increased iron accumulation. Increased iron induces increased oxidative stress and increased GSH consumption. Decreased GSH produces further complex I inhibition (From Núñez et al. (2012) with permission from the publisher)

In this scheme, neuronal death linked to complex I dysfunction is brought about by a positive feedback loop in which complex I inhibition results in decreased Fe–S cluster synthesis, IRP1 activation, increased DMT1 and TfR expression, and iron accumulation. Complex I dysfunction and increased cellular iron result in decreased GSH levels. Both increased oxidative stress and low GSH levels further inhibit complex I activity. Another input to this cycle is contributed by inflammatory cytokines that induce hepcidin synthesis, which by inducing FPN1 degradation results in increased neuronal iron content. Inflammatory cytokines also transcriptionally regulate DMT1, FPN1, and Zip 14 synthesis and activate IRPs (see text). Central to this scheme is the deregulation of iron homeostasis since iron chelators effectively block cell death and prevent early events in neurodegeneration such as neuritic tree shortening (Zhu et al. 2007; Gómez et al. 2010).

9 Conclusion

Diminished activity of mitochondrial complex I, iron accumulation, oxidative stress, and inflammation are pathognomonic signs of sporadic PD. It is possible that the initiation of any one of these processes will initiate and enhance the others through the generation of a positive feedback loop that will produce apoptotic neuronal death. Intervention of this positive loop should result in prolonged life of the affected neurons. Still unanswered is the question of why SN pars compacta neurons are so particular prone to this deregulation.

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References

Aguirre, P., Mena, N., Tapia, V., Arredondo, M., & Núñez, M. T. (2005). Iron homeostasis in neuronal cells: A role for IREG1. *BMC Neuroscience, 6*, 3.

Aguirre, P., Urrutia, P., Tapia, V., Villa, M., Paris, I., et al. (2012). The dopamine metabolite aminochrome inhibits mitochondrial complex I and modifies the expression of iron transporters DMT1 and FPN1. *Biometals, 25*, 795–803.

Andolfo, I., De Falco, L., Asci, R., Russo, R., Colucci, S., et al. (2010). Regulation of divalent metal transporter 1 (DMT1) non-IRE isoform by the microRNA Let-7d in erythroid cells. *Haematologica, 95*, 1244–1252.

Arriagada, C., Paris, I., Sanchez de las Matas, M. J., Martinez-Alvarado, P., Cardenas, S., et al. (2004). On the neurotoxicity mechanism of leukoaminochrome o-semiquinone radical derived from dopamine oxidation: mitochondria damage, necrosis, and hydroxyl radical formation. *Neurobiology of Disease, 16*, 468–477.

Banerjee, R., Starkov, A. A., Beal, M. F., & Thomas, B. (2009). Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis. *Biochimica et Biophysica Acta, 1792*, 651–663.

Barnum, C. J., & Tansey, M. G. (2010). Modeling neuroinflammatory pathogenesis of Parkinson's disease. *Progress in Brain Research, 184*, 113–132.

Bartzokis, G., Sultzer, D., Cummings, J., Holt, L. E., Hance, D. B., et al. (2000). In vivo evaluation of brain iron in Alzheimer disease using magnetic resonance imaging. *Archives of General Psychiatry, 57*, 47–53.

Bautista, J., Corpas, R., Ramos, R., Cremades, O., Gutierrez, J. F., et al. (2000). Brain mitochondrial complex I inactivation by oxidative modification. *Biochemical and Biophysical Research Communications, 275*, 890–894.

Beard, J. L., Wiesinger, J. A., Li, N., & Connor, J. R. (2005). Brain iron uptake in hypotransferrinemic mice: Influence of systemic iron status. *Journal of Neuroscience Research, 79*, 254–261.

Beinert, H., Emptage, M. H., Dreyer, J. L., Scott, R. A., Hahn, J. E., et al. (1983). Iron-sulfur stoichiometry and structure of iron-sulfur clusters in three-iron proteins: Evidence for [3Fe-4S] clusters. *Proceedings of the National Academy of Sciences of the United States of America, 80*, 393–396.

Berg, D., & Youdim, M. B. (2006). Role of iron in neurodegenerative disorders. *Topics in Magnetic Resonance Imaging, 17*, 5–17.

Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., et al. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neuroscience, 3*, 1301–1306.

Blackinton, J., Ahmad, R., Miller, D. W., van der Brug, M. P., Canet-Aviles, R. M., et al. (2005). Effects of DJ-1 mutations and polymorphisms on protein stability and subcellular localization. *Brain Research. Molecular Brain Research, 134*, 76–83.

Boka, G., Anglade, P., Wallach, D., Javoy-Agid, F., Agid, Y., et al. (1994). Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neuroscience Letters, 172*, 151–154.

Boserup, M. W., Lichota, J., Haile, D., & Moos, T. (2011). Heterogenous distribution of ferroportin-containing neurons in mouse brain. *Biometals, 24*, 357–375.

Bouton, C. (1999). Nitrosative and oxidative modulation of iron regulatory proteins. *Cellular and Molecular Life Sciences, 55*, 1043–1053.

Broom, L., Marinova-Mutafchieva, L., Sadeghian, M., Davis, J. B., Medhurst, A. D., et al. (2011). Neuroprotection by the selective iNOS inhibitor GW274150 in a model of Parkinson disease. *Free Radical Biology & Medicine, 50*, 633–640.

Burdo, J. R., Menzies, S. L., Simpson, I. A., Garrick, L. M., Garrick, M. D., et al. (2001). Distribution of divalent metal transporter 1 and metal transport protein 1 in the normal and Belgrade rat. *Journal of Neuroscience Research, 66*, 1198–1207.

Cairo, G., & Recalcati, S. (2007). Iron-regulatory proteins: Molecular biology and pathophysiological implications. *Expert Reviews in Molecular Medicine, 9*, 1–13.

Campillos, M., Cases, I., Hentze, M. W., & Sanchez, M. (2010). SIREs: Searching for iron-responsive elements. *Nucleic Acids Research, 38*, W360–W367.

Castellani, R., Hirai, K., Aliev, G., Drew, K. L., Nunomura, A., et al. (2002). Role of mitochondrial dysfunction in Alzheimer's disease. *Journal of Neuroscience Research, 70*, 357–360.

Chaston, T., Chung, B., Mascarenhas, M., Marks, J., Patel, B., et al. (2008). Evidence for differential effects of hepcidin in macrophages and intestinal epithelial cells. *Gut, 57*, 374–382.

Cheah, J. H., Kim, S. F., Hester, L. D., Clancy, K. W., Patterson, S. E., 3rd, et al. (2006). NMDA receptor-nitric oxide transmission mediates neuronal iron homeostasis via the GTPase Dexras1. *Neuron, 51*, 431–440.

Chinopoulos, C., & Adam-Vizi, V. (2001). Mitochondria deficient in complex I activity are depolarized by hydrogen peroxide in nerve terminals: Relevance to Parkinson's disease. *Journal of Neurochemistry, 76*, 302–306.

Chinta, S. J., & Andersen, J. K. (2006). Reversible inhibition of mitochondrial complex I activity following chronic dopaminergic glutathione depletion in vitro: Implications for Parkinson's disease. *Free Radical Biology & Medicine, 41*, 1442–1448.

Clementi, E., Brown, G. C., Feelisch, M., & Moncada, S. (1998). Persistent inhibition of cell respiration by nitric oxide: Crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 7631–7636.

Cmejla, R., Ptackova, P., Petrak, J., Savvulidi, F., Cerny, J., et al. (2010). Human MRCKalpha is regulated by cellular iron levels and interferes with transferrin iron uptake. *Biochemical and Biophysical Research Communications, 395*, 163–167.

Davis, M. R., Shawron, K. M., Rendina, E., Peterson, S. K., Lucas, E. A., et al. (2011). Hypoxia inducible factor-2 alpha is translationally repressed in response to dietary iron deficiency in Sprague–Dawley rats. *The Journal of Nutrition, 141*, 1590–1596.

De Domenico, I., McVey Ward, D., & Kaplan, J. (2008). Regulation of iron acquisition and storage: Consequences for iron-linked disorders. *Nature Reviews Molecular Cell Biology, 9*, 72–81.

De Domenico, I., Vaughn, M. B., Li, L., Bagley, D., Musci, G., et al. (2006). Ferroportin-mediated mobilization of ferritin iron precedes ferritin degradation by the proteasome. *The EMBO Journal, 25*, 5396–5404.

Devalia, V., Carter, K., Walker, A. P., Perkins, S. J., Worwood, M., et al. (2002). Autosomal dominant reticuloendothelial iron overload associated with a 3-base pair deletion in the ferroportin 1 gene (SLC11A3). *Blood, 100*, 695–697.

Ding, H., Yan, C. Z., Shi, H., Zhao, Y. S., Chang, S. Y., et al. (2011). Hepcidin is involved in iron regulation in the ischemic brain. *PloS One, 6*, e25324.

Double, K. L., Gerlach, M., Schunemann, V., Trautwein, A. X., Zecca, L., et al. (2003). Iron-binding characteristics of neuromelanin of the human substantia nigra. *Biochemical Pharmacology, 66*, 489–494.

Dringen, R., Gutterer, J. M., & Hirrlinger, J. (2000). Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. *European Journal of Biochemistry, 267*, 4912–4916.

Du, F., Qian, C., Qian, Z. M., Wu, X. M., Xie, H., et al. (2011). Hepcidin directly inhibits transferrin receptor 1 expression in astrocytes via a cyclic AMP-protein kinase a pathway. *Glia, 59*, 936–945.

Du, F., Qian, Z. M., Zhu, L., Wu, X. M., Yung, W. H., et al. (2009). L-DOPA neurotoxicity is mediated by up-regulation of DMT1-IRE expression. *PloS One, 4*, e4593.

Faucheux, B. A., Hirsch, E. C., Villares, J., Selimi, F., Mouatt-Prigent, A., et al. (1993). Distribution of 125I-ferrotransferrin binding sites in the mesencephalon of control subjects and patients with Parkinson's disease. *Journal of Neurochemistry, 60*, 2338–2341.

Frazier, M. D., Mamo, L. B., Ghio, A. J., & Turi, J. L. (2011). Hepcidin expression in human airway epithelial cells is regulated by interferon-gamma. *Respiratory Research, 12*, 100.

Gainetdinov, R. R., Fumagalli, F., Jones, S. R., & Caron, M. G. (1997). Dopamine transporter is required for in vivo MPTP neurotoxicity: Evidence from mice lacking the transporter. *Journal of Neurochemistry, 69*, 1322–1325.

Garrick, M. D., & Garrick, L. M. (2009). Cellular iron transport. *Biochimica et Biophysica Acta, 1790*, 309–325.

Gerlach, M., Riederer, P., & Double, K. L. (2008). Neuromelanin-bound ferric iron as an experimental model of dopaminergic neurodegeneration in Parkinson's disease. *Parkinsonism & Related Disorders, 14*(Suppl 2), S185–S188.

Girijashanker, K., He, L., Soleimani, M., Reed, J. M., Li, H., et al. (2008). Slc39a14 gene encodes ZIP14, a metal/bicarbonate symporter: Similarities to the ZIP8 transporter. *Molecular Pharmacology, 73*, 1413–1423.

Gnana-Prakasam, J. P., Martin, P. M., Mysona, B. A., Roon, P., Smith, S. B., et al. (2008). Hepcidin expression in mouse retina and its regulation via lipopolysaccharide/Toll-like receptor-4 pathway independent of Hfe. *The Biochemical Journal, 411*, 79–88.

Gómez, F. J., Aguirre, P., Gonzalez-Billault, C., & Núñez, M. T. (2010). Iron mediates neuritic tree collapse in mesencephalic neurons treated with 1-methyl-4-phenylpyridinium (MPP+). *Journal of Neural Transmission, 118*, 421–431.

Gorell, J. M., Ordidge, R. J., Brown, G. G., Deniau, J. C., Buderer, N. M., et al. (1995). Increased iron-related MRI contrast in the substantia nigra in Parkinson's disease. *Neurology, 45*, 1138–1143.

Graf, E., Mahoney, J. R., Bryant, R. G., & Eaton, J. W. (1984). Iron-catalyzed hydroxyl radical formation. Stringent requirement for free iron coordination site. *The Journal of Biological Chemistry, 259*, 3620–3624.

Gunshin, H., Mackenzie, B., Berger, U. V., Gunshin, Y., Romero, M. F., et al. (1997). Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature, 388*, 482–488.

Guo, B., Phillips, J. D., Yu, Y., & Leibold, E. A. (1995). Iron regulates the intracellular degradation of iron regulatory protein 2 by the proteasome. *The Journal of Biological Chemistry, 270*, 21645–21651.

Haeger, P., Alvarez, A., Leal, N., Adasme, T., Núñez, M. T., et al. (2010). Increased hippocampal expression of the divalent metal transporter 1 (DMT1) mRNA variants 1B and +IRE and DMT1 protein after NMDA-receptor stimulation or spatial memory training. *Neurotoxicity Research, 17*, 238–247.

Han, J., Day, J. R., Connor, J. R., & Beard, J. L. (2003). Gene expression of transferrin and transferrin receptor in brains of control vs. iron-deficient rats. *Nutritional Neuroscience, 6*, 1–10.

Hanson, E. S., Foot, L. M., & Leibold, E. A. (1999). Hypoxia post-translationally activates iron-regulatory protein 2. *The Journal of Biological Chemistry, 274*, 5047–5052.

Harding, C., Heuser, J., & Stahl, P. (1983). Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *The Journal of Cell Biology, 97*, 329–339.

Harrison, P. M., & Arosio, P. (1996). The ferritins: Molecular properties, iron storage function and cellular regulation. *Biochimica et Biophysica Acta, 1275*, 161–203.

Hattingen, E., Magerkurth, J., Pilatus, U., Mozer, A., Seifried, C., et al. (2009). Phosphorus and proton magnetic resonance spectroscopy demonstrates mitochondrial dysfunction in early and advanced Parkinson's disease. *Brain, 132*, 3285–3297.

Hellman, N. E., & Gitlin, J. D. (2002). Ceruloplasmin metabolism and function. *Annual Review of Nutrition, 22*, 439–458.

Herrera, A. J., Castano, A., Venero, J. L., Cano, J., & Machado, A. (2000). The single intranigral injection of LPS as a new model for studying the selective effects of inflammatory reactions on dopaminergic system. *Neurobiology of Disease, 7*, 429–447.

Hidalgo, C., & Núñez, M. T. (2007). Calcium, iron and neuronal function. *IUBMB Life, 59*, 280–285.

Hirsch, E. C., Brandel, J. P., Galle, P., Javoy-Agid, F., & Agid, Y. (1991). Iron and aluminum increase in the substantia nigra of patients with Parkinson's disease: An X-ray microanalysis. *Journal of Neurochemistry, 56*, 446–451.

Hirsch, E. C., Hunot, S., Damier, P., & Faucheux, B. (1998). Glial cells and inflammation in Parkinson's disease: A role in neurodegeneration? *Annals of Neurology, 44*, S115–S120.

Hubert, N., & Hentze, M. W. (2002). Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: Implications for regulation and cellular function. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 12345–12350.

Humphries, K. M., Yoo, Y., & Szweda, L. I. (1998). Inhibition of NADH-linked mitochondrial respiration by 4-hydroxy-2-nonenal. *Biochemistry, 37*, 552–557.

Hunot, S., Brugg, B., Ricard, D., Michel, P. P., Muriel, M. P., et al. (1997). Nuclear translocation of NF-kappaB is increased in dopaminergic neurons of patients with parkinson disease. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 7531–7536.

Jellinger, K. A. (1999). The role of iron in neurodegeneration: Prospects for pharmacotherapy of Parkinson's disease. *Drugs & Aging, 14*, 115–140.

Jenner, P. (1991). Oxidative stress as a cause of Parkinson's disease. *Acta Neurologica Scandinavica. Supplementum, 136*, 6–15.

Jiang, H., Song, N., Xu, H., Zhang, S., Wang, J., et al. (2010). Up-regulation of divalent metal transporter 1 in 6-hydroxydopamine intoxication is IRE/IRP dependent. *Cell Research, 20*, 345–356.

Kakhlon, O., & Cabantchik, Z. I. (2002). The labile iron pool: Characterization, measurement, and participation in cellular processes(1). *Free Radical Biology & Medicine, 33*, 1037–1046.

Kaur, D., Lee, D., Ragapolan, S., & Andersen, J. K. (2009). Glutathione depletion in immortalized midbrain-derived dopaminergic neurons results in increases in the labile iron pool: Implications for Parkinson's disease. *Free Radical Biology & Medicine, 46*, 593–598.

Kaur, D., Yantiri, F., Rajagopalan, S., Kumar, J., Mo, J. Q., et al. (2003). Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: A novel therapy for Parkinson's disease. *Neuron, 37*, 899–909.

Kell, D. B. (2010). Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. *Archives of Toxicology, 84*, 825–889.

Knutson, M. D., Vafa, M. R., Haile, D. J., & Wessling-Resnick, M. (2003). Iron loading and erythrophagocytosis increase ferroportin 1 (FPN1) expression in J774 macrophages. *Blood, 102*, 4191–4197.

Kovtunovych, G., Eckhaus, M. A., Ghosh, M. C., Ollivierre-Wilson, H., & Rouault, T. A. (2010). Dysfunction of the heme recycling system in heme oxygenase 1-deficient mice: Effects on macrophage viability and tissue iron distribution. *Blood, 116*, 6054–6062.

Krause, A., Neitz, S., Magert, H. J., Schulz, A., Forssmann, W. G., et al. (2000). LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Letters, 480*, 147–150.

Kruszewski, M. (2003). Labile iron pool: The main determinant of cellular response to oxidative stress. *Mutation Research, 531*, 81–92.

Langston, J. W., Ballard, P., Tetrud, J. W., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science, 219*, 979–980.

Latunde-Dada, G. O., Xiang, L., Simpson, R. J., & McKie, A. T. (2011). Duodenal cytochrome b (Cybrd 1) and HIF-2alpha expression during acute hypoxic exposure in mice. *European Journal of Nutrition, 50*, 699–704.

LaVaute, T., Smith, S., Cooperman, S., Iwai, K., Land, W., et al. (2001). Targeted deletion of the gene encoding iron regulatory protein-2 causes misregulation of iron metabolism and neurodegenerative disease in mice. *Nature Genetics, 27*, 209–214.

Lee, D. W., Kaur, D., Chinta, S. J., Rajagopalan, S., & Andersen, J. K. (2009). A disruption in iron-sulfur center biogenesis via inhibition of mitochondrial dithiol glutaredoxin 2 may contribute to mitochondrial and cellular iron dysregulation in mammalian glutathione-depleted dopaminergic cells: Implications for Parkinson's disease. *Antioxidants & Redox Signaling, 11*, 2083–2094.

Lee, P., Peng, H., Gelbart, T., Wang, L., & Beutler, E. (2005). Regulation of hepcidin transcription by interleukin-1 and interleukin-6. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 1906–1910.

Li, L., Holscher, C., Chen, B. B., Zhang, Z. F., & Liu, Y. Z. (2011). Hepcidin treatment modulates the expression of Divalent Metal Transporter-1, Ceruloplasmin, and Ferroportin-1 in the Rat Cerebral Cortex and Hippocampus. *Biological Trace Element Research, 143*, 1581–1593.

Lichten, L. A., Liuzzi, J. P., & Cousins, R. J. (2009). Interleukin-1beta contributes via nitric oxide to the upregulation and functional activity of the zinc transporter Zip14 (Slc39a14) in murine hepatocytes. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 296*, G860–G867.

Lill, R., Dutkiewicz, R., Elsasser, H. P., Hausmann, A., Netz, D. J., et al. (2006). Mechanisms of iron-sulfur protein maturation in mitochondria, cytosol and nucleus of eukaryotes. *Biochimica et Biophysica Acta, 1763*, 652–667.

Lill, R., & Muhlenhoff, U. (2008). Maturation of iron-sulfur proteins in eukaryotes: Mechanisms, connected processes, and diseases. *Annual Review of Biochemistry, 77*, 669–700.

Lis, A., Paradkar, P. N., Singleton, S., Kuo, H. C., Garrick, M. D., et al. (2005). Hypoxia induces changes in expression of isoforms of the divalent metal transporter (DMT1) in rat pheochromocytoma (PC12) cells. *Biochemical Pharmacology, 69*, 1647–1655.

Liu, X., & Theil, E. C. (2005). Ferritins: dynamic management of biological iron and oxygen chemistry. *Accounts of Chemical Research, 38*, 167–175.

Liuzzi, J. P., Lichten, L. A., Rivera, S., Blanchard, R. K., Aydemir, T. B., et al. (2005). Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 6843–6848.

Ludwiczek, S., Aigner, E., Theurl, I., & Weiss, G. (2003). Cytokine-mediated regulation of iron transport in human monocytic cells. *Blood, 101*, 4148–4154.

Ludwiczek, S., Theurl, I., Muckenthaler, M. U., Jakab, M., Mair, S. M., et al. (2007). Ca^{2+} channel blockers reverse iron overload by a new mechanism via divalent metal transporter-1. *Nature Medicine, 13*, 448–454.

Lv, Z., Jiang, H., Xu, H., Song, N., & Xie, J. (2011). Increased iron levels correlate with the selective nigral dopaminergic neuron degeneration in Parkinson's disease. *Journal of Neural Transmission, 118*, 361–369.

Marques, F., Falcao, A. M., Sousa, J. C., Coppola, G., Geschwind, D., et al. (2009). Altered iron metabolism is part of the choroid plexus response to peripheral inflammation. *Endocrinology, 150*, 2822–2828.

Martelli, A., Wattenhofer-Donze, M., Schmucker, S., Bouvet, S., Reutenauer, L., et al. (2007). Frataxin is essential for extramitochondrial Fe-S cluster proteins in mammalian tissues. *Human Molecular Genetics, 16*, 2651–2658.

Martin, I., Dawson, V. L., & Dawson, T. M. (2011). Recent advances in the genetics of Parkinson's disease. *Annual Review of Genomics and Human Genetics, 12*, 301–325.

Martins, E. A., Robalinho, R. L., & Meneghini, R. (1995). Oxidative stress induces activation of a cytosolic protein responsible for control of iron uptake. *Archives of Biochemistry and Biophysics, 316*, 128–134.

McGeer, P. L., Itagaki, S., Boyes, B. E., & McGeer, E. G. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology, 38*, 1285–1291.

McKie, A. T., Barrow, D., Latunde-Dada, G. O., Rolfs, A., Sager, G., et al. (2001). An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science, 291*, 1755–1759.

McKie, A. T., Marciani, P., Rolfs, A., Brennan, K., Wehr, K., et al. (2000). A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Molecular Cell, 5*, 299–309.

Mehlhase, J., Sandig, G., Pantopoulos, K., & Grune, T. (2005). Oxidation-induced ferritin turnover in microglial cells: Role of proteasome. *Free Radical Biology & Medicine, 38*, 276–285.

Meister, A., & Anderson, M. E. (1983). Glutathione. *Annual Review of Biochemistry, 52*, 711–760.

Mena, N. P., Bulteau, A. L., Salazar, J., Hirsch, E. C., & Núñez, M. T. (2011). Effect of mitochondrial complex I inhibition on Fe-S cluster protein activity. *Biochemical and Biophysical Research Communications, 409*, 241–246.

Meyron-Holtz, E. G., Ghosh, M. C., Iwai, K., LaVaute, T., Brazzolotto, X., et al. (2004). Genetic ablations of iron regulatory proteins 1 and 2 reveal why iron regulatory protein 2 dominates iron homeostasis. *The EMBO Journal, 23*, 386–395.

Mizuno, Y., Sone, N., & Saitoh, T. (1987). Effects of 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine and 1-methyl-4-phenylpyridinium ion on activities of the enzymes in the electron transport system in mouse brain. *Journal of Neurochemistry, 48*, 1787–1793.

Mogi, M., Harada, M., Kondo, T., Riederer, P., Inagaki, H., et al. (1994). Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. *Neuroscience Letters, 180*, 147–150.

Moos, T., & Morgan, E. H. (1998). Evidence for low molecular weight, non-transferrin-bound iron in rat brain and cerebrospinal fluid. *Journal of Neuroscience Research, 54*, 486–494.

Moos, T., & Rosengren, N. T. (2006). Ferroportin in the postnatal rat brain: implications for axonal transport and neuronal export of iron. *Seminars in Pediatric Neurology, 13*, 149–157.

Moos, T., Rosengren Nielsen, T., Skjorringe, T., & Morgan, E. H. (2007). Iron trafficking inside the brain. *Journal of Neurochemistry, 103*, 1730–1740.

Mulero, V., & Brock, J. H. (1999). Regulation of iron metabolism in murine J774 macrophages: role of nitric oxide-dependent and -independent pathways following activation with gamma interferon and lipopolysaccharide. *Blood, 94*, 2383–2389.

Muller, T., Blum-Degen, D., Przuntek, H., & Kuhn, W. (1998). Interleukin-6 levels in cerebrospinal fluid inversely correlate to severity of Parkinson's disease. *Acta Neurologica Scandinavica, 98*, 142–144.

Munch, G., Luth, H. J., Wong, A., Arendt, T., Hirsch, E., et al. (2000). Crosslinking of alpha-synuclein by advanced glycation endproducts–an early pathophysiological step in Lewy body formation? *Journal of Chemical Neuroanatomy, 20*, 253–257.

Nagatsu, T. (2002). Parkinson's disease: Changes in apoptosis-related factors suggesting possible gene therapy. *Journal of Neural Transmission, 109*, 731–745.

Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. B., Donovan, A., et al. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science, 306*, 2090–2093.

Nicolas, G., Bennoun, M., Devaux, I., Beaumont, C., Grandchamp, B., et al. (2001). Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 8780–8785.

Núñez, M. T., Gallardo, V., Munoz, P., Tapia, V., Esparza, A., et al. (2004). Progressive iron accumulation induces a biphasic change in the glutathione content of neuroblastoma cells. *Free Radical Biology & Medicine, 37*, 953–960.

Núñez, M. T., Urrutia, P., Mena, N. P., Aguirre, P., Tapia, V., & Salazar, J. (2012). Iron toxicity in neurodegeneration. *Biometals, 25*, 761–776.

Ohgami, R. S., Campagna, D. R., Greer, E. L., Antiochos, B., McDonald, A., et al. (2005). Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. *Nature Genetics, 37*, 1264–1269.

Ohgami, R. S., Campagna, D. R., McDonald, A., & Fleming, M. D. (2006). The Steap proteins are metalloreductases. *Blood, 108*, 1388–1394.

Paradkar, P. N., & Roth, J. A. (2006a). Nitric oxide transcriptionally down-regulates specific isoforms of divalent metal transporter (DMT1) via NF-kappaB. *Journal of Neurochemistry, 96*, 1768–1777.

Paradkar, P. N., & Roth, J. A. (2006b). Post-translational and transcriptional regulation of DMT1 during P19 embryonic carcinoma cell differentiation by retinoic acid. *The Biochemical Journal, 394*, 173–183.

Patel, S. A., & Simon, M. C. (2008). Biology of hypoxia-inducible factor-2alpha in development and disease. *Cell Death and Differentiation, 15*, 628–634.

Pelizzoni, I., Macco, R., Morini, M. F., Zacchetti, D., Grohovaz, F., et al. (2011). Iron handling in hippocampal neurons: Activity-dependent iron entry and mitochondria-mediated neurotoxicity. *Aging Cell, 10*, 172–183.

Perry, G., Taddeo, M. A., Petersen, R. B., Castellani, R. J., Harris, P. L., et al. (2003). Adventitiously-bound redox active iron and copper are at the center of oxidative damage in Alzheimer disease. *Biometals, 16*, 77–81.

Perry, T. L., Godin, D. V., & Hansen, S. (1982). Parkinson's disease: A disorder due to nigral glutathione deficiency? *Neuroscience Letters, 33*, 305–310.

Peyssonnaux, C., Zinkernagel, A. S., Schuepbach, R. A., Rankin, E., Vaulont, S., et al. (2007). Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). *The Journal of Clinical Investigation, 117*, 1926–1932.

Pigeon, C., Ilyin, G., Courselaud, B., Leroyer, P., Turlin, B., et al. (2001). A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *The Journal of Biological Chemistry, 276*, 7811–7819.

Pinto, J. P., Ribeiro, S., Pontes, H., Thowfeequ, S., Tosh, D., et al. (2008). Erythropoietin mediates hepcidin expression in hepatocytes through EPOR signaling and regulation of C/EBPalpha. *Blood, 111*, 5727–5733.

Qian, Z. M., Wu, X. M., Fan, M., Yang, L., Du, F., et al. (2011). Divalent metal transporter 1 is a hypoxia-inducible gene. *Journal of Cellular Physiology, 226*, 1596–1603.

Remor, A. P., de Matos, F. J., Ghisoni, K., da Silva, T. L., Eidt, G., et al. (2011). Differential effects of insulin on peripheral diabetes-related changes in mitochondrial bioenergetics: Involvement of advanced glycosylated end products. *Biochimica et Biophysica Acta, 1812*, 1460–1471.

Rouault, T. A., Zhang, D. L., & Jeong, S. Y. (2009). Brain iron homeostasis, the choroid plexus, and localization of iron transport proteins. *Metabolic Brain Disease, 24*, 673–684.

Salazar, J., Mena, N., Hunot, S., Prigent, A., Alvarez-Fischer, D., et al. (2008). Divalent metal transporter 1 (DMT1) contributes to neurodegeneration in animal models of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 18578–18583.

Salgado, J. C., Olivera-Nappa, A., Gerdtzen, Z. P., Tapia, V., Theil, E. C., et al. (2010). Mathematical modeling of the dynamic storage of iron in ferritin. *BMC Systems Biology, 4*, 147.

Sanchez, M., Galy, B., Schwanhaeusser, B., Blake, J., Bahr-Ivacevic, T., et al. (2011). Iron regulatory protein-1 and −2: transcriptome-wide definition of binding mRNAs and shaping of the cellular proteome by IRPs. *Blood, 118*, e168–e179.

Sayre, L. M., Perry, G., Atwood, C. S., & Smith, M. A. (2000). The role of metals in neurodegenerative diseases. *Cellular and Molecular Biology (Noisy-le-Grand, France), 46*, 731–741.

Schafer, F. Q., & Buettner, G. R. (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radical Biology & Medicine, 30*, 1191–1212.

Schalinske, K. L., & Eisenstein, R. S. (1996). Phosphorylation and activation of both iron regulatory proteins 1 and 2 in HL-60 cells. *The Journal of Biological Chemistry, 271*, 7168–7176.

Schapira, A. H. (2010). Complex I: Inhibitors, inhibition and neurodegeneration. *Experimental Neurology, 224*, 331–335.

Schapira, A. H., Cooper, J. M., Dexter, D., Clark, J. B., Jenner, P., et al. (1990). Mitochondrial complex I deficiency in Parkinson's disease. *Journal of Neurochemistry, 54*, 823–827.

Schapira, A. H., & Gegg, M. (2011). Mitochondrial contribution to Parkinson's disease pathogenesis. *Parkinsons Disease, 2011*, 159160.

Scotcher, K. P., Irwin, I., DeLanney, L. E., Langston, J. W., & Di Monte, D. (1990). Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-phenylpyridinium ion on ATP levels of mouse brain synaptosomes. *Journal of Neurochemistry, 54*, 1295–1301.

Segura-Aguilar, J., Metodiewa, D., & Welch, C. J. (1998). Metabolic activation of dopamine o-quinones to o-semiquinones by NADPH cytochrome P450 reductase may play an important role in oxidative stress and apoptotic effects. *Biochimica et Biophysica Acta, 1381*, 1–6.

Shachar, D. B., Kahana, N., Kampel, V., Warshawsky, A., & Youdim, M. B. (2004). Neuroprotection by a novel brain permeable iron chelator, VK-28, against 6-hydroxydopamine lession in rats. *Neuropharmacology, 46*, 254–263.

Shaw, G. C., Cope, J. J., Li, L., Corson, K., Hersey, C., et al. (2006). Mitoferrin is essential for erythroid iron assimilation. *Nature, 440*, 96–100.

Sheftel, A., Stehling, O., & Lill, R. (2010). Iron-sulfur proteins in health and disease. *Trends in Endocrinology and Metabolism, 21*, 302–314.

Sian, J., Dexter, D. T., Lees, A. J., Daniel, S., Agid, Y., et al. (1994). Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Annals of Neurology, 36*, 348–355.

Sims, N. R., Anderson, M. F., Hobbs, L. M., Kong, J. Y., Phillips, S., et al. (2000). Impairment of brain mitochondrial function by hydrogen peroxide. *Brain Research. Molecular Brain Research, 77*, 176–184.

Snyder, A. M., & Connor, J. R. (2009). Iron, the substantia nigra and related neurological disorders. *Biochimica et Biophysica Acta, 1790*, 606–614.

Sofic, E., Riederer, P., Heinsen, H., Beckmann, H., Reynolds, G. P., et al. (1988). Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. *Journal of Neural Transmission, 74*, 199–205.

Song, N., Wang, J., Jiang, H., & Xie, J. (2010). Ferroportin 1 but not hephaestin contributes to iron accumulation in a cell model of Parkinson's disease. *Free Radical Biology & Medicine, 48*, 332–341.

Symons, M. C. R., & Gutteridge, J. M. C. (1998). *Free radicals and iron: Chemistry, biology, and medicine*. Oxford/New York: Oxford University Press.

Tacchini, L., Bianchi, L., Bernelli-Zazzera, A., & Cairo, G. (1999). Transferrin receptor induction by hypoxia. HIF-1-mediated transcriptional activation and cell-specific post-transcriptional regulation. *The Journal of Biological Chemistry, 274*, 24142–24146.

Tacchini, L., Gammella, E., De Ponti, C., Recalcati, S., & Cairo, G. (2008). Role of HIF-1 and NF-kappaB transcription factors in the modulation of transferrin receptor by inflammatory and anti-inflammatory signals. *The Journal of Biological Chemistry, 283*, 20674–20686.

Tretter, L., Sipos, I., & Adam-Vizi, V. (2004). Initiation of neuronal damage by complex I deficiency and oxidative stress in Parkinson's disease. *Neurochemical Research, 29*, 569–577.

Tulpule, K., Robinson, S. R., Bishop, G. M., & Dringen, R. (2010). Uptake of ferrous iron by cultured rat astrocytes. *Journal of Neuroscience Research, 88*, 563–571.

Unciuleac, M., Warkentin, E., Page, C. C., Boll, M., & Ermler, U. (2004). Structure of a xanthine oxidase-related 4-hydroxybenzoyl-CoA reductase with an additional [4Fe-4S] cluster and an inverted electron flow. *Structure, 12*, 2249–2256.

Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M., Harvey, K., et al. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science, 304*, 1158–1160.

Vargas, J. D., Herpers, B., McKie, A. T., Gledhill, S., McDonnell, J., et al. (2003). Stromal cell-derived receptor 2 and cytochrome b561 are functional ferric reductases. *Biochimica et Biophysica Acta, 1651*, 116–123.

Vulpe, C. D., Kuo, Y. M., Murphy, T. L., Cowley, L., Askwith, C., et al. (1999). Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nature Genetics, 21*, 195–199.

Vymazal, J., Righini, A., Brooks, R. A., Canesi, M., Mariani, C., et al. (1999). T1 and T2 in the brain of healthy subjects, patients with Parkinson disease, and patients with multiple system atrophy: relation to iron content. *Radiology, 211*, 489–495.

Wallander, M. L., Leibold, E. A., & Eisenstein, R. S. (2006). Molecular control of vertebrate iron homeostasis by iron regulatory proteins. *Biochimica et Biophysica Acta, 1763*, 668–689.

Wang, D., Wang, L. H., Zhao, Y., Lu, Y. P., & Zhu, L. (2010a). Hypoxia regulates the ferrous iron uptake and reactive oxygen species level via divalent metal transporter 1 (DMT1) Exon1B by hypoxia-inducible factor-1. *IUBMB Life, 62*, 629–636.

Wang, S. M., Fu, L. J., Duan, X. L., Crooks, D. R., Yu, P., et al. (2010b). Role of hepcidin in murine brain iron metabolism. *Cellular and Molecular Life Sciences, 67*, 123–133.

Wang, J., & Pantopoulos, K. (2011). Regulation of cellular iron metabolism. *The Biochemical Journal, 434*, 365–381.

Wang, Q., Du, F., Qian, Z. M., Ge, X. H., Zhu, L., et al. (2008). Lipopolysaccharide induces a significant increase in expression of iron regulatory hormone hepcidin in the cortex and substantia nigra in rat brain. *Endocrinology, 149*, 3920–3925.

Wang, X., Garrick, M. D., Yang, F., Dailey, L. A., Piantadosi, C. A., et al. (2005). TNF, IFN-gamma, and endotoxin increase expression of DMT1 in bronchial epithelial cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 289*, L24–L33.

Wardrop, S. L., & Richardson, D. R. (2000). Interferon-gamma and lipopolysaccharide regulate the expression of Nramp2 and increase the uptake of iron from low relative molecular mass complexes by macrophages. *European Journal of Biochemistry, 267*, 6586–6593.

Weinreb, O., Mandel, S., Bar-Am, O., & Amit, T. (2011). Iron-chelating backbone coupled with monoamine oxidase inhibitory moiety as novel pluripotential therapeutic agents for Alzheimer's disease: A tribute to Moussa Youdim. *Journal of Neural Transmission, 118*, 479–492.

Williams, K., Wilson, M. A., & Bressler, J. (2000). Regulation and developmental expression of the divalent metal-ion transporter in the rat brain. *Cellular and Molecular Biology (Noisy-le-Grand, France), 46*, 563–571.

Wilson, R. B. (2006). Iron dysregulation in Friedreich ataxia. *Seminars in Pediatric Neurology, 13*, 166–175.

Wong, S. S., Li, R. H., & Stadlin, A. (1999). Oxidative stress induced by MPTP and MPP(+): Selective vulnerability of cultured mouse astrocytes. *Brain Research, 836*, 237–244.

Wright, R. O., & Baccarelli, A. (2007). Metals and neurotoxicology. *The Journal of Nutrition, 137*, 2809–2813.

Wrighting, D. M., & Andrews, N. C. (2006). Interleukin-6 induces hepcidin expression through STAT3. *Blood, 108*, 3204–3209.

Yang, F., Liu, X. B., Quinones, M., Melby, P. C., Ghio, A., et al. (2002). Regulation of reticuloendothelial iron transporter MTP1 (Slc11a3) by inflammation. *The Journal of Biological Chemistry, 277*, 39786–39791.

Ye, H., & Rouault, T. A. (2010). Erythropoiesis and iron sulfur cluster biogenesis. *Advances in Hematology*, *2010*.

Youdim, M. B., Ben-Shachar, D., & Riederer, P. (1989). Is Parkinson's disease a progressive siderosis of substantia nigra resulting in iron and melanin induced neurodegeneration? *Acta Neurologica Scandinavica. Supplementum, 126*, 47–54.

Youdim, M. B., & Buccafusco, J. J. (2005). Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. *Trends in Pharmacological Sciences, 26*, 27–35.

Youdim, M. B., Stephenson, G., & Ben, S. D. (2004). Ironing iron out in Parkinson's disease and other neurodegenerative diseases with iron chelators: A lesson from 6-hydroxydopamine and iron chelators, desferal and VK-28. *Annals of the New York Academy of Sciences, 1012*, 306–325.

Zecca, L., Tampellini, D., Gatti, A., Crippa, R., Eisner, M., et al. (2002). The neuromelanin of human substantia nigra and its interaction with metals. *Journal of Neural Transmission, 109*, 663–672.

Zecca, L., Youdim, M. B., Riederer, P., Connor, J. R., & Crichton, R. R. (2004). Iron, brain ageing and neurodegenerative disorders. *Nature Reviews Neuroscience, 5*, 863–873.

Zechel, S., Huber-Wittmer, K., & von Bohlen und Halbach, O. (2006). Distribution of the iron-regulating protein hepcidin in the murine central nervous system. *Journal of Neuroscience Research, 84*, 790–800.

Zhang, D. L., Hughes, R. M., Ollivierre-Wilson, H., Ghosh, M. C., & Rouault, T. A. (2009). A ferroportin transcript that lacks an iron-responsive element enables duodenal and erythroid precursor cells to evade translational repression. *Cell Metabolism, 9*, 461–473.

Zhang, Y., Marcillat, O., Giulivi, C., Ernster, L., & Davies, K. J. (1990). The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *The Journal of Biological Chemistry, 265*, 16330–16336.

Zheng, H., Youdim, M. B., & Fridkin, M. (2010). Site-activated chelators targeting acetylcholinesterase and monoamine oxidase for Alzheimer's therapy. *ACS Chemical Biology, 5*, 603–610.

Zhu, W., Xie, W., Pan, T., Xu, P., Fridkin, M., et al. (2007). Prevention and restoration of lactacystin-induced nigrostriatal dopamine neuron degeneration by novel brain-permeable iron chelators. *The FASEB Journal, 21*, 3835–3844.

Zoccarato, F., Toscano, P., & Alexandre, A. (2005). Dopamine-derived dopaminochrome promotes H(2)O(2) release at mitochondrial complex I: Stimulation by rotenone, control by Ca(2+), and relevance to Parkinson disease. *The Journal of Biological Chemistry, 280*, 15587–15594.

Links Between Paraquat and Parkinson's Disease

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Contents

Abstract

Parkinson's disease (PD) is currently regarded as the most common degenerative disorder of the aging brain after Alzheimer's dementia. Much progress has been made in identifying the genes involved in familial, or inherited, PD. However,

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the majority of cases are sporadic (not inherited) and their origin(s) still remain largely undetermined. The environment is a key contributor to human health and disease. Epidemiological evidence suggests that environmental factors play a role in the etiology of neurodegenerative diseases. Particularly, paraquat (PQ) has been largely demonstrated to induce cell death in a variety of cell types and tissues associated with PD. The study of PQ-induced neurotoxicity has provided valuable insight into the mechanisms regulating neuronal cell death by environmental toxicants. However, to date, the molecular mechanisms involved in neuronal cell death by PQ have not been completely identified. This article presents a comprehensive review of the published epidemiologic and toxicologic literature and critically evaluates whether a relationship exists between PQ exposure and PD.

List of Abbreviations

ARE	Antioxidant responsive element
ASK1	Apoptosis signal-regulating kinase 1
CMA	Chaperone-mediated autophagy
DA	Dopamine
DOPAC	3,4-Dihydroxyphenylacetic acid
DβHB	D-β-hydroxybutyrate
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
GSH	Glutathione
HVA	Homovanillic acid
IRE1	Inositol-requiring enzyme 1
JNK	c-Jun NH_2-terminal kinase
LAMP-2A	Lysosomal-associated membrane protein-2A
LD	Lethal dose
MAO-B	Monoamine oxidase B
Mn^{2+}-EB-DTC	Mn^{2+}-ethylene-bis-dithiocarbamate
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
PD	Parkinson's disease
PEG 400	Polyethylene glycol 400
PKC	Protein kinase C
PQ	Paraquat
REP1	Dinucleotide repeat sequence
ROS	Reactive oxygen species
SAPK	Stress-activated protein kinase
SOD	Superoxide dismutase
TH	Tyrosine hydroxylase
Trx	Thioredoxin
WT	Wild-type

1 Introduction

Paraquat (PQ, methyl viologen), 1,1′-dimethyl-4,4′-bipyridinium (Fig. 1), is a widely used potent herbicide. It was first synthesized in 1882 by Weidel and Russo, as recorded by Haley in his review (Haley 1979). Its redox properties were discovered by Michaelis and Hill in 1933 (Haley 1979). At first, it was used as an indicator of oxidation reduction, since, in the absence of molecular oxygen, donating an electron to PQ ($PQ2^+$) generated a monocationically stable violet or blue form, and is, thus, commonly known as methyl viologen (Dinis-Oliveira et al. 2008). But its properties as a herbicide were not discovered until 1955 and, some years later, in 1962, it was actively introduced to the global markets. PQ is registered and used in approximately 100 countries worldwide, being the second most widely used herbicide in the world after glyphosate. Despite this, today, its use is banned in the European Union (EU), unlike the import of products from outside the EU that have been treated with PQ. In its recommended rating of "pesticides by risk," the OMS (World Health Organization) considers composite PQ to be moderately toxic (Category II). The ECB (European Chemicals Bureau) classifies PQ by the route of exposure, so it is considered very toxic (R26) by inhalation, toxic (R25) orally, and moderately toxic (R24) dermally. PQ is included in the family of herbicides known as bipyridines. It is a contact herbicide, non-selective, systemic, and does not act on the leaves of green plants. Among its advantages, it is rapidly absorbed by the leaves of plants that are sprayed, exerting its effect very quickly, as the clay soil, which makes it biologically inactive.

There is controversy surrounding the use of PQ in agriculture because, even though its herbicidal properties are excellent, otherwise, it has clear toxicity to humans and the environment, especially proper precautions are not taken. In this sense, the structural similarity between PQ and the active metabolite of the neurotoxin called MPTP (MPP^+, Fig. 1), widely accepted as a model of parkinsonism, together with some correlations observed in epidemiological studies between the use of PQ and the development of Parkinson's disease (PD) (Tanner et al. 2011), led to the postulation of the existence of a relationship between the pesticide and the origin of the disease (Costello et al. 2009; Di Monte et al. 1986; Hertzman et al. 1990;

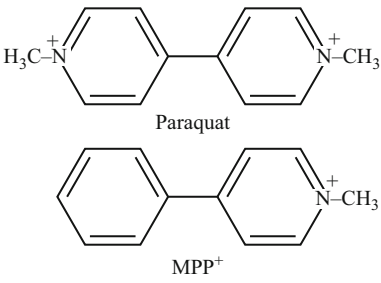

Fig. 1 Chemical structures of PQ and MPP^+

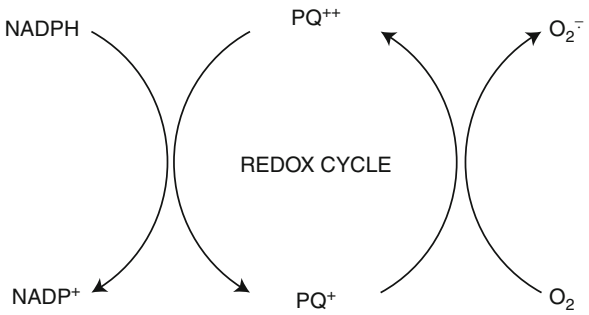

Fig. 2 Redox cycle of PQ

Liou et al. 1997). Both show neurotoxic effects based on the generation of oxidative stress, but by different routes (Richardson et al. 2005). PQ is currently considered as a valid model for studying neurotoxicity based on oxidative stress, as in the case of MPP^+, and investigations are ongoing concerning the relationship that may exist between application and exposure to this pesticide and the development of PD, which is a widely accepted fact in the case of MPP^+, leading to an accumulation of very strong and important evidence in the case of PQ, oxidative stress, and cell death. The same active ingredient that makes PQ a perfect herbicide also makes it a perfect toxic for any mammalian cell. The redox cycling of PQ (Fig. 2) on biological systems has two important implications: one is the generation of reactive oxygen species (ROS) and the other is the depletion of reducing equivalents (NADH, NADPH, etc.), which are necessary for the proper functioning thereof, affecting different cellular processes, such as the synthesis of fatty acids. Like inside plant cells, within the model's neurons, PQ needs a donor of electrons in order to be reduced. The potential standard reduction (E°) of a compound indicates the affinity of this structure to accept electrons. PQ has an E° of -0.45 V. The potential E° of the redox couples NAD^+/NADH and $NADP^+$/NADPH are -0.32 V and -0.324 V, respectively, which means that PQ, under physiological conditions and with the aid of diaphorase cells, could accept electrons from either of the reducing agents. The potential E° of MPP^+ is -1.18 V, which indicates that PQ has a greater ability to accept electrons than MPP^+ (Drechsel and Patel 2008). Among the cellular enzymes that can give electrons to PQ (PQ enzymes with diaphorase), those that can be highlighted are the following: mitochondrial complex I (NADH: ubiquinone reductase complex) (Fukushima et al. 1993), thioredoxin reductase (Gray et al. 2007), NADPH:ferredoxin oxidoreductase (Liochev et al. 1994), NADPH oxidase (Bonneh-Barkay et al. 2005), and NOS (Patel et al. 1996), as well as others. The mitochondria, therefore, becomes a major source of ROS generation within the PQ-induced mechanism, which may induce PQ-diaphorase activity at one or more points during the breathing cycle (Drechsel and Patel 2008). Once the PQ has been reduced, it can then be oxidized by a molecule of oxygen and generate a superoxide molecule ($O_2^{\bullet-}$), which occurs in cell oxidative stress and will switch to different routes that trigger cell damage in different components and

initiate the activation of different cellular mechanisms, such as apoptosis or autophagy. Apoptosis induced by PQ has been demonstrated to involve mainly the intrinsic mitochondrial pathway. The intrinsic pathway of apoptosis, also referred to as the mitochondrial pathway, is activated by a wide variety of cytotoxic stimuli or environmental stressors. Although the mechanisms by which these stimuli trigger apoptosis differ between them, they convey the release of pro-apoptotic proteins from the mitochondria, including cytochrome c. However, the exact mechanisms mediating cytochrome c release are still a matter of debate (Grimm and Brdiczka 2007; Ravagnan et al. 2002). In PD, cell death by apoptosis has been proposed to result from mitochondrial dysfunction, leading to an increase in oxidative stress and a decline in ATP production. PQ-induced cytotoxicity has been suggested to be mediated through the extrinsic pathway of apoptosis by activation of the Fas receptor (Vogt et al. 1998), but its relevance to the progression of PD remains unclear. On the other hand, in recent years, converging evidence suggests that the impairment of homeostasis mechanisms processing unwanted and misfolded proteins plays a central role in the pathogenesis of PD (Olanow 2007). Impairment of the autophagy-lysosomal pathway was related to the development of PD (Pan et al. 2008). The activation of autophagy was also identified within peripheral blood mononuclear cells from PD patients (Prigione et al. 2010). In this sense, PQ is able to also induce autophagy in dopaminergic cellular models (González-Polo et al. 2007b). Therefore, PQ is considered as one of the most useful models for studying neurotoxicity based on the generation of oxidative stress (such as PD), playing a key and fundamental role in the production of superoxide anions in the redox cycling of the herbicide, which can induce both apoptosis and autophagy processes.

2 Epidemiologic Evidence

As evidence emerges that several genes are involved in the pathogenesis of PD, the role of environmental chemicals in the etiology of this disease has become intensely debated. Many chemicals that are toxicologically or structurally similar to MPTP, an agent known to induce Parkinsonian symptoms in humans (Langston and Ballard 1983), could be a risk factor for PD. Several studies reported increased risk associated with exposure to either insecticides or herbicides (Butterfield et al. 1993; Firestone et al. 2005; Gorell et al. 2004; Semchuk et al. 1992) and organochloride, organophosphate, and carbamate insecticides (Seidler et al. 1996). The relationship between exposure duration and PD risk was investigated in several studies and showed a positive correlation with the duration of exposure to, and high doses of, herbicides and insecticides (Brown et al. 2006; Seidler et al. 1996). In these studies, PQ exposure was shown to be significantly associated with PD (Hertzman et al. 1990; Liou et al. 1997). A case–control study of environmental risk factors and PD in Taiwan reported evidence of a strong correlation between PD incidence and the level of PQ exposure (Liou et al. 1997). Another report from Dinis-Oliveira and coworkers showed that

occupational exposure to PQ was associated with parkinsonism in a study in British Columbia (Dinis-Oliveira et al. 2006).

PQ has been shown to interact synergistically with other pesticides and insecticides. It has been reported that exposure to a combination of maneb (a dithiocarbamate fungicide) and PQ increases PD risk, particularly in younger subjects and/or when exposure occurs at younger ages (Costello et al. 2009; Thiruchelvam et al. 2000). Combined exposure of rotenone and PQ has been linked experimentally to pathophysiological mechanisms implicated in human PD (Tanner et al. 2011). Another study in Central Valley, California, that combined ambient exposure to ziram and PQ as well as combined ambient exposure to maneb and PQ at both workplaces and places of residence showed substantially increased risk of PD. Those exposed to ziram, maneb, and PQ together experienced the greatest increase in PD risk (Wang et al. 2011).

Different groups of pesticides have also shown positive associations with PD. Case reports and post-mortem studies have described PD in persons exposed to organophosphate insecticides; organochlorine insecticides, including dieldrin; herbicides, including glyphosate and diquat; fungicides, including maneb and other dithiocarbamates (Corrigan et al. 1998; Firestone et al. 2005; Fleming et al. 1994; Kamel and Hoppin 2004; Wechsler et al. 1991). However, other studies have not found a significant association, although PD risk was still elevated (Firestone et al. 2005; Hertzman et al. 1990; Kamel and Hoppin 2004; Li et al. 2005a; Wirdefeldt et al. 2011). In addition, potential confounding factors include age, well-water consumption, farming, rural living, head trauma, smoking and caffeine intake, and infectious diseases such as nocardiosis, as all of these have been suggested to affect the incidence of PD (Berry et al. 2010). The Agricultural Health Study carried out a cohort investigation of over 79,500 agricultural workers from North Carolina and Iowa. The researchers considered various exposures, including acute poisoning episodes and chronic exposures, and took account of several confounding factors and the different farming practices. They found that the prevalence of neurological symptoms was associated with cumulative life-time exposure to pesticides, notably, organophosphates and organochlorines, and that this prevalence was mainly associated with chronic moderate exposure (Berry et al. 2010; Kamel et al. 2007).

3 Relation Between Environmental and Genetic Factors in Parkinson's Disease: The Paraquat and PARK Genes Connection

The etiology of PD is unknown, but it likely has a multifactorial origin, involving both genetic and environmental factors, such as exposure to PQ. The interaction of both factors is possibly responsible for the selective death of dopaminergic neurons observed in this disease. Outside of the studies that have identified human mutations as a basis for disease, the high number of individuals with sporadic PD have an unknown etiology. Although this interaction is not clear, there exist many studies with results that indicate the direct interaction of PQ with PARK genes (Table 1).

Table 1 Summary of genetic ("PARK") loci associated with a monogenetic form of PD

Gene	Locus	Protein name	Inheritance	Function
PARK 1/4	4q21.3-q22	α-synuclein	AD	LB component
PARK 2	6q25-27	Parkin	AR	E3 ubiquitin ligase
PARK 3	2p13	¿?	AD	¿?
PARK 5	4p14	UCHL-1	AD	Ubiquitin C-terminal hydrolase
PARK 6	1p35-36	PINK1	AR	Mitochondrial kinase
PARK 7	1p36	DJ-1	AR	Antioxidant protein
PARK 8	12q12	LRRK2	AD	Kinase, GTPase
PARK 9	1p36	ATP13A2	AR	ATPase, cationic transport
PARK 10	1p32	¿?	AD	¿?
PARK 11	2q36-q37	GIGYF2	AD	Receptor tyrosine kinase signaling
PARK 12	Xq21-q25	¿?	X-linked	¿?
PARK 13	2p13	HtrA2/Omi	AD	Serine protease
PARK 14	22q13.1	PLA2G6	AR	Phospholipase A2
PARK 15	22q11.2	FBXO7	AR	E3 ubiquitin ligase
PARK 16	1q32	RAB7L1	¿?	¿?
PARK 17	4p	VPS35 (2011)	¿?	¿?
PARK 18	6p	EIF4G1 (2011)	¿?	¿?

¿? - unknown

One of the most important genes related to PD is *PARK 1/4*, which codifies to α-synuclein and plays an important role in the LB formation, and could establish interactions with toxicants. In this sense, in 2001, Vladimir N. Uversky and colleagues (2001) showed how the presence of PQ produced the fibril formation of recombinant α-synuclein in vitro, being the first approach that linked pesticides with PD. Interestingly, this fact is accelerated in a dose-dependent manner and the exposure to PQ increased the α-synuclein levels in the ventral mesencephalon and frontal cortex of mice (Manning-Bog et al. 2002). Moreover, that laboratory provided evidence that the α-synuclein overexpression protects front dopaminergic cell PQ-dependent degeneration in transgenic mice, for both human WT α-synuclein and human Ala53Thr mutant α-synuclein (Manning-Bog et al. 2003). Furthermore, the PQ exposure leads to the nuclear localization of α-synuclein in mice midbrain, and it co-localizes with histone H3, and this event could be a mechanism to decrease the pool of free histones (Goers et al. 2003). Under macromolecular crowding conditions, the presence of PQ accelerates the α-synuclein fibrillation compared with only the addition of polymers, such as PEG 400 (Munishkina et al. 2004). In an in vivo study, Thiruchelvam and colleagues (Thiruchelvam et al. 2004) treated male mice over a period of 7 weeks with combined PQ and Mn^{2+}-ethylene-bis-dithiocarbamate (Mn^{2+}-EB-DTC), observing that the mice which expressed human doubly-mutated α-synuclein (A53T and A30P) presented a significant reduction in locomotor activity, dopamine (DA) levels, and TH+ neurons, as well as small decrease in

3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels, but the DA turnover was elevated relative to saline counterparts. In HEK293 cells, G209A mutant α-synuclein expressing cells were partially protected from the PQ oxidative stress, but less than that for WT α-synuclein cells (Orth et al. 2004). On the other hand, a report based in the nematode *C. elegans* (Ved et al. 2005) demonstrated that WT α-synuclein and A53T mutant showed no difference in terms of sensitivity to PQ compared with the nontransgenic line, and the co-treatment with PQ and probucol increased the survival levels, but not when using D-β-hydroxybutyrate (DβHB), indicating that the free radical generation contributes to PQ toxicity, and, in this model, it is independent of the complex I inhibition. In astrocytes, the expression of PEP-1–SOD fusion protein with Tat-α-synuclein increased the cell viability compared with cells pretreated with mutant proteins (A30P and A53T) after the PQ exposure, and the neuroprotective effect was synergistically higher than the expression of Tat–α-synuclein when measuring the SOD activity assay. Also, in that study, when PEP-1–SOD was cotransduced with WT Tat-α-synuclein, the number of TH^+ neurons and the Hsp70 protein levels were enhanced compared with PQ-treated cells, but not when they overexpressed A53T mutant Tat-α-synuclein (Choi et al. 2006). A study by Fernagut et al. (2007), using mice overexpressing human WT α-synuclein under the Thy-1 promoter (Thy1-αSYN), demonstrated how they had several impaired postural adjustments (such as errors per step, time to orient downward, etc.), which were unchanged after the PQ treatment. However, this herbicide induced a significant increase of the proteinase K-resistant α-synuclein inclusions in the substantia nigra of Thy1-αSYN mice, and their diameter were higher relative to the saline treatment. This fact could be due to an increased level of the protein or to the α-synuclein nitration, allowing its resistance to proteolysis (Uversky et al. 2005). Studies in human neuroblastoma SH-SY5Y cells (Chau et al. 2009) have shown that PQ produced similar cell death and free radical generation in control and WT α-synuclein cells; however, this rate is higher in cells overexpressing A53T mutant α-synuclein. Also, the caspase-3 activity was highest in the mutant cells, in line with the mitochondrial membrane potential reduction, indicating apoptosis mediated by PQ. Otherwise, the co-incubation with cabergoline (dopamine agonist) decreased this pattern of cell death, having better protection in A53T mutant cells. Another neuroprotector was used to prevent PQ toxicity (Chau et al. 2010), rasagiline (irreversible MAO-B oxidase inhibitor), demonstrating that it ameliorated the PQ cell death in SH-SY5Y cells overexpressing or not WT or A53T mutant α-synuclein, as well as decreasing the PQ-induced caspase-3 activation and free radical production. In the same way, the A53T mutant mice suffered a TH+ neuronal loss in a synergic manner with PQ, combined or not with iron, and this event is age-dependent, just as the increase of the protein oxidation levels. However, the dopaminergic neuronal death and the nitrated α-synuclein can be partly rescued using the antioxidant EUK-189 (Peng et al. 2010). A recent epidemiologic report showed an approach between α-synuclein and environmental factors, like PQ, because of the low risk of PD development in subjects who were either homozygous or heterozygous for the α-synuclein REP1 259 genotype, and subjects who were homozygous or

heterozygous for the REP1 263 genotype have a higher risk, but in PQ exposure areas, the protective effect of the shorter repeat REP1 could be lost, and the longer REP1 genotype may enhance this susceptibility and promote a higher risk in younger populations (Gatto et al. 2010). An important item could be if there is a mechanism to maintain the α-synuclein levels in the cell, and some studies (Mak et al. 2010) have shown how α-synuclein is associated with lysosomes in the midbrain of PQ-treated mice to uptake and internalize this protein for later clearance in this organelle via chaperone-mediated autophagy (CMA), because the PQ exposure increased the hsc70 and LAMP-2A levels and their interaction with α-synuclein. To establish a relationship between CMA and α-synuclein, the investigators used transgenic mice overexpressing mouse α-synuclein, observing higher levels of LAMP-2A and hsc70. A new report showed that lithium protects against neuronal cell death and prevents nitrated/oxidized α-synuclein accumulation in human A53T mutant-expressing mice chronically exposed to PQ/maneb in several brain regions (Kim et al. 2011).

Another gene involved in PD is *parkin* (*PARK2*), and several studies have demonstrated that the environmental stress also modulates the corresponding protein. In this way, a report by Pesah and colleagues showed how *parkin* mutant flies have enhanced sensitivity to PQ toxicity (Pesah et al. 2004), claiming that parkin could have a protective role. Also, PQ changes the parkin solubility and promotes the formation of intracellular parkin inclusions in SH-SY5Y cells expressing FLAG-parkin (Wang et al. 2005). In a previously cited study (Ved et al. 2005), in *C. elegans*, the *KO8E3.7*/*Parkin KO* did not show a higher sensitivity to the PQ treatment, and the same pattern was demonstrated in the co-treatment with probucol and DβHB as in the previous paragraph. In *parkin* mutant human fibroblasts, the treatment with PQ induced significant loss of mitochondrial membrane potential, slight increase in protein oxidation, and reduction of mitochondrial branching (Grünewald et al. 2010).

Following the above, *PINK1* (*PARK6*) is a gene that is strongly related to PD. In this case, in *PINK1* silenced cells, PQ generated an important reduction in cell viability and a higher rate of apoptosis cell death (Gegg et al. 2009). In primary fibroblasts cultures, the mitochondrial membrane potential was unaffected in control and mutant cells after PQ exposure, but the glutathione (GSH) levels were increased in missense culture to a greater extent (Grünewald et al. 2009). A report realized in *C. elegans* (Sämann et al. 2009) suggested a functional linkage between the *lrrk-1* and *pink-1* homologs of human *LRRK2* (*PARK8*) and *PINK1* genes, showing how the loss of *pink-1* function produced a higher sensitivity to PQ, whereas the genetic deletion of *lrrk-1* could compensate for both of these deficiencies.

Finally, DJ-1 (CAP1/RS/PARK7) is a protein related to early-onset PD, for which patients respond to treatment with dopamine (Bonifati et al. 2003). Although the function of DJ-1 is unclear, several studies suggest their possible involvement in the cellular response against oxidative stress, because DJ-1 has the capacity to eliminate ROS by auto-oxidation (Bonifati et al. 2003; Kinumi et al. 2004; Taira et al. 2004). It has been observed that, if DJ-1 shows mutations in C106, it is unable

to protect the cell under conditions of oxidative stress caused by MPP^+ (an inhibitor of mitochondrial complex I) (Canet-Avilés et al. 2004). These findings suggest that mutations in the DJ-1 protein can cause mitochondrial susceptibility against toxins such as MPP^+. There are many cellular and animal models available to investigate the combined effect of genetic and environmental factors involved in PD and to study the interaction between DJ-1 and the herbicide PQ. The idea that DJ-1 stimulates the autophagy response against PQ and reduces apoptotic cell death response could open the door to the development of new treatment strategies against PD (González-Polo et al. 2010).

4 Cellular Models in Paraquat Neurotoxicity

In PD, cell death by apoptosis has been proposed to result from mitochondrial dysfunction, leading to an increase in oxidative stress and a decline in ATP production. Some studies have shown that PQ induces cytochrome c release (Fei et al. 2008; González-Polo et al. 2004) and caspase-9 activation, which are preceded by the induction/activation of pro-apoptotic Bax and Bak (Fei et al. 2008; Yang and Tiffany-Castiglioni 2008). The evaluation of Bcl-2 family members showed that PQ induces high levels of Bak, Bid, BNip3, and Noxa (Fei et al. 2008). Interestingly, there are many works which demonstrate that, individually, PQ and maneb (and other pesticides related to PD) activate Bak, but, together, they trigger Bax-dependent cell death. Focusing on mechanisms responsible for this synergy, the study showed that maneb + PQ increases the expression of three strong Bak inhibitors, Bfl-1, Bcl-xL and Mcl-1, and, also, induces Bax activators that included Bik and Bim (Fei and Ethell 2008). The induction of pro-apoptotic Bax and apoptosis in response to PQ has also been reported to be dependent on p53 (Rossi et al. 2001; Yang and Tiffany-Castiglioni 2008). PQ neurotoxicity has also been reported to require the activation of stress-activated protein kinases (SAPKs) (Chun et al. 2001; Klintworth et al. 2007; Niso-Santano et al. 2006, 2010; Ramachandiran et al. 2007; Yang et al. 2009).

Although the mitochondrial pathway of apoptosis has been largely linked to PQ-induced cell death, other signaling pathways have also been implicated in PQ toxicity. Recently, PQ has been shown to induce DNA damage and endoplasmic reticulum (ER) stress. ER stress is associated with the activation of inositol-requiring enzyme 1 (IRE1), apoptosis signal-regulating kinase 1 (ASK1), and c-Jun NH_2-terminal kinase (JNK) (Niso-Santano et al. 2010; Yang et al. 2009). In this sense, PQ-induced oxidation of Trx has been reported as a possible mechanism for the activation of the ASK1/JNK signaling pathways (Niso-Santano et al. 2010; Ramachandiran et al. 2007; Yang et al. 2009). NF-E2-related factor-2 (Nrf2)-dependent regulation of antioxidant responsive element (ARE)-mediated gene expression has been shown to protect against PQ (Minelli et al. 2009). Accordingly, Nrf2-dependent regulation of Trx levels determines the sensitivity of PQ toxicity by the activation of ASK1/JNKp38 signaling (Niso-Santano et al. 2010). PQ-induced tyrosine nitration and lipid peroxidation have been

demonstrated (Schmuck et al. 2002). However, the molecular targets for these signaling events remain to be elucidated. It was recently demonstrated that oxidative stress induced by PQ generates protein aggregation of the plasma membrane Ca^{2+} -ATPase and its degradation by calpain (Zaidi et al. 2009).

Recent studies have demonstrated that low concentrations of PQ induce autophagy, which is followed by apoptosis (González-Polo et al. 2007a, b, 2009) and modulated by DJ-1. Because the inhibition of autophagy potentiated apoptosis induced by PQ, it was proposed that autophagy might be acting as a protective mechanism against cell death progression.

The fact that PQ induces the accumulation of autophagic vacuoles and increases the degradation of proteins in the cytoplasm of SH-SY5Y cells (González-Polo et al. 2007a) indicates that the increased oxidative stress can activate autophagy in the initial stages of mitochondrial dysfunction, having a protective role of PQ-induced cell death (González-Polo et al. 2007a, b). Moreover, it has been shown that PQ exposure induces an early ER (endoplasmic reticulum) stress response that is correlated with this adaptive activation of autophagy, with hallmarks such as the accumulation of autophagic vacuoles, activation of beclin-1, accumulation of LC3-II, p62 degradation, and mTOR dephosphorylation (González-Polo et al. 2007a, b; Niso-Santano et al. 2011). This response is accelerated in cells that overexpress WT of the ASK1 protein. The inhibition of autophagy caused, in this model, an exacerbation of the apoptosis induced by WT ASK1 overexpression with or without PQ. These results suggest that autophagy has an important role in the cell death/survival events produced by PQ and ASK1 that contribute to neuronal degeneration.

Therefore, increased autophagy might be a new strategy for the treatment of neurodegenerative diseases (Menzies et al. 2006). However, it is encouraging to consider enhancing autophagic capacity as a therapeutic strategy in the prevention of neurodegeneration because, as mentioned above, there is evidence showing that the abnormal regulation of autophagic pathways may lead to apoptosis and cell death (Walls et al. 2010).

Most of the studies regarding the molecular mechanisms of PQ-induced apoptosis and autophagy have been directed towards neuronal cell types (Table 2). However, it is obvious that the complexity of brain tissue organization is given by the interaction of different neurons with glial cell types. Compared to neurons, astrocytes have been demonstrated to be more resistant to PQ-induced oxidative stress by the responsive induction of a variety of antioxidant systems, including catalase and SODs (Olesen et al. 2008; Röhrdanz et al. 2001; Schmuck et al. 2002). Recent studies have demonstrated that microglial cells react to pro-oxidant conditions, suggesting that they can contribute to environmental stress-induced neurotoxicity (Vogt et al. 1998). PQ induces microglial activation, which seems to precede PD neurodegeneration (Purisai et al. 2007). Low PQ concentrations have recently been demonstrated to be toxic to neurons only in the presence of microglial cells. In this study, it was observed that, in neuron–microglia cultures exposed to PQ, microglia was a source of PQ-derived oxidative stress (Wu et al. 2005). Interestingly, NADPH oxidase from glial cells has been proposed to mediate the

Table 2 List of studies on the induction of apoptosis and/or autophagy by PQ

Model	Apoptotic major findings	References
Cerebellar granule cells	Caspase 3 activation	González-Polo et al. 2004
	Cytochrome c release	
	DNA fragmentation	
Rat brain neuroblasts, E18 cells	ERK1/2 activation	Niso-Santano et al. 2006
	JNK activation	
	PKB activation	
Pheochromocytoma PC12 cells	JNK/p38 activation	Klintworth et al. 2007
Rat primary cultured dopaminergic neurons	JNK activation	Klintworth et al. 2007
SK-N-SH neuroblastoma cells	Bak activation	Fei et al. 2008
	Cytochrome c release	
	Caspase 3 activation	
	Thioredoxin oxidized	Ramachandiran et al. 2007
	JNK activation	
	Caspase 3 activation	
***Substantia nigra*-derived dopaminergic SN4741 cell line**	JNK/MAPK activation	Chun et al. 2001
	Caspases 1 and 3 activation	
SH-SY5Y human neuroblastoma cells	JNK/p38 activation	Klintworth et al. 2007
	Mitochondrial intrinsic pathway associated with p53	Yang and Tiffany-Castiglioni 2008
	IRE1/ASK1/JNK activation	Yang et al. 2009
	ASK1/JNK activation	Niso-Santano et al. 2010
	ASK1/p38 activation	
	Caspase 3 activation	
Autophagic major findings		
SH-SY5Y human neuroblastoma cells	Accumulation of autophagic vacuoles	González-Polo et al. 2007a, b
	Recruitment of an LC3-GFP fusion protein to autophagic vacuoles	
	Long-lived protein degradation regulated by mTOR signaling	
	ER stress response	Niso-Santano et al. 2011
	Beclin-1 activation	
	p62 degradation	
	Autophagic events DJ-1 dependent	González-Polo et al. 2009

generation of ROS (Kim et al. 2008; Purisai et al. 2007; Schmuck et al. 2002), which seems to be regulated by the activation of protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) signaling pathways (Kim et al. 2008; Miller et al. 2007).

5 Animal Models in Paraquat Neurotoxicity

Animal models are an important tool for studying different human diseases, including PD. Animals do not develop PD spontaneously, but it is possible mimic the symptoms of this disease, either by surgery or the use of chemicals to damage the tissues of the body in a way that reflects the changing disease. Animal models allow scientists prove what is causing parkinsonism symptoms and to test different treatments that cannot be tested in people. Therefore, despite these models never being able to fully mimic the human model, they are vital in order to understand the molecular and biochemical features of PD (Orth and Tabrizi 2003).

Using drugs to alter the animals is the main method used in research on Parkinson's disease. MPTP, rotenone, or PQ are used to cause parkinsonian symptoms in different animal models. The animal models commonly used for PD investigations are monkey, rat, or mice, but animals like rabbits, fruit flies, dogs, nematodes, zebrafish, pigs, or guinea pigs are also used, although to a lesser extent.

These models have highlighted the possibility that environmental chemicals used in everyday situations may be responsible for contributing to the development of PD. Behavioral effects and loss of neurones in the substantia nigra were observed and the depletion of dopamine was reported in many, but not all, of these studies.

The following is a review of the different animal models used for the studies of parkinsonian symptoms associated with exposure to PQ.

5.1 Rat Animal Model

The use of a rat animal model to study parkinsonian symptoms is very common. Exposure to PQ in rats induces much damage at different levels in rats, and presents lung and renal toxicity and toxicity to the liver. At higher doses, observations include decreased body weight gain, clinical signs like dyspnea, increased respiratory sounds, swellings and sores in the genital area, hematological changes, and effects on organ weight, as well as increased mortality.

In addition, a large number of studies have been performed in rat brains to observe animal behavior after being treated with PQ. It has been shown that there are changes in behavior as well as neuropathological effects in rats which received both systemic and intrahippocampal injections of PQ dichloride (Corasaniti et al. 1992). After treatment with PQ by unilateral injection into the substantia nigra, there was loss of Nissl substance, glial reaction, and loss of neurones in the substantia nigra, and, neurochemically, there was dopamine depletion (Liou et al. 1996). The neuropathological findings in another study showed that PQ possesses marked neurotoxicity, with neuropathological lesions culminating in neuronal necrosis (Calò et al. 1990). Another study showed that PQ induces oxidative stress, neuronal loss in the substantia nigra region, and parkinsonism in adult rats (Somayajulu-Niţu et al. 2009) and lipid peroxidation in rat brains (Yumino et al. 2002). This neurodegeneration is observed after exposure to PQ, which might occur via an early inflammatory response in young adult animals (Cicchetti et al. 2005).

Therefore, in rats, it has been shown that PQ induces neuronal damage and changes in the behavior of these animals. Furthermore, this damage could be caused by oxidative stress, lipid peroxidation, and inflammatory responses after stimulation with PQ.

5.2 Mouse Animal Model

Many in vivo studies in mice have shown that acute PQ administration may elicit a distinct decrease in the level of dopamine and spontaneous motor activity in mice (Kang et al. 2009; Li et al. 2005b) and there are studies that relate directly the PQ exposure in mice with a decrease in motor activity (Brooks et al. 1999). However, equivocal results have been reported by studies investigating the effect of exposure to PQ on selective nigrostriatal degeneration in rodents. There are studies which show that PQ does not induce Parkinson's disease symptoms in mice (Rojo et al. 2007), but other works show that PQ administration triggers an apoptotic cell death program through oxidative stress-mediated activation of the JNK signaling pathway, suggesting a possible mechanism for selective dopaminergic neuron loss (Peng et al. 2004). There has been a study on C57BL/6 mice exposed to PQ, maneb, or a PQ + maneb mixture, a combination that produces PD symptoms in mice (Thiruchelvam et al. 2000).

With a better understanding of the genetic alterations involved in familial PD, several transgenic mouse models have been generated that can reproduce many features of parkinsonism. Animal models generated by mutations or deletions of the relevant Parkinson's-related genes have also been used to examine the effects of PQ; for example, studies have shown that the overexpression of α-synuclein protects against PQ toxicity (Manning-Bog et al. 2002, 2003).

5.3 Monkey Animal Model

Monkey species, like *Saimiri sciureus* or *Macaca fascicularis*, are normally used in studies with PQ treatments. There have been studies carried out to characterize and compare the toxicity, clinical syndromes, and pathology of PQ, using an LD_{50} in these animals of normally 50–70 mg/kg body weight. Many decades ago, there were studies indicating that the oral administration of PQ in this species induced clinical signs of intoxication, such as anorexia, adipsia, diarrhea, hyperpnea, dyspnea, and tachycardia, which depend on the duration of exposure and the concentration of PQ (Murray and Gibson 1972; Purser and Rose 1979). However, there are few studies of monkeys concerning the neuronal damage caused by PQ exposition, although recent studies on PQ administration in monkeys have demonstrated that it produces a depletion of dopamine in the striatum of these animals (O'Leary et al. 2008).

5.4 Fruit Fly Animal Model

Drosophila melanogaster is a popular study species in science. This particular species has become an invaluable tool to scientists, who have been studying this insect for over a century. The fruit fly *D. melanogaster* has a similar number of genes to humans and, therefore, it is often used in genetic changes in order to observe the effects on the progression of disease. A large number of human genes, including those implicated in PD such as parkin, UCH-L1, PINK1, DJ-1, and LRRK2, have highly conserved homologs in *D. melanogaster* (Whitworth et al. 2006).

The exists many models of fruit flies to study genetic changes, which has shown dysfunction of DA neurons, locomotor deficits, and retinal degeneration after treatment with PQ. The α-synuclein (Takahashi et al. 2003) or parkin models in flies show that PQ induces DA neuron loss, mitochondrial pathology, locomotor deficits, LB-like protein aggregates, and sensitivity to oxidative stress (Greene et al. 2005). Another study in flies indicates that DJ-1 plays a critical role in the survival of dopaminergic neurons and response to oxidative stress in PQ treatment (Menzies et al. 2005). In *D. melanogaster*, there are also studies that show damage at the level of oxidative stress which occurs with PQ exposure, along with neuronal damage that this entails (Rzezniczak et al. 2011).

5.5 Nematode Animal Model

Caenorhabditis elegans is a nematode animal model used commonly in scientific studies. *C. elegans* models have a well-characterized genome and the adult wild-type worm comprises 1,000 cells, about a third of which are neurons, exactly eight of which are dopaminergic neurons. It also has all the dopamine transmission mechanisms of a human model (Nass et al. 2001). These dopamine neurons of *C. elegans* are sensitive to neurotoxins and pesticides like 6-OHDA (Nass et al. 2002), MPP^+ (Braungart et al. 2004), and rotenone or PQ (Ved et al. 2005). This has led to a search for genetic patterns among nematodes of resistance to the toxicity of PQ in genes that seem to be involved in the function of chemosensory cilia (Fujii et al. 2004, 2005) or with antioxidant genes (Yanase et al. 2002).

5.6 Zebrafish Animal Model

The *Danio rerio* species is a 3–4-cm-long zebrafish used to study development and gene function as a potential model of PD. In zebrafish, dopaminergic neurons are anatomically comparable with the nigrostriatal tract in mammals. These neurons are sensitive to some of the classical PD model toxins, namely, 6-OHDA and MPTP, showing reduced brain levels of dopamine after treatment (Anichtchik et al. 2004).

A study among zebrafish with PQ showed that, in adult zebrafish, there was no induced effect after a long period of exposure to pesticide PQ, and in larval zebrafish, only a trend of reduction in diencephalic DA neurons was shown, which did not reach statistical significance (Bretaud et al. 2004).

5.7 Dog Animal Model

In many studies with dogs, principally the Beagle dog breed, the animals received PQ as a dietary supplement and the results indicate that levels of pulmonary capillary and pulmonary vascular resistance were increased, mean arterial pressure and cardiac output were decreased, while the total vascular resistance and pulmonary vascular resistance were increased (Sone et al. 1989). Another study showed the appearance of dyspnea, and, often, emaciation or inappetence, with some body weight loss. However, there are no conclusive studies on the neurological damage produced in dogs after exposure to PQ.

5.8 Rabbit Animal Model

Rabbits are another animal model used to probe the toxicity of PQ. After PQ exposition, damage by pneumonitis has been detected in the animals (Seidenfeld et al. 1985), as well as damage to the eyes, liver, kidneys, heart, and lungs (Hassan et al. 1989), but there exists no studies demonstrating damage at the neurological level.

5.9 Pig Animal Model

Pigs have been used on rare occasions for PQ toxicity studies, showing that high concentrations of and prolonged exposure to PQ may have deleterious effects on epidermal morphology in the absence of significant percutaneous absorption (Srikrishna et al. 1992), but there has been no study neuronal damage in these animal models.

6 Conclusion

Toxicologic studies have suggested that multiple genetic and environmental factors could be involved in the etiology of PD. Studies with transgenic mice suggest that the genetic background may also lead to increased vulnerability to the neurotoxic effects of pesticides such as PQ. The weight of evidence is sufficient to conclude that a generic association between PQ exposure and PD exists, but it is not sufficient to conclude that this is a causal relationship. In addition, the multifactorial etiology of PD hampers unequivocally establishment of the role of any individual contributory causal factor. Further research is needed in order to identify long-term biomarkers of exposure, improve methods for estimating pesticide exposure, and

undertake prospective cohort studies of PQ-exposed people. It is also necessary to increase the volume of studies on the molecular basis of PQ toxicity in order to establish a clear relationship between its neurotoxic effects and in vivo neuronal loss that leads to PD.

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References

Anichtchik, O. V., Kaslin, J., Peitsaro, N., Scheinin, M., & Panula, P. (2004). Neurochemical and behavioural changes in zebrafish Danio rerio after systemic administration of 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Journal of Neurochemistry, 88*(2), 443–453.

Berry, C., La Vecchia, C., & Nicotera, P. (2010). Paraquat and Parkinson's disease. *Cell Death and Differentiation, 17*(7), 1115–1125.

Bonifati, V., Rizzu, P., Squitieri, F., Krieger, E., Vanacore, N., van Swieten, J. C., Brice, A., van Duijn, C. M., Oostra, B., Meco, G., & Heutink, P. (2003). DJ-1 (PARK7), a novel gene for autosomal recessive, early onset parkinsonism. *Neurological Science, 24*(3), 159–160.

Bonneh-Barkay, D., Langston, W. J., & Di Monte, D. A. (2005). Toxicity of redox cycling pesticides in primary mesencephalic cultures. *Antioxidants & Redox Signaling, 7*(5–6), 649–653.

Braungart, E., Gerlach, M., Riederer, P., Baumeister, R., & Hoener, M. C. (2004). Caenorhabditis elegans MPP^+ model of Parkinson's disease for high-throughput drug screenings. *Neurodegenerative Diseases, 1*(4–5), 175–183.

Bretaud, S., Lee, S., & Guo, S. (2004). Sensitivity of zebrafish to environmental toxins implicated in Parkinson's disease. *Neurotoxicology and Teratology, 26*(6), 857–864.

Brooks, A. I., Chadwick, C. A., Gelbard, H. A., Cory-Slechta, D. A., & Federoff, H. J. (1999). Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. *Brain Research, 823*(1–2), 1–10.

Brown, T. P., Rumsby, P. C., Capleton, A. C., Rushton, L., & Levy, L. S. (2006). Pesticides and Parkinson's disease–is there a link? *Environmental Health Perspectives, 114*(2), 156–164.

Butterfield, P. G., Valanis, B. G., Spencer, P. S., Lindeman, C. A., & Nutt, J. G. (1993). Environmental antecedents of young-onset Parkinson's disease. *Neurology, 43*(6), 1150–1158.

Calò, M., Iannöne, M., Passafaro, M., & Nisticò, G. (1990). Selective vulnerability of hippocampal CA3 neurones after microinfusion of paraquat into the rat substantia nigra or into the ventral tegmental area. *Journal of Comparative Pathology, 103*(1), 73–78.

Canet-Avilés, R. M., Wilson, M. A., Miller, D. W., Ahmad, R., McLendon, C., Bandyopadhyay, S., Baptista, M. J., Ringe, D., Petsko, G. A., & Cookson, M. R. (2004). The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine–sulfinic acid-driven mitochondrial localization. *Proceedings of the National Academy of Sciences of the United States of America, 101*(24), 9103–9108.

Chau, K. Y., Korlipara, L. V., Cooper, J. M., & Schapira, A. H. (2009). Protection against paraquat and A53T alpha-synuclein toxicity by cabergoline is partially mediated by dopamine receptors. *Journal of Neurological Sciences, 278*(1–2), 44–53.

Chau, K. Y., Cooper, J. M., & Schapira, A. H. (2010). Rasagiline protects against alpha-synuclein induced sensitivity to oxidative stress in dopaminergic cells. *Neurochemistry International, 57*(5), 525–529.

Choi, H. S., An, J. J., Kim, S. Y., Lee, S. H., Kim, D. W., Yoo, K. Y., Won, M. H., Kang, T. C., Kwon, H. J., Kang, J. H., Cho, S. W., Kwon, O. S., Park, J., Eum, W. S., & Choi, S. Y. (2006). PEP-1-SOD fusion protein efficiently protects against paraquat-induced dopaminergic neuron damage in a Parkinson disease mouse model. *Free Radical Biology & Medicine, 41*(7), 1058–1068.

Chun, H. S., Gibson, G. E., DeGiorgio, L. A., Zhang, H., Kidd, V. J., & Son, J. H. (2001). Dopaminergic cell death induced by MPP(+), oxidant and specific neurotoxicants shares the common molecular mechanism. *Journal of Neurochemistry, 76*(4), 1010–1021.

Cicchetti, F., Lapointe, N., Roberge-Tremblay, A., Saint-Pierre, M., Jimenez, L., Ficke, B. W., & Gross, R. E. (2005). Systemic exposure to paraquat and maneb models early Parkinson's disease in young adult rats. *Neurobiology of Disease, 20*(2), 360–371.

Corasaniti, M. T., Bagetta, G., Rodinò, P., Gratteri, S., & Nisticò, G. (1992). Neurotoxic effects induced by intracerebral and systemic injection of paraquat in rats. *Human and Experimental Toxicology, 11*(6), 535–539.

Corrigan, F. M., Murray, L., Wyatt, C. L., & Shore, R. F. (1998). Diorthosubstituted polychlorinated biphenyls in caudate nucleus in Parkinson's disease. *Experimental Neurology, 150*(2), 339–342.

Costello, S., Cockburn, M., Bronstein, J., Zhang, X., & Ritz, B. (2009). Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. *American Journal of Epidemiology, 169*(8), 919–926.

Di Monte, D., Sandy, M. S., Ekström, G., & Smith, M. T. (1986). Comparative studies on the mechanisms of paraquat and 1-methyl-4-phenylpyridine (MPP^+) cytotoxicity. *Biochemical and Biophysical Research Communications, 137*(1), 303–309.

Dinis-Oliveira, R. J., Remião, F., Carmo, H., Duarte, J. A., Navarro, A. S., Bastos, M. L., & Carvalho, F. (2006). Paraquat exposure as an etiological factor of Parkinson's disease. *Neurotoxicology, 27*(6), 1110–1122.

Dinis-Oliveira, R. J., Duarte, J. A., Sánchez-Navarro, A., Remião, F., Bastos, M. L., & Carvalho, F. (2008). Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. *Critical Reviews in Toxicology, 38*(1), 13–71.

Drechsel, D. A., & Patel, M. (2008). Role of reactive oxygen species in the neurotoxicity of environmental agents implicated in Parkinson's disease. *Free Radical Biology & Medicine, 44*(11), 1873–1886.

Fei, Q., & Ethell, D. W. (2008). Maneb potentiates paraquat neurotoxicity by inducing key Bcl-2 family members. *Journal of Neurochemistry, 105*(6), 2091–2097.

Fei, Q., McCormack, A. L., Di Monte, D. A., & Ethell, D. W. (2008). Paraquat neurotoxicity is mediated by a Bak-dependent mechanism. *Journal of Biological Chemistry, 283*(6), 3357–3364.

Fernagut, P. O., Hutson, C. B., Fleming, S. M., Tetreaut, N. A., Salcedo, J., Masliah, E., & Chesselet, M. F. (2007). Behavioral and histopathological consequences of paraquat intoxication in mice: Effects of alpha-synuclein over-expression. *Synapse, 61*(12), 991–1001.

Firestone, J. A., Smith-Weller, T., Franklin, G., Swanson, P., Longstreth, W. T., Jr., & Checkoway, H. (2005). Pesticides and risk of Parkinson disease: A population-based case–control study. *Archives of Neurology, 62*(1), 91–95.

Fleming, L., Mann, J. B., Bean, J., Briggle, T., & Sánchez-Ramos, J. R. (1994). Parkinson's disease and brain levels of organochlorine pesticides. *Annals of Neurology, 36*(1), 100–103.

Fujii, M., Matsumoto, Y., Tanaka, N., Miki, K., Suzuki, T., Ishii, N., & Ayusawa, D. (2004). Mutations in chemosensory cilia cause resistance to paraquat in nematode *Caenorhabditis elegans*. *Journal of Biological Chemistry, 279*(19), 20277–20282.

Fujii, M., Tanaka, N., Miki, K., Hossain, M. N., Endoh, M., & Ayusawa, D. (2005). Uncoupling of longevity and paraquat resistance in mutants of the nematode *Caenorhabditis elegans*. *Bioscience, Biotechnology, and Biochemistry, 69*(10), 2015–2018.

Fukushima, T., Yamada, K., Isobe, A., Shiwaku, K., & Yamane, Y. (1993). Mechanism of cytotoxicity of paraquat. I. NADH oxidation and paraquat radical formation via complex I. *Experimental and Toxicologic Pathology, 45*(5–6), 345–349.

Gatto, N. M., Rhodes, S. L., Manthripragada, A. D., Bronstein, J., Cockburn, M., Farrer, M., & Ritz, B. (2010). Alpha-synuclein gene may interact with environmental factors in increasing risk of Parkinson's disease. *Neuroepidemiology, 35*(3), 191–195.

Gegg, M. E., Cooper, J. M., Schapira, A. H., & Taanman, J. W. (2009). Silencing of PINK1 expression affects mitochondrial DNA and oxidative phosphorylation in dopaminergic cells. *PLoS One, 4*(3), e4756.

Goers, J., Manning-Bog, A. B., McCormack, A. L., Millett, I. S., Doniach, S., Di Monte, D. A., Uversky, V. N., & Fink, A. L. (2003). Nuclear localization of alpha-synuclein and its interaction with histones. *Biochemistry, 42*(28), 8465–8471.

González-Polo, R. A., Rodríguez-Martín, A., Morán, J. M., Niso, M., Soler, G., & Fuentes, J. M. (2004). Paraquat-induced apoptotic cell death in cerebellar granule cells. *Brain Research, 1011*(2), 170–176.

González-Polo, R. A., Niso-Santano, M., Ortíz-Ortíz, M. A., Gómez-Martín, A., Morán, J. M., García-Rubio, L., Francisco-Morcillo, J., Zaragoza, C., Soler, G., & Fuentes, J. M. (2007a). Inhibition of paraquat-induced autophagy accelerates the apoptotic cell death in neuroblastoma SH-SY5Y cells. *Toxicological Sciences, 97*(2), 448–458.

González-Polo, R. A., Niso-Santano, M., Ortíz-Ortíz, M. A., Gómez-Martín, A., Morán, J. M., García-Rubio, L., Francisco-Morcillo, J., Zaragoza, C., Soler, G., & Fuentes, J. M. (2007b). Relationship between autophagy and apoptotic cell death in human neuroblastoma cells treated with paraquat: Could autophagy be a "brake" in paraquat-induced apoptotic death? *Autophagy, 3*(4), 366–367.

González-Polo, R., Niso-Santano, M., Morán, J. M., Ortiz-Ortiz, M. A., Bravo-San Pedro, J. M., Soler, G., & Fuentes, J. M. (2009). Silencing DJ-1 reveals its contribution in paraquat-induced autophagy. *Journal of Neurochemistry, 109*(3), 889–898.

González-Polo, R. A., Niso-Santano, M., Gómez-Sánchez, R., Bravo-San Pedro, J. M., & Fuentes, J. M. (2010). DJ-1 as a modulator of autophagy: An hypothesis. *The Scientific World Journal, 10*, 1574–1579.

Gorell, J. M., Peterson, E. L., Rybicki, B. A., & Johnson, C. C. (2004). Multiple risk factors for Parkinson's disease. *Journal of Neurological Sciences, 217*(2), 169–174.

Gray, J. P., Heck, D. E., Mishin, V., Smith, P. J., Hong, J. Y., Thiruchelvam, M., Cory-Slechta, D. A., Laskin, D. L., & Laskin, J. D. (2007). Paraquat increases cyanide-insensitive respiration in murine lung epithelial cells by activating an NAD(P)H: Paraquat oxidoreductase: Identification of the enzyme as thioredoxin reductase. *Journal of Biological Chemistry, 282*(11), 7939–7949.

Greene, J. C., Whitworth, A. J., Andrews, L. A., Parker, T. J., & Pallanck, L. J. (2005). Genetic and genomic studies of Drosophila parkin mutants implicate oxidative stress and innate immune responses in pathogenesis. *Human Molecular Genetics, 14*(6), 799–811.

Grimm, S., & Brdiczka, D. (2007). The permeability transition pore in cell death. *Apoptosis, 12*(5), 841–855.

Grünewald, A., Gegg, M. E., Taanman, J. W., King, R. H., Kock, N., Klein, C., & Schapira, A. H. (2009). Differential effects of PINK1 nonsense and missense mutations on mitochondrial function and morphology. *Experimental Neurology, 219*(1), 266–273.

Grünewald, A., Voges, L., Rakovic, A., Kasten, M., Vandebona, H., Hemmelmann, C., Lohmann, K., Orolicki, S., Ramirez, A., Schapira, A. H., Pramstaller, P. P., Sue, C. M., & Klein, C. (2010). Mutant Parkin impairs mitochondrial function and morphology in human fibroblasts. *PLoS One, 5*(9), e12962.

Haley, T. J. (1979). Review of the toxicology of paraquat (1,1′-dimethyl-4,4′-bipyridinium chloride). *Clinical Toxicology, 14*(1), 1–46.

Hassan, R. A., Afzal, M., Ali, M., & Gubler, C. J. (1989). Effect of paraquat administered intraperitoneally on the nonpolar lipids of rabbits. *Ecotoxicology and Environmental Safety, 17*(1), 47–58.

Hertzman, C., Wiens, M., Bowering, D., Snow, B., & Calne, D. (1990). Parkinson's disease: A case–control study of occupational and environmental risk factors. *American Journal of Industrial Medicine, 17*(3), 349–355.

Kamel, F., & Hoppin, J. A. (2004). Association of pesticide exposure with neurologic dysfunction and disease. *Environmental Health Perspectives, 112*(9), 950–958.

Kamel, F., Tanner, C., Umbach, D., Hoppin, J., Alavanja, M., Blair, A., Comyns, K., Goldman, S., Korell, M., Langston, J., Ross, G., & Sandler, D. (2007). Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. *American Journal of Epidemiology, 165*(4), 364–374.

Kang, M. J., Gil, S. J., & Koh, H. C. (2009). Paraquat induces alternation of the dopamine catabolic pathways and glutathione levels in the substantia nigra of mice. *Toxicology Letters, 188*(2), 148–152.

Kim, S., Hwang, J., Lee, W. H., Hwang, D. Y., & Suk, K. (2008). Role of protein kinase Cdelta in paraquat-induced glial cell death. *Journal of Neuroscience Research, 86*(9), 2062–2070.

Kim, Y. H., Rane, A., Lussier, S., & Andersen, J. K. (2011). Lithium protects against oxidative stress-mediated cell death in alpha-synuclein-overexpressing in vitro and in vivo models of Parkinson's disease. *Journal of Neuroscience Research, 89*(10), 1666–1675.

Kinumi, T., Kimata, J., Taira, T., Ariga, H., & Niki, E. (2004). Cysteine-106 of DJ-1 is the most sensitive cysteine residue to hydrogen peroxide-mediated oxidation in vivo in human umbilical vein endothelial cells. *Biochemical and Biophysical Research Communications, 317*(3), 722–728.

Klintworth, H., Newhouse, K., Li, T., Choi, W. S., Faigle, R., & Xia, Z. (2007). Activation of c-Jun N-terminal protein kinase is a common mechanism underlying paraquat- and rotenone-induced dopaminergic cell apoptosis. *Toxicological Sciences, 97*(1), 149–162.

Langston, J. W., & Ballard, P. A., Jr. (1983). Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *The New England Journal of Medicine, 309*(5), 310.

Li, A. A., Mink, P. J., McIntosh, L. J., Teta, M. J., & Finley, B. (2005a). Evaluation of epidemiologic and animal data associating pesticides with Parkinson's disease. *Journal of Occupational and Environmental Medicine, 47*(10), 1059–1087.

Li, X., Yin, J., Cheng, C. M., Sun, J. L., Li, Z., & Wu, Y. L. (2005b). Paraquat induces selective dopaminergic nigrostriatal degeneration in aging C57BL/6 mice. *Chinese Medical Journal, 118*(16), 1357–1361.

Liochev, S. I., Hausladen, A., Beyer, W. F., Jr., & Fridovich, I. (1994). NADPH: Ferredoxin oxidoreductase acts as a paraquat diaphorase and is a member of the soxRS regulon. *Proceedings of the National Academy of Sciences of the United States of America, 91*(4), 1328–1331.

Liou, H. H., Chen, R. C., Tsai, Y. F., Chen, W. P., Chang, Y. C., & Tsai, M. C. (1996). Effects of paraquat on the substantia nigra of the Wistar rats: Neurochemical, histological, and behavioral studies. *Toxicology and Applied Pharmacology, 137*(1), 34–41.

Liou, H. H., Tsai, M. C., Chen, C. J., Jeng, J. S., Chang, Y. C., Chen, S. Y., & Chen, R. C. (1997). Environmental risk factors and Parkinson's disease: A case–control study in Taiwan. *Neurology, 48*(6), 1583–1588.

Mak, S. K., McCormack, A. L., Manning-Bog, A. B., Cuervo, A. M., & Di Monte, D. A. (2010). Lysosomal degradation of alpha-synuclein in vivo. *Journal of Biological Chemistry, 285*(18), 13621–13629.

Manning-Bog, A. B., McCormack, A. L., Li, J., Uversky, V. N., Fink, A. L., & Di Monte, D. A. (2002). The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: Paraquat and alpha-synuclein. *Journal of Biological Chemistry, 277*(3), 1641–1644.

Manning-Bog, A. B., McCormack, A. L., Purisai, M. G., Bolin, L. M., & Di Monte, D. A. (2003). Alpha-synuclein overexpression protects against paraquat-induced neurodegeneration. *Journal of Neuroscience, 23*(8), 3095–3099.

Menzies, F. M., Yenisetti, S. C., & Min, K. T. (2005). Roles of Drosophila DJ-1 in survival of dopaminergic neurons and oxidative stress. *Current Biology, 15*(17), 1578–1582.

Menzies, F. M., Ravikumar, B., & Rubinsztein, D. C. (2006). Protective roles for induction of autophagy in multiple proteinopathies. *Autophagy, 2*(3), 224–225.

Miller, R. L., Sun, G. Y., & Sun, A. Y. (2007). Cytotoxicity of paraquat in microglial cells: Involvement of PKCdelta- and ERK1/2-dependent NADPH oxidase. *Brain Research, 1167*, 129–139.

Minelli, A., Conte, C., Grottelli, S., Bellezza, I., Emiliani, C., & Bolaños, J. P. (2009). Cyclo(His-Pro) up-regulates heme oxygenase 1 via activation of Nrf2-ARE signalling. *Journal of Neurochemistry, 111*(4), 956–966.

Munishkina, L. A., Cooper, E. M., Uversky, V. N., & Fink, A. L. (2004). The effect of macromolecular crowding on protein aggregation and amyloid fibril formation. *Journal of Molecular Recognition, 17*(5), 456–464.

Murray, R. E., & Gibson, J. E. (1972). A comparative study of paraquat intoxication in rats, guinea pigs and monkeys. *Experimental and Molecular Pathology, 17*(3), 317–325.

Nass, R., Miller, D. M., & Blakely, R. D. (2001). *C. elegans*: A novel pharmacogenetic model to study Parkinson's disease. *Parkinsonism & Related Disorders, 7*(3), 185–191.

Nass, R., Hall, D. H., Miller, D. M., 3rd, & Blakely, R. D. (2002). Neurotoxin-induced degeneration of dopamine neurons in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America, 99*(5), 3264–3269.

Niso-Santano, M., Morán, J. M., García-Rubio, L., Gómez-Martín, A., González-Polo, R. A., Soler, G., & Fuentes, J. M. (2006). Low concentrations of paraquat induces early activation of extracellular signal-regulated kinase 1/2, protein kinase B, and c-Jun N-terminal kinase 1/2 pathways: Role of c-Jun N-terminal kinase in paraquat-induced cell death. *Toxicological Sciences, 92*(2), 507–515.

Niso-Santano, M., González-Polo, R. A., Bravo-San Pedro, J. M., Gómez-Sánchez, R., Lastres-Becker, I., Ortiz-Ortiz, M. A., Soler, G., Morán, J. M., Cuadrado, A., & Fuentes, J. M. (2010). Activation of apoptosis signal-regulating kinase 1 is a key factor in paraquat-induced cell death: Modulation by the Nrf2/Trx axis. *Free Radical Biology & Medicine, 48*(10), 1370–1381.

Niso-Santano, M., Bravo-San Pedro, J. M., Gómez-Sánchez, R., Climent, V., Soler, G., Fuentes, J. M., & González-Polo, R. A. (2011). ASK1 overexpression accelerates paraquat-induced autophagy via endoplasmic reticulum stress. *Toxicological Sciences, 119*(1), 156–168.

Olanow, C. W. (2007). The pathogenesis of cell death in Parkinson's disease–2007. *Movement Disorders, 22*(Suppl 17), S335–S342.

O'Leary, K. T., Parameswaran, N., Johnston, L. C., McIntosh, J. M., Di Monte, D. A., & Quik, M. (2008). Paraquat exposure reduces nicotinic receptor-evoked dopamine release in monkey striatum. *Journal of Pharmacology and Experimental Therapeutics, 327*(1), 124–129.

Olesen, B. T., Clausen, J., & Vang, O. (2008). Characterization of the transcriptional profile in primary astrocytes after oxidative stress induced by paraquat. *Neurotoxicology, 29*(1), 13–21.

Orth, M., & Tabrizi, S. J. (2003). Models of Parkinson's disease. *Movement Disorders, 18*(7), 729–737.

Orth, M., Tabrizi, S. J., Tomlinson, C., Messmer, K., Korlipara, L. V., Schapira, A. H., & Cooper, J. M. (2004). G209A mutant alpha synuclein expression specifically enhances dopamine induced oxidative damage. *Neurochemistry International, 45*(5), 669–676.

Pan, T., Kondo, S., Le, W., & Jankovic, J. (2008). The role of autophagy-lysosome pathway in neurodegeneration associated with Parkinson's disease. *Brain, 131*(Pt 8), 1969–1978.

Patel, M., Day, B. J., Crapo, J. D., Fridovich, I., & McNamara, J. O. (1996). Requirement for superoxide in excitotoxic cell death. *Neuron, 16*(2), 345–355.

Peng, J., Mao, X. O., Stevenson, F. F., Hsu, M., & Andersen, J. K. (2004). The herbicide paraquat induces dopaminergic nigral apoptosis through sustained activation of the JNK pathway. *Journal of Biological Chemistry, 279*(31), 32626–32632.

Peng, J., Oo, M. L., & Andersen, J. K. (2010). Synergistic effects of environmental risk factors and gene mutations in Parkinson's disease accelerate age-related neurodegeneration. *Journal of Neurochemistry, 115*(6), 1363–1373.

Pesah, Y., Pham, T., Burgess, H., Middlebrooks, B., Verstreken, P., Zhou, Y., Harding, M., Bellen, H., & Mardon, G. (2004). Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development, 131*(9), 2183–2194.

Prigione, A., Piazza, F., Brighina, L., Begni, B., Galbussera, A., Difrancesco, J. C., Andreoni, S., Piolti, R., & Ferrarese, C. (2010). Alpha-synuclein nitration and autophagy response are induced in peripheral blood cells from patients with Parkinson disease. *Neuroscience Letters, 477*(1), 6–10.

Purisai, M. G., McCormack, A. L., Cumine, S., Li, J., Isla, M. Z., & Di Monte, D. A. (2007). Microglial activation as a priming event leading to paraquat-induced dopaminergic cell degeneration. *Neurobiology of Disease, 25*(2), 392–400.

Purser, D. A., & Rose, M. S. (1979). The toxicity and renal handling of paraquat in cynomolgus monkeys. *Toxicology, 15*(1), 31–41.

Ramachandiran, S., Hansen, J. M., Jones, D. P., Richardson, J. R., & Miller, G. W. (2007). Divergent mechanisms of paraquat, MPP^+, and rotenone toxicity: Oxidation of thioredoxin and caspase-3 activation. *Toxicological Sciences, 95*(1), 163–171.

Ravagnan, L., Roumier, T., & Kroemer, G. (2002). Mitochondria, the killer organelles and their weapons. *Journal of Cellular Physiology, 192*(2), 131–137.

Richardson, J. R., Quan, Y., Sherer, T. B., Greenamyre, J. T., & Miller, G. W. (2005). Paraquat neurotoxicity is distinct from that of MPTP and rotenone. *Toxicological Sciences, 88*(1), 193–201.

Röhrdanz, E., Schmuck, G., Ohler, S., & Kahl, R. (2001). The influence of oxidative stress on catalase and MnSOD gene transcription in astrocytes. *Brain Research, 900*(1), 128–136.

Rojo, A. I., Cavada, C., de Sagarra, M. R., & Cuadrado, A. (2007). Chronic inhalation of rotenone or paraquat does not induce Parkinson's disease symptoms in mice or rats. *Experimental Neurology, 208*(1), 120–126.

Rossi, L., Marchese, E., Lombardo, M. F., Rotilio, G., & Ciriolo, M. R. (2001). Increased susceptibility of copper-deficient neuroblastoma cells to oxidative stress-mediated apoptosis. *Free Radical Biology & Medicine, 30*(10), 1177–1187.

Rzezniczak, T. Z., Douglas, L. A., Watterson, J. H., & Merritt, T. J. (2011). Paraquat administration in Drosophila for use in metabolic studies of oxidative stress. *Analytical Biochemistry, 419*(2), 345–347.

Sämann, J., Hegermann, J., von Gromoff, E., Eimer, S., Baumeister, R., & Schmidt, E. (2009). *Caenorhabditits elegans* LRK-1 and PINK-1 act antagonistically in stress response and neurite outgrowth. *Journal of Biological Chemistry, 284*(24), 16482–16491.

Schmuck, G., Röhrdanz, E., Tran-Thi, Q. H., Kahl, R., & Schlüter, G. (2002). Oxidative stress in rat cortical neurons and astrocytes induced by paraquat in vitro. *Neurotoxicity Research, 4*(1), 1–13.

Seidenfeld, J. J., Sobonya, R. E., & Toyoshima, J. M. (1985). Recovery from paraquat pneumonitis. *British Journal of Industrial Medicine, 42*(3), 178–183.

Seidler, A., Hellenbrand, W., Robra, B. P., Vieregge, P., Nischan, P., Joerg, J., Oertel, W. H., Ulm, G., & Schneider, E. (1996). Possible environmental, occupational, and other etiologic factors for Parkinson's disease: A case–control study in Germany. *Neurology, 46*(5), 1275–1284.

Semchuk, K. M., Love, E. J., & Lee, R. G. (1992). Parkinson's disease and exposure to agricultural work and pesticide chemicals. *Neurology, 42*(7), 1328–1335.

Somayajulu-Niţu, M., Sandhu, J. K., Cohen, J., Sikorska, M., Sridhar, T. S., Matei, A., Borowy-Borowski, H., & Pandey, S. (2009). Paraquat induces oxidative stress, neuronal loss in substantia nigra region and parkinsonism in adult rats: Neuroprotection and amelioration of symptoms by water-soluble formulation of coenzyme Q10. *BMC Neuroscience, 10*, 88.

Sone, T., Talbot, A., Harada, T., Ikuo, T., Kato, T., & Uematsu, H. (1989). The effects of 24% paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride) on hemodynamics, blood gases, plasma lactate and plasma catecholamines in dogs. *Veterinary and Human Toxicology, 31*(2), 149–153.

Srikrishna, V., Riviere, J. E., & Monteiro-Riviere, N. A. (1992). Cutaneous toxicity and absorption of paraquat in porcine skin. *Toxicology and Applied Pharmacology, 115*(1), 89–97.

Taira, T., Saito, Y., Niki, T., Iguchi-Ariga, S. M., Takahashi, K., & Ariga, H. (2004). DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Reports, 5*(2), 213–218.

Takahashi, M., Kanuka, H., Fujiwara, H., Koyama, A., Hasegawa, M., Miura, M., & Iwatsubo, T. (2003). Phosphorylation of alpha-synuclein characteristic of synucleinopathy lesions is recapitulated in alpha-synuclein transgenic Drosophila. *Neuroscience Letters, 336*(3), 155–158.

Tanner, C. M., Kamel, F., Ross, G. W., Hoppin, J. A., Goldman, S. M., Korell, M., Marras, C., Bhudhikanok, G. S., Kasten, M., Chade, A. R., Comyns, K., Richards, M. B., Meng, C., Priestley, B., Fernandez, H. H., Cambi, F., Umbach, D. M., Blair, A., Sandler, D. P., & Langston, J. W. (2011). Rotenone, paraquat, and Parkinson's disease. *Environmental Health Perspectives, 119*(6), 866–872.

Thiruchelvam, M., Brockel, B. J., Richfield, E. K., Baggs, R. B., & Cory-Slechta, D. A. (2000). Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: Environmental risk factors for Parkinson's disease? *Brain Research, 873*(2), 225–234.

Thiruchelvam, M. J., Powers, J. M., Cory-Slechta, D. A., & Richfield, E. K. (2004). Risk factors for dopaminergic neuron loss in human alpha-synuclein transgenic mice. *European Journal of Neuroscience, 19*(4), 845–854.

Uversky, V. N., Li, J., & Fink, A. L. (2001). Pesticides directly accelerate the rate of alpha-synuclein fibril formation: A possible factor in Parkinson's disease. *FEBS Letters, 500*(3), 105–108.

Uversky, V. N., Yamin, G., Munishkina, L. A., Karymov, M. A., Millett, I. S., Doniach, S., Lyubchenko, Y. L., & Fink, A. L. (2005). Effects of nitration on the structure and aggregation of alpha-synuclein. *Brain Research. Molecular Brain Research, 134*(1), 84–102.

Ved, R., Saha, S., Westlund, B., Perier, C., Burnam, L., Sluder, A., Hoener, M., Rodrigues, C. M., Alfonso, A., Steer, C., Liu, L., Przedborski, S., & Wolozin, B. (2005). Similar patterns of mitochondrial vulnerability and rescue induced by genetic modification of alpha-synuclein, parkin, and DJ-1 in *Caenorhabditis elegans*. *Journal of Biological Chemistry, 280*(52), 42655–42668.

Vogt, M., Bauer, M. K., Ferrari, D., & Schulze-Osthoff, K. (1998). Oxidative stress and hypoxia/reoxygenation trigger CD95 (APO-1/Fas) ligand expression in microglial cells. *FEBS Letters, 429*(1), 67–72.

Walls, K. C., Ghosh, A. P., Franklin, A. V., Klocke, B. J., Ballestas, M., Shacka, J. J., Zhang, J., & Roth, K. A. (2010). Lysosome dysfunction triggers Atg7-dependent neural apoptosis. *Journal of Biological Chemistry, 285*(14), 10497–10507.

Wang, C., Ko, H. S., Thomas, B., Tsang, F., Chew, K. C., Tay, S. P., Ho, M. W., Lim, T. M., Soong, T. W., Pletnikova, O., Troncoso, J., Dawson, V. L., Dawson, T. M., & Lim, K. L. (2005). Stress-induced alterations in parkin solubility promote parkin aggregation and compromise parkin's protective function. *Human Molecular Genetics, 14*(24), 3885–3897.

Wang, A., Costello, S., Cockburn, M., Zhang, X., Bronstein, J., & Ritz, B. (2011). Parkinson's disease risk from ambient exposure to pesticides. *European Journal of Epidemiology, 26*(7), 547–555.

Wechsler, L. S., Checkoway, H., Franklin, G. M., & Costa, L. G. (1991). A pilot study of occupational and environmental risk factors for Parkinson's disease. *Neurotoxicology, 12*(3), 387–392.

Whitworth, A. J., Wes, P. D., & Pallanck, L. J. (2006). Drosophila models pioneer a new approach to drug discovery for Parkinson's disease. *Drug Discovery Today, 11*(3–4), 119–126.

Wirdefeldt, K., Adami, H. O., Cole, P., Trichopoulos, D., & Mandel, J. (2011). Epidemiology and etiology of Parkinson's disease: A review of the evidence. *European Journal of Epidemiology, 26*(Suppl 1), S1–S58.

Wu, X. F., Block, M. L., Zhang, W., Qin, L., Wilson, B., Zhang, W. Q., Veronesi, B., & Hong, J. S. (2005). The role of microglia in paraquat-induced dopaminergic neurotoxicity. *Antioxidants & Redox Signaling, 7*(5–6), 654–661.

Yanase, S., Yasuda, K., & Ishii, N. (2002). Adaptive responses to oxidative damage in three mutants of *Caenorhabditis elegans* (age-1, mev-1 and daf-16) that affect life span. *Mechanisms of Ageing and Development, 123*(12), 1579–1587.

Yang, W., & Tiffany-Castiglioni, E. (2008). Paraquat-induced apoptosis in human neuroblastoma SH-SY5Y cells: Involvement of p53 and mitochondria. *Journal of Toxicology and Environmental Health. Part A, 71*(4), 289–299.

Yang, W., Tiffany-Castiglioni, E., Koh, H. C., & Son, I. H. (2009). Paraquat activates the IRE1/ASK1/JNK cascade associated with apoptosis in human neuroblastoma SH-SY5Y cells. *Toxicology Letters, 191*(2–3), 203–210.

Yumino, K., Kawakami, I., Tamura, M., Hayashi, T., & Nakamura, M. (2002). Paraquat- and diquat-induced oxygen radical generation and lipid peroxidation in rat brain microsomes. *Journal of Biochemistry, 131*(4), 565–570.

Zaidi, A., Fernandes, D., Bean, J. L., & Michaelis, M. L. (2009). Effects of paraquat-induced oxidative stress on the neuronal plasma membrane Ca(2+)-ATPase. *Free Radical Biology & Medicine, 47*(10), 1507–1514.

Manganese Neurotoxicity

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Abstract

Over the past years there has been considerable progress in our understanding of manganese (Mn)-induced neurotoxicity and its mechanisms. These studies led to changes in Mn safety assessment around the world. However, manganism continues to represent a health concern, especially considering the recent findings linking manganism to Parkinson's disease (PD). Animal models have been invaluable in these investigations. Findings from these studies are discussed in this chapter within the context of Mn-induced neurotoxicity mechanisms and its role in the etiology of parkinsonism.

Keywords

Dopamine • Manganese • Neurotoxicity • Parkinson's disease

1 Introduction

Over the past several decades, there has been considerable progress in our basic knowledge regarding the mechanisms and factors regulating Mn neurotoxicity. The disorder known as manganism is associated with the preferential accumulation of Mn in the globus pallidus of the basal ganglia, which is generally considered to be the major and initial site of injury. Because the area of the central nervous system (CNS) comprising the basal ganglia is complex and dependent on the precise functioning and balance between several neurotransmitters, it is not surprising that symptoms of manganism overlap with those of Parkinson's disease. The fact that neurological symptoms and onset of Mn toxicity are quite broad and can vary unpredictably probably reflects specific genetic variance of the physiological and biochemical makeup within the basal ganglia; however, there is evidence suggesting that persistent exposure can predispose individuals to acquire dystonic movements associated with Idiopathic Parkinson's disease (IPD). This chapter provides a review on Mn-induced neurotoxicity with specific focus on data using various animal models that provided some key findings in an attempt to unravel mechanisms that lead to these syndromes and what they have in common.

2 Manganese Essentiality

Mn is an essential nutrient necessary for a variety of metabolic functions including those involved in normal human development, activation of certain metalloenzymes, energy metabolism, immunological system function, nervous system function, reproductive hormone function, and in antioxidant enzymes that protects cells from damage due to free radicals (ATSDR 2000). In the brain, Mn is an important cofactor for a variety of enzymes, including those involved in neurotransmitter synthesis and metabolism (Erikson and Aschner 2003). The most important source of Mn is the diet, although there is no consensus regarding safe and adequate levels of this nutrient for various age groups.

Mn deficiencies, although not frequently reported, can result in several biochemical and structural defects in experimental animals (Keen et al. 1999). Thus, taking into account the variety of enzymatic processes which are dependent upon Mn, an inadequate daily supply of the metal may cause a variety of health repercussions, ranging from generalized growth impairment, birth defects, reduced fertility, and impaired bone formation to altered metabolism of lipids, proteins, and carbohydrates (Freeland-Graves and Lin 1991; Keen et al. 1999). Mn is a required cofactor for several enzymes; however, it can be replaced by iron, magnesium, or copper. Notably, the majority of Mn-dependent enzymes known are found in bacteria and plants. The well known Mn-dependent mammalian enzymes include arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and manganese superoxide dismutase (MnSOD).

3 Manganese Toxicity

3.1 Sources of Manganese Exposure

There are many sources of Mn exposure which have been of interest in causing neurotoxicity. Table 1 summarizes the most important sources of Mn intoxication.

3.2 Main Routes of Manganese Exposure

3.2.1 Pulmonary Tract

Mn is readily absorbed by the pulmonary tract as it lacks the homeostatic control systems that are present in the GI tract. The bioavailability of the inspired Mn depends on several factors, such as the size, mass, and density of the particle, the site of Mn deposition, the composition of Mn within the particulate matter, and how efficiently the metal is solubilized by macrophages within the lung (Roth 2006). It is believed that, at least in part, three characteristics contribute to Mn's absorption via the pulmonary tract: (1) transport into the olfactory or trigeminal presynaptic nerve endings located in the nasal mucosa, (2) transport across the pulmonary epithelial lining fluid, and/or (3) mucociliary elevator for the lung clearance (Roth 2006).

3.2.2 Gastrointestinal (GI) Tract

Oral intake of Mn is the major physiological route of absorption, occurring mainly through the consumption of water, fruits, and vegetables (Khan et al. 2008). The mechanism by which Mn is absorbed by this route is not completely understood; however, it is believed that both passive and active transport are involved (Garcia-Aranda et al. 1983). In general, the concentration of Mn in the diet influences the amount absorbed through GI tract as well as the amount which is eliminated in the bile. When dietary Mn levels are high, adaptive changes include

Table 1 Sources containing Mn that may lead to intoxication

Source	Examples	Nontoxic doses	Reference
Water		<150 μg/L	Ljung and Vahter 2007; Wasserman et al. 2006
Diet	Teas, vegetables, dietary supplements	0.9–10 mg/day	Finley and Davis 1999; ATSDR 2000
Airborne	Welding fumes, MMT[a], steel foundries, ferromanganese production	<2 μg/day	Lynam et al. 1999; Roth 2009; Zayed et al. 1999
Milk and infant formulas		<105 mg/day	Stastny et al. 1984
Parenteral exposure	TPN[b], radiological contrast	100 μg/L	Bertinet et al. 2000; Ono et al. 1995
Pesticides	Maneb, Mancozeb		Aschner 2000
Drugs	Methcathinone		Sikk et al. 2011

[a]Methylcyclopentadienyl manganese tricarbonyl
[b]Total parenteral nutrition

reduced GI absorption, enhanced Mn liver metabolism, and increased biliary and pancreatic excretion of the metal (Dorman et al. 2001, 2006). Additionally, several factors can influence Mn absorption and retention. For example, dietary intake is influenced by the presence of other trace minerals, phytate, ascorbic acid, and other dietary constituents (Aschner and Gannon 1994; Davidsson et al. 1989).

3.3 Manganese Pharmacokinetics

3.3.1 Absorption

The major source of Mn in humans is via dietary ingestion (Au et al. 2009). Approximately 3–5 % of ingested Mn is absorbed through the intestinal wall, and the rest is excreted in feces, representing a precise homeostatic control over Mn absorption. Its uptake is tightly regulated and any excess of ingested Mn is readily excreted via the bile. In contrast, both pulmonary uptake and particulate transport via the olfactory bulb (Verity 1999; Zayed et al. 1999) can lead to Mn deposition in the striatum and cerebellum and inflammation of the nasal epithelium (Roth 2009).

Iron (Fe) has a strong influence on Mn homeostasis as both metals share physiological transporter [transferrin (Tf)], binding and uptake via the Tf transporter and the divalent metal transporter, DMT1/NRAMP2. In rodent models, Fe deficiency is associated with increased Mn absorption across the gastrointestinal tract, as well increased Mn deposition in the brain (Fitsanakis et al. 2008; Freeland-Graves and Lin 1991; Garcia et al. 2007).

3.3.2 Transport

The identity of the carrier(s) involved in Mn transport into the brain is still controversial. It is believed that at normal plasma concentrations,

Mn enters into the CNS primarily across the capillary endothelium, whereas at high plasma concentrations, transport across the choroid plexus predominates (Murphy et al. 1991). How and in what chemical form Mn is transported across the blood–brain barrier (BBB) has been addressed in series of studies. Mn is absorbed in the GI tract as Mn^{2+}, is oxidized to Mn^{3+} by liver and plasma ceruloplasmin, and transported by transferrin (Tf) (Aschner and Gannon 1994; Takeda et al. 1995). Tf-dependent Mn transport across the BBB has been documented (Aschner and Gannon 1994), but the majority of BBB Mn transport occurs via the DMT1.

A critical regulator of brain Mn levels is the divalent metal transporter, DMT-1/NRAMP-2. DMT-1 (also referred to as the DCT or divalent cation transporter) is known to shuttle both Mn and Fe ions in the (+2) valence, as well as other divalent metals. Mutations in *DMT-1* in the rat or mouse result in significantly lower brain levels and uptake of both Mn and Fe (Chua and Morgan 1997; Fleming et al. 1998). Notably, a recent study (Salazar et al. 2008) has shown that DMT-1 contributes to neurodegeneration in an experimental model of PD.

Additional brain Mn transporters include the Mn-citrate transporters (MCT) and the Mn-bicarbonate symporters (Crossgrove et al. 2003). However, the relevance of these genes to Mn transport in vivo is not completely understood. The Mn-bicarbonate symporters, ZIP-8 and ZIP-14, have been identified as members of the solute carrier-39 and are expressed on brain capillaries (He et al. 2006). These symporters utilize a HCO_3^- gradient as the driving force for Mn uptake across the plasma membrane.

Other possible mechanisms for Mn transport include dopamine transporters (DAT). It is believed that DAT facilitates Mn transport into dopaminergic striatal neurons and that Mn accumulates in the globus pallidus via axonal transport (Anderson et al. 2007). As a result, blockage of the DAT in the striatum attenuates Mn accumulation in striatal neurons and decreases Mn concentrations in the globus pallidus (Anderson et al. 2007). Finally, Mn transport via voltage-regulated channels (Lucaciu et al. 1997), store-operated channels (Riccio et al. 2002), ionotropic glutamate receptor channels (Kannurpatti et al. 2000) (all Ca^{2+} channels), and choline transporters (Lockman et al. 2001) has also been described.

3.3.3 Distribution

Under basal conditions, brain Mn concentrations are heterogeneous, with the highest values reported in the palladium and putamen in humans and in the hypothalamus in rats (Bird et al. 1984; Dorman et al. 2001). The basal ganglia accumulate the highest Mn levels upon excessive exposure to this metal, although the precise physiological basis for this result remains unclear. However, the high expression of DMT1 in several nuclei of the basal ganglia likely contributes to the propensity of Mn to accumulate in this particular region of the brain (Huang et al. 2004). Similarly, high levels of Mn within the olfactory bulb have been associated with the high expression of DMT1 in this region in rats (Fitsanakis et al. 2008). However, further characterization of the absorption and elimination kinetics, as well as Mn uptake and export pathways are necessary to better understand the basis of differential Mn accumulation

in different brain regions. It is worth noting that recent pharmacokinetic modeling of Mn accumulation in the rat required different diffusion constants between brain regions to account for the asymmetry in Mn levels across the brain (Nong et al. 2008).

It is noteworthy that no information exists on the putative extracellular transport mechanisms of Mn; thus, there is no knowledge regarding conditions that affect the removal of Mn from the brain. However, ferroportin-1 protein expression, the only known iron exporter, is induced by Mn exposure in HEK293T cells (and mouse brain), attenuating Mn accumulation and cytotoxicity and strongly suggesting that ferroportin-1 is a putative Mn exporter (Yin et al. 2010).

3.3.4 Excretion

The physiological half-life of Mn in the adult rat and primate brain is approximately 51–74 days (Cotzias et al. 1968; Newland et al. 1987; Takeda et al. 1995). The main excretion mechanism for Mn depends on normal liver function. Indeed, blood Mn concentrations are increased during the active phase of acute hepatitis as well as in post hepatic cirrhosis, and a significant correlation exists between blood Mn and the activities of liver enzymes in patients with hepatitis and cirrhosis (Spahr et al. 1996). Corroborating this hypothesis, magnetic resonance imaging (MRI) has consistently shown signal hyperintensity in the globus pallidus of at least 80 % of cirrhotic patients (Butterworth et al. 1995). Furthermore, direct measurements in pallidal samples obtained from the autopsies of cirrhotic patients revealed several-fold increases of Mn concentrations, and histopathologic evaluations showed Alzheimer's type II astrocytosis (Weissenborn et al. 1995). In fact, hepatic encephalopathy results in cognitive, psychiatric, and motor impairments, which could be related to manganism (Hazell and Butterworth 1999).

4 Manganese Neurotoxicity

It has been known for more than 150 years that Mn is a neurotoxic agent. Mn toxicity has been predominantly observed in occupational settings, following the accidental ingestion of large quantities or after chronic inhalation of high levels (Mergler et al. 1994). The brain is particularly susceptible to excess of this metal, but the mechanisms of toxicity are poorly understood. In humans, it has been postulated that there is a spectrum of neurobehavioral and neurophysiological effects associated with Mn toxicity, including both subclinical and clinical symptoms (Mergler et al. 1994).

Mn was first considered to be neurotoxic when five workers employed in grinding the black oxide of Mn developed an unsteady gait and muscular weakness (Couper 1837). Traditionally, Mn neurotoxicity has been most commonly associated with occupations such as Mn mining and smelting, battery manufacturing, and steel production (Santamaria et al. 2007). Nowadays industrial environments, occupations in battery production, welding operations, and mining represent the main sources of Mn exposure and intoxication (Huang et al. 1989; Roth 2009). Furthermore, non-occupational exposures from contaminated drinking water, milk and infant formulas, total parenteral nutrition (TPN) and air as a result of the combustion of the gasoline

additive, methylcyclopentadienyl manganese tricarbonyl (MMT), also present contemporary health concerns (Aschner and Dorman 2006; Aschner et al. 2009; Santamaria et al. 2007).

Mn neurotoxicity, or *locura manganica*, also referred to as manganism, is a neurologic disorder characterized by psychological and neurological abnormalities which resemble Parkinson's disease (PD) (Barbeau 1984; Huang et al. 1989; Mena et al. 1967), Mn also damages brain areas distinct from those that are affected in PD (Calne et al. 1994; Olanow 2004). The similarities between the clinical manifestations of PD and manganism include the presence of generalized bradykinesia and widespread rigidity, while dissimilarities include less frequent resting tremor, more frequent dystonia, symmetry of effects, a particular propensity to fall backward, a characteristic "cock-walk," in which patients walk on their toes with elbows flexed and spine erect (Calne et al. 1994). There are also differences with respect to treatment response – although there may be an initial response to levodopa, the primary treatment option for PD, there is typically a failure to achieve a sustained therapeutic response in patients with manganism (Aschner et al. 2009; Calne et al. 1994). The similarities between the two disorders can be partially explained by the fact that the basal ganglia accumulate most of the excess Mn compared with other brain regions in manganism, and dysfunction in the basal ganglia is involved in PD (Dobson et al. 2004). PD is primarily associated with the loss of dopaminergic neurons within the substantia nigra, allowing the caudate and putamen to become overly active and possibly cause continuous output of excitatory signals to the corticospinal motor control system (Dobson et al. 2004). The substantia nigra is spared in manganism, which is linked to the degeneration of GABAergic neurons within the globus pallidus in pathways postsynaptic to the nigrostriatal system (Pal et al. 1999).

The symptoms observed in Mn-exposed subjects are believed to be caused by the preferential accumulation of Mn in brain areas which are rich in dopaminergic neurons (DAergic) and are largely involved in motor control. Perturbation of striatal DA levels likely explains the biphasic syndrome experienced by manganism patients. Upon Mn intoxication, an initial phase of increased DA production is associated with psychotic episodes commonly observed in psychiatric patients (Aschner et al. 2009; Roth 2009). As Mn poisoning progresses, catecholamine levels decrease, most likely due to the loss of nigrostriatal DAergic neurons and, consequently, the parkinsonian-like symptoms ensue (Benedetto et al. 2009).

4.1 Manganese and Mitochondria

Different studies point to the mitochondria as the main cellular targets of Mn toxicity. Nominally, mitochondria have been posited to play a central role in the etiology of both Parkinson's disease (PD) and manganism. Due to transport mechanisms that favor the influx of Mn over its efflux, the metal accumulates in this organelle and thus can readily disturb its function (Gavin et al. 1990). Using

imaging techniques, Morello et al. (2008) reported that prior to any ultrastructural damage to neurons and astrocytes, the largest rate of Mn increase was noted in the mitochondrial cytoplasmic matrix of these cells (Morello et al. 2008).

Mn^{2+} is sequestered by mitochondria via the Ca^{2+} uniporter (Chance 1965). Once inside, Mn^{2+} can trigger mitochondrial dysfunction through several mechanisms, primarily involving oxidative stress. Oxidative stress can also occur as a consequence of mitochondrial dysfunction caused by Mn. Studies of postmortem PD brains show damage to the substantia nigra (SN) consistent with generation of reactive oxygen and nitrogen species (ROS, RNS), characterized by lipid peroxidation, protein oxidation, 3-nitrotyrosine formation, DNA oxidation, DNA breaks and a decrease in the activities of the ROS scavenging enzymes glutathione peroxidase and superoxide dismutase (Bowman et al. 2011). Mn can also impair mitochondrial function by inhibiting the mitochondrial electron transfer chain. Zhang et al. (2004) reported that Mn^{2+} affects the activity of respiratory chain enzymes, inhibiting complexes I–IV and interfering with oxidative phosphorylation (Zhang et al. 2004). Furthermore, it has been shown that Mn can bind to a specific Mg^{2+} site on the F1-ATPase, slowing its activity (Smith et al. 1985). Such effects result in a decline of ATP production, leading to increased leakage of electrons and increased O^{2-} production, thereby causing the eventual collapse of cellular function, which may be followed by cell death (Scholte 1988).

Mn also alters calcium homeostasis in mitochondria by inhibiting its efflux and increasing its levels in the organelle (Gavin et al. 1990). It has been widely reported that elevated calcium levels associated with ROS production result in the opening of the mitochondrial permeability transition pore (MPT), increasing the permeability to protons, ions and solutes (Zoratti and Szabo 1995). As a consequence, there is loss of the inner membrane potential ($\Delta\psi m$), leading to mitochondrial swelling, impairment of oxidative phosphorylation and ATP synthesis (Gunter and Pfeiffer 1990). Indeed, Mn has been found to decrease $\Delta\psi m$ in a concentration dependent manner, indicating that this calcium-dependent process may occur during Mn neurotoxicity (Gunter and Pfeiffer 1990; Milatovic et al. 2007; Yin et al. 2008).

Additionally, apoptotic mechanisms may be also related to Mn neurotoxicity. Calcium-induced MPT opening leads to the activation of the Bcl-2 family of proteins, especially Bax/Bac, culminating with the release of cytochrome c (Cyt c) (Green and Reed 1998; Korsmeyer et al. 2000). Cyt c activates, via the ERK pathway, the cysteine protease caspase-3, the executioner protein, which is responsible for mediating apoptosis, chromatin condensation and DNA fragmentation (Petit et al. 1996). Indeed, Yin et al. (2008) demonstrated that Mn exposure in astrocytes results in ERK and caspase-3 activation. Further, Malecki (2001) observed DNA strand breakage at Mn concentrations as low as 5 μM, reinforcing this mechanistic hypothesis.

In addition, the overexpression of wild-type human α-synuclein in *C. elegans* increases vulnerability to mitochondrial complex I inhibitors, such as rotenone, fenperoximate, pyridaben, and stigmatellin (Weissenborn et al. 1995). α-Synuclein overexpression has also been recently shown to enhance Mn-induced neurotoxicity via the NF-kB-mediated pathway (Hazell and Butterworth 1999) in a mesencephalic cell line (MES 23.5). Mn also induces the overexpression of α-synuclein in

PC12 cells via ERK activation (Zlotkin et al. 1995) and chronic exposure to Mn decreases striatal dopamine turnover in human α-synuclein transgenic mice (Butterworth et al. 1995).

4.2 Dopaminergic System

Dopamine (DA) is one of the most prevalent catecholamines within the brain; its signaling helps control locomotion, emotion and neuroendrocrine secretion (Cooper et al. 1996). In this regard, research conducted on Mn toxicity either focuses on the effect on the DA transporter (DAT) or the DA receptor. Postnatal Mn exposure causes a decline in presynaptic DAergic functioning, such as DAT protein expression and DA uptake in the striatum, and a long-term decrease in DA efflux (Huang et al. 2003; McDougall et al. 2008). In adult animal models, there is strong evidence to suggest that exposure to Mn can inhibit DA neurotransmission and deplete striatal DA (Chen et al. 2006; Pal et al. 1999; Reichel et al. 2006), thereby resulting in the observed motor deficits (see review, Guilarte 2010). Importantly, chronic exposure to Mn also decreases striatal DA turnover in human alpha-synuclein transgenic mice (Peneder et al. 2011).

The two main families of DA receptors responsible for DA regulation are D1- and D2-receptors. D1-receptors are G-protein-coupled receptors that stimulate adenylate cyclase activity, while D2-receptors inhibit adenylate cyclase activity (Baldessarini and Tarazi 1996; Hazelwood et al. 2008). A recent study has demonstrated that striatal D2-receptors are not affected by Mn intoxication in monkeys (Eriksson et al. 1992); nevertheless, other studies have shown that the density of D2-receptors is lowered in developing rats treated with Mn (Seth and Chandra 1984).

The direct interaction between Mn and DA is also noteworthy. Both Mn^{2+} and Mn^{3+} are known to react with DA via the Fenton‘s reaction, catalyzing the auto-oxidation of DA and generating reactive oxygen species (ROS), thereby leading to oxidative damage (Donaldson et al. 1981, 1982; Lloyd 1995; Stokes et al. 1999). Mn-induced DA auto-oxidation relies on various mechanisms involving semi-quinone and aminochrome intermediates, L-cysteine or copper (Cu) and NADH facilitation (Graumann et al. 2002; Lloyd 1995). Mn oxidant generation is believed to depend on its oxidation state, and oxidative damage is more pronounced for Mn^{3+} versus Mn^{2+} oxidation state (Reaney and Smith 2005).

5 Manganese and Parkinson’s Disease

Idiopathic Parkinson’s disease (IPD) is a progressive neurodegenerative disorder with a slow onset, and compared with the familial forms of the disease, it is associated with advanced age (>55 years old), tremors at rest, bradykinesia, rigidity, and postural instability (Jankovic 2008; Lees et al. 2009; Tolosa et al. 2006).

Molecular imaging is a useful strategy for the diagnosis of PD and has provided extensive evidence that PD patients exhibit decreased levels of presynaptic dopamine neuron terminal markers in the striatum (Felicio et al. 2009). This is consistent with the loss of DA terminals as a result of degeneration of neuronal cell bodies in the substantia nigra pars compacta. Single positron emission tomography studies have shown that PD patients exhibit decreased levels of DA transporters (DATs) and vesicular monoamine transporter type 2(VMAT2) and reduced activity of dopa decarboxylase as measured by the conversion of L-DOPA to dopamine in the striatum (Felicio et al. 2009).

As previously described in this chapter, Mn causes oxidative stress, mitochondrial dysfunction, DAergic degeneration, among other molecular damages, which culminate with an extrapyramidal syndrome called parkinsonism, because of the similarities with PD. While the underlying etiology of the majority of cases of IPD is unknown, there is evidence that exposure to Mn, even at relatively low levels, can result in a spectrum of neurologic impairment including parkinsonism and perhaps IPD itself (Meeker et al. 2007). Manganism like PD is associated with altered neurological dysfunction of the basal ganglia, and because symptoms often overlap between the two, incorrect diagnosis can occur. This is especially true in the latter stages of the disorder when it is feasible that manganism may actually manifest in more Parkinson's-like features in susceptible individuals (Roth 2009).

Characterization of this neurological disorder and investigation of the mechanisms was possible due to research of Mn neurotoxicity in different animal models. A suitable animal model for parkinsonism is histopathologically characterized by the progressive and significant loss of DA neurons and the loss of some non-DA neurons. Moreover, animal models readily mimic the clinical manifestations of the human descriptions.

5.1 Studies in *Drosophila melanogaster*

Drosophila has a relatively simple central nervous system, showing progressive degeneration of DAergic neurons, formation of Lewy bodies, and impairment of climbing activity in PD models (Barone et al. 2011; Feany and Bender 2000). There are about 200 DAergic neurons in the flies; however, they are responsible for modulating a wide range of behaviors ranging from sleep and locomotion to courtship and learning (Van Swinderen and Andretic 2011).

Under chronic Mn exposure, flies show reduced survival, life-span, and reduced locomotor activity, with Mn showing greater toxic effects than iron and copper (Bonilla-Ramirez et al. 2011). Mn accumulates in the head of the flies, and the number of DAergic neuronal clusters labeled with green fluorescent protein (GFP) is reduced upon Mn treatment (Bonilla-Ramirez et al. 2011). This study also showed that Mn-induced DAergic degeneration was associated with impairment in the climbing behavior and mobility shortage analogous to fly PD models.

5.2 Studies in *Caenorhabditis elegans*

The nematode *Caenorhabditis elegans* offers several advantages as a tool for studying neurotoxicity, including its relatively short life-span, lasting 20 days on average, and low costs in growing and maintaining large colonies (Link 2006). Genetic, genomic, and chemical mutant screens are more easily performed in *C. elegans* than in most other experimental species. Compound screening using *C. elegans* is also less time-consuming, due to fast reproduction and a high progeny number (Schmidt et al. 2007). The basic cell biology and biochemistry of nematodes overlap with mammals, including similar ion channels, neurotransmitters (e.g., DA, serotonin, acetylcholine, GABA), vesicular transporters, receptors, and synaptic components, particularly the ones that form the DAergic system (Bargmann 1998). The wiring diagram of the nematode nervous system consists of a defined set of 302 neurons, of which eight are dopaminergic (Gitler et al. 2009). Several worm behaviors are related to DAergic function which also enables to evaluate the effects of the degeneration in this model.

Mn exposure in worms has been associated with morphological alterations, reduced longevity, oxidative stress, and behavioral alterations, such as decreased head trash and body-bend frequencies (Au et al. 2009; Benedetto et al. 2010; Settivari et al. 2009; Wang and Xing 2008). Particularly, DAergic degenerations were observed after acute exposure to this metal (Benedetto et al. 2010; Settivari et al. 2009). Benedetto et al., making use of various genetic strains of *C. elegans* in conjunction with the fluorescent-tagging of different classes of neuron types, have demonstrated that Mn causes a dose-dependent and specific degeneration of DAergic neurons (Benedetto et al. 2010). This effect was absent in GABAergic, serotonergic, or glutamatergic neurons. A novel finding also showed that this Mn-induced degeneration requires the presence of the reuptake transporter, dopamine transporter 1 (DAT-1), as *dat-1* loss-of-function abolished the Mn-induced DAergic loss in the *dat-1*;Pdat-1::GFP worms (Benedetto et al. 2010). In addition, Mn toxicity was reduced by the loss of tyrosine hydroxylase (TH)/CAT-2 function in the double knockout strain, *cat-2(e1112);dat-1(ok157)*, establishing that proper DA synthesis is required for the DAT-1-dependent Mn-induced toxicity. Furthermore, the absence of vesicular monoamine transporter (VMAT2)/CAT-1 in the *cat-1(e1111)* mutants, where the DAergic neurons are unable to release DA at the synaptic cleft, resulted in increased tolerance to Mn exposure, indicating that extracellular, but not intracellular DA, is involved in mediating Mn-induced toxicity (Benedetto et al. 2010). Corroborating this finding, loss-of-function of the three DA receptors [*dop-1(vs100), dop-2(vs105)*, *dop-3(vs106)*] led to increase in extracellular DA, exacerbating Mn toxicity (Benedetto et al. 2010).

The ability of Mn to cause specific DAergic degeneration was linked to the presence in worms of orthologues of the mammalian divalent metal transporter-1 (DMT-1)/NRAMP2 (natural resistance-associated macrophage protein 2). Two of three gene orthologues in worms (*smf-1* and *smf-3)* are mostly localized in the digestive system but are also present in DAergic neurons (Au et al. 2009; Settivari et al. 2009). Decreased susceptibility to Mn and its accumulation in *smf-1* and *smf-3*

knockout worms demonstrated the involvement of SMF-1 and SMF-3 in Mn uptake in *C. elegans*. Work by Settivari et al. also indicated a role for *smf* in Mn neurotoxicity, demonstrating that genetic knockdown or deletion of *smf-1* and *smf-2* partially inhibits DAergic neuronal death (Settivari et al. 2009).

Pesticides containing Mn are also a source of exposure. Mancozeb (MZ), whose active ingredient is Mn/zinc (Zn)-ethylene-bis-dithiocarbamate (Mn/Zn-EBDC) caused mitochondrial dysfunction and GABAergic and DAergic degeneration in worms (Negga et al. 2011a, b). The use of MZ in the crops has been increasing, yet it has been linked to PD and parkinsonism in humans (Israeli et al. 1983).

5.3 Studies in Rodents

Knowledge regarding Mn neurotoxicity and its relation to IPD has been largely derived from rodent studies. A welding fume exposure in rats that corresponded to ~5 years for humans led to alterations in reduced tyrosine hydroxylase levels in the striatum and, more interestingly, to alterations in PD-related proteins expression (Sriram et al. 2010). For instance, there was decrease in the expression of Park5 and Park7. Park5 is involved in the ubiquitin-proteasome system and compromised function or reduced expression of this protein has been associated to IPD (Choi et al. 2004). Park7 mutations account for ~1–2 % of early onset cases of PD, and its reduction may be related to the decrease in mitochondrial integrity (Sriram et al. 2010).

Another PD-related gene that has been investigated is α-synuclein. The deposition of this small protein in Lewy bodies due to three point mutations in the α-synuclein gene is a molecular hallmark of familial PD (Ulmer and Bax 2005). It has been reported that Mn can induce overexpression of α-synuclein in PC12 cells, which reduces cellular viability (Cai et al. 2010). Furthermore, Mn exposure in rat-derived primary cerebellar neuron culture increased levels of α- synuclein associated to increased oxidative stress (Tong et al. 2009). Furthermore, rat mesencephalic cells expressing α-synuclein showed higher neurotoxicity to Mn, an effect that occurred through NF-κB pathway activation (Prabhakaran et al. 2011). Combined, these important studies link PD-related genes and manganism, indicating that Mn may increase susceptibility to PD-inherent mutations.

5.4 Studies in Nonhuman Primates

The use of nonhuman primates to study Mn-induced neurotoxicity is of great relevance, considering that they are closely related to humans in relation to genetics, biology, neurotransmission systems, and particularly, behavior. Historically, nonhuman primate studies can be divided into two categories. The studies from the 1960s to the early 1990s used Mn doses that were considerably higher than those used in studies from 1995 to 2008 (for a review, see Guilarte (2010)). Most studies before 1995 used cumulative doses >300 mg/kg body weight. These early studies did not use

neuroimaging techniques because of their more recent availability, with the exception of Newland and Weiss (1992), who administered very low doses of Mn and used T1-weighted magnetic resonance imaging (MRI) (Newland and Weiss 1992).

Administration of exceedingly high doses of Mn (approximately 300 mg/kg/day) caused loss of postural stability, excitability, hypoactivity, falling, muscular rigidity, tremors, unsteady gait and loss of power in limbs (Eriksson et al. 1992; Neff et al. 1969; Pentschew et al. 1963). Notably, chronic exposure to exceedingly high doses (10 and 15 mg/kg/day) of Mn for >272 days can also cause serious cognitive, behavioral, and motor impairments (Schneider et al. 2006). For instance, videotaped animals showed increased frequency in certain stereotyped behaviors, such as increase in grooming and licking fingers and decrease in climbing in the cage (Schneider et al. 2006).

Decreased DA levels in monkeys brains were usually described (Chandra et al. 1979; Dorman et al. 2006; Newland et al. 1987). However, depending on the exposure design and route of exposure, the areas of decreased dopamine vary from one report to another. Consistently, basal ganglia (especially striatum and globus pallidus) are the brain regions that are more often described as having decreased levels of the neurotransmitter (Bird et al. 1984; Chandra et al. 1979; Eriksson et al. 1987), associated with higher Mn accumulation (Dorman et al. 2006, 2008; Erikson et al. 2007).

5.5 Studies in Humans

As pointed out by Perl and Olanow, a very limited number of autopsy studies have been performed on workers who were exposed to Mn or on persons with other related conditions in which Mn would be increased in the brain (Perl and Olanow 2007). Recently, noninvasive imaging techniques to measure Mn effects in the brain have been tested with relative success. For instance, PET (positron emission tomography) scan imaging with 6-[(18)F]fluoro-l-dopa (FDOPA) showed that asymptomatic welders have presynaptic nigrostriatal dopaminergic dysfunction with different anatomical localization from the symptomatic IPD patients analyzed (Criswell et al. 2011). In addition, specific tests showed that these asymptomatic welders were showing motor impairment (Criswell et al. 2011).

Several other authors have proposed that occupational exposure to Mn is a risk factor for parkinsonism (Gorell et al. 1999; Kim et al. 1999; Racette et al. 2001, 2005). This is also verified in reports demonstrating increased frequency of parkinsonism in communities near these occupational sources, becoming an environmental issue. An epidemiological study investigated the risk of Parkinson's disease in the cities of Toronto and Hamilton, Canada, in relationship with industrial emissions of Mn as well as the use of MMT as a gasoline additive (Finkelstein and Jerrett 2007). Subjects were assessed based on residence location to traffic-generated air pollution and neighborhood levels of ambient Mn. Results of this study found an association between ambient levels of airborne Mn in Hamilton and the risk of physician-diagnosed Parkinson's disease. Their statistical modeling

suggests that exposure to ambient Mn correlates with early onset of parkinsonism, consistent with the premise that chronic exposure to Mn adds to the inherent loss of neurons triggered by the normal aging process. A more recent study by Lucchini et al. (2007) evaluated the incidence of Parkinson's disease in pre-Alps Valcamonica valley area adjacent to three ferroalloy plants (Lucchini et al. 2007). These authors indicate that the prevalence of parkinsonian disorders in this area was higher than elsewhere in Italy and Europe having a high crude (296/100,000) and sex- and age-standardized prevalence (407/100,000) when compared to other national and international data (overall prevalence of parkinsonism of 108–257/100,000 in Europe; 157.7/100,000 in Italy) (von Campenhausen et al. 2005).

Mn neurotoxicity is also of great concern. Case reports have been published on patients with hepatic diseases, such as cirrhosis and hepatitis. These patients develop encephalopathy and parkinsonian behavior (Dharmasaroja 2010; Mendieta Zeron et al. 2011; Olanow 2004), associated with reduced liver Mn clearance and increased Mn blood levels, as well as neuronal accumulation, as indicated by the increased t1-weighted signals in MRI (Chalela et al. 2011; Kulisevsky et al. 1993; Rivera-Mancia et al. 2011). Parenteral nutrition has also been linked to encephalopathy and extra-pyramidal symptoms, as the daily doses administered in these preparations are 5–6-fold higher than the required dose (Chalela et al. 2011; Hardy 2009).

6 Conclusion

Over the past few decades considerable progress has been made in understanding Mn neurotoxicity and its mechanisms. Given that Mn accumulates and affects neurons in the basal ganglia, it is not surprising that symptoms of Mn neurotoxicity overlap with those of PD. The studies reviewed herein illustrate that Mn can accelerate the onset of parkinsonism in exposed individuals and that the accumulated Mn in the basal ganglia, which includes both the globus pallidus and the substantia nigra, can lead to DAergic neuronal degeneration and parkinsonism. Furthermore, some subpopulations may display increased vulnerability to Mn-induced neurological deficits becoming most prevalent in late age. The question on whether chronic exposure to Mn directly leads to the development of IPD is difficult to resolve and will require additional studies.

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References

Anderson, J. G., Cooney, P. T., & Erikson, K. M. (2007). Brain manganese accumulation is inversely related to gamma-amino butyric acid uptake in male and female rats. *Toxicological Sciences, 95*, 188–195.

Aschner, M. (2000). Manganese: Brain transport and emerging research needs. *Environmental Health Perspectives, 108*(Suppl 3), 429–432.

Aschner, M., & Gannon, M. (1994). Manganese (Mn) transport across the rat blood–brain barrier: Saturable and transferrin-dependent transport mechanisms. *Brain Research Bulletin, 33*, 345–349.

Aschner, M., & Dorman, D. C. (2006). Manganese: Pharmacokinetics and molecular mechanisms of brain uptake. *Toxicological Reviews, 25*, 147–154.

Aschner, M., Erikson, K. M., Herrero Hernandez, E., & Tjalkens, R. (2009). Manganese and its role in Parkinson's disease: From transport to neuropathology. *Neuromolecular Medicine, 11*, 252–266.

ATSDR (Agency of Toxic Substances and Disease Registry). (2000). *Toxicological profile for manganese*. Atlanta: U.S. Department of Health and Human Services Public Health Service.

Au, C., Benedetto, A., Anderson, J., Labrousse, A., Erikson, K., Ewbank, J. J., & Aschner, M. (2009). SMF-1, SMF-2 and SMF-3 DMT1 orthologues regulate and are regulated differentially by manganese levels in *C. elegans*. *PloS One, 4*, e7792.

Baldessarini, R. J., & Tarazi, F. I. (1996). Brain dopamine receptors: A primer on their current status, basic and clinical. *Harvard Review of Psychiatry, 3*, 301–325.

Barbeau, A. (1984). Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C. Cotzias). *Neurotoxicology, 5*, 13–35.

Bargmann, C. I. (1998). Neurobiology of the *Caenorhabditis elegans* genome. *Science, 282*, 2028–2033.

Barone, M. C., Sykiotis, G. P., & Bohmann, D. (2011). Genetic activation of Nrf2 signaling is sufficient to ameliorate neurodegenerative phenotypes in a Drosophila model of Parkinson's disease. *Disease Models & Mechanisms, 4*, 701–707.

Benedetto, A., Au, C., & Aschner, M. (2009). Manganese-induced dopaminergic neurodegeneration: Insights into mechanisms and genetics shared with Parkinson's disease. *Chemical Reviews, 109*, 4862–4884.

Benedetto, A., Au, C., Avila, D. S., Milatovic, D., & Aschner, M. (2010). Extracellular dopamine potentiates mn-induced oxidative stress, lifespan reduction, and dopaminergic neurodegeneration in a BLI-3-dependent manner in *Caenorhabditis elegans*. *PLoS Genetics, 6*. pii: e1001084. doi:10.1371/journal.pgen.1001084.

Bertinet, D. B., Tinivella, M., Balzola, F. A., de Francesco, A., Davini, O., Rizzo, L., Massarenti, P., Leonardi, M. A., & Balzola, F. (2000). Brain manganese deposition and blood levels in patients undergoing home parenteral nutrition. *JPEN. Journal of Parenteral and Enteral Nutrition, 24*, 223–227.

Bird, E. D., Anton, A. H., & Bullock, B. (1984). The effect of manganese inhalation on basal ganglia dopamine concentrations in rhesus monkey. *Neurotoxicology, 5*, 59–65.

Bonilla-Ramirez, L., Jimenez-Del-Rio, M., & Velez-Pardo, C. (2011). Acute and chronic metal exposure impairs locomotion activity in *Drosophila melanogaster*: A model to study Parkinsonism. *Biometals, 24*, 1045–1057.

Bowman, A. B., Kwakye, G. F., Herrero Hernandez, E., & Aschner, M. (2011). Role of manganese in neurodegenerative diseases. *Journal of Trace Elements in Medicine and Biology, 25*(4), 191–203.

Butterworth, R. F., Spahr, L., Fontaine, S., & Layrargues, G. P. (1995). Manganese toxicity, dopaminergic dysfunction and hepatic encephalopathy. *Metabolic Brain Disease, 10*, 259–267.

Cai, T., Yao, T., Zheng, G., Chen, Y., Du, K., Cao, Y., Shen, X., Chen, J., & Luo, W. (2010). Manganese induces the overexpression of alpha-synuclein in PC12 cells via ERK activation. *Brain Research, 1359*, 201–207.

Calne, D. B., Chu, N. S., Huang, C. C., Lu, C. S., & Olanow, W. (1994). Manganism and idiopathic parkinsonism: Similarities and differences. *Neurology, 44*, 1583–1586.

Chalela, J. A., Bonillha, L., Neyens, R., & Hays, A. (2011). Manganese encephalopathy: An under-recognized condition in the intensive care unit. *Neurocritical Care, 14*, 456–458.

Chance, B. (1965). The energy-linked reaction of calcium with mitochondria. *Journal of Biological Chemistry, 240*, 2729–2748.

Chandra, S. V., Srivastava, R. S., & Shukla, G. S. (1979). Regional distribution of metals and biogenic amines in the brain of monkeys exposed to manganese. *Toxicology Letters, 4*, 189–192.

Chen, M. K., Lee, J. S., McGlothan, J. L., Furukawa, E., Adams, R. J., Alexander, M., Wong, D. F., & Guilarte, T. R. (2006). Acute manganese administration alters dopamine transporter levels in the non-human primate striatum. *Neurotoxicology, 27*, 229–236.

Choi, J., Levey, A. I., Weintraub, S. T., Rees, H. D., Gearing, M., Chin, L. S., & Li, L. (2004). Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. *Journal of Biological Chemistry, 279*, 13256–13264.

Chua, A. C., & Morgan, E. H. (1997). Manganese metabolism is impaired in the Belgrade laboratory rat. *Journal of Comparative Physiology. B, 167*, 361–369.

Cooper, J., Bloom, F., & Roth, R. (1996). *Dopamine*. New York: Oxford University Press.

Cotzias, G. C., Horiuchi, K., Fuenzalida, S., & Mena, I. (1968). Chronic manganese poisoning. Clearance of tissue manganese concentrations with persistance of the neurological picture. *Neurology, 18*, 376–382.

Couper, J. (1837). On the effects of black oxide of manganese when inhaled into the lungs. *British Annals of Medicine and Pharmacology, 1*, 41–42.

Criswell, S. R., Perlmutter, J. S., Videen, T. O., Moerlein, S. M., Flores, H. P., Birke, A. M., & Racette, B. A. (2011). Reduced uptake of [(1)F] FDOPA PET in asymptomatic welders with occupational manganese exposure. *Neurology, 76*, 1296–1301.

Crossgrove, J. S., Allen, D. D., Bukaveckas, B. L., Rhineheimer, S. S., & Yokel, R. A. (2003). Manganese distribution across the blood–brain barrier. I. Evidence for carrier-mediated influx of managanese citrate as well as manganese and manganese transferrin. *Neurotoxicology, 24*, 3–13.

Davidsson, L., Cederblad, A., Lonnerdal, B., & Sandstrom, B. (1989). Manganese retention in man: A method for estimating manganese absorption in man. *American Journal of Clinical Nutrition, 49*, 170–179.

Dharmasaroja, P. (2010). Signal intensity loss on T2-weighted gradient-recalled echo magnetic resonance images in the basal ganglia in a patient with chronic hepatic encephalopathy. *The Neurologist, 16*, 265–268.

Dobson, A. W., Erikson, K. M., & Aschner, M. (2004). Manganese neurotoxicity. *Annals of the New York Academy of Sciences, 1012*, 115–128.

Donaldson, J., LaBella, F. S., & Gesser, D. (1981). Enhanced autoxidation of dopamine as a possible basis of manganese neurotoxicity. *Neurotoxicology, 2*, 53–64.

Donaldson, J., McGregor, D., & LaBella, F. (1982). Manganese neurotoxicity: A model for free radical mediated neurodegeneration? *Canadian Journal of Physiology and Pharmacology, 60*, 1398–1405.

Dorman, D. C., Struve, M. F., James, R. A., McManus, B. E., Marshall, M. W., & Wong, B. A. (2001). Influence of dietary manganese on the pharmacokinetics of inhaled manganese sulfate in male CD rats. *Toxicological Sciences, 60*, 242–251.

Dorman, D. C., Struve, M. F., Marshall, M. W., Parkinson, C. U., James, R. A., & Wong, B. A. (2006). Tissue manganese concentrations in young male rhesus monkeys following subchronic manganese sulfate inhalation. *Toxicological Sciences, 92*, 201–210.

Dorman, D. C., Struve, M. F., Norris, A., & Higgins, A. J. (2008). Metabolomic analyses of body fluids after subchronic manganese inhalation in rhesus monkeys. *Toxicological Sciences, 106*, 46–54.

Erikson, K. M., & Aschner, M. (2003). Manganese neurotoxicity and glutamate-GABA interaction. *Neurochemistry International, 43*, 475–480.

Erikson, K. M., Dorman, D. C., Lash, L. H., & Aschner, M. (2007). Manganese inhalation by rhesus monkeys is associated with brain regional changes in biomarkers of neurotoxicity. *Toxicological Sciences, 97*, 459–466.

Eriksson, H., Magiste, K., Plantin, L. O., Fonnum, F., Hedstrom, K. G., Theodorsson-Norheim, E., Kristensson, K., Stalberg, E., & Heilbronn, E. (1987). Effects of manganese oxide on monkeys as revealed by a combined neurochemical, histological and neurophysiological evaluation. *Archives of Toxicology, 61*, 46–52.

Eriksson, H., Tedroff, J., Thuomas, K. A., Aquilonius, S. M., Hartvig, P., Fasth, K. J., Bjurling, P., Langstrom, B., Hedstrom, K. G., & Heilbronn, E. (1992). Manganese induced brain lesions in *Macaca fascicularis* as revealed by positron emission tomography and magnetic resonance imaging. *Archives of Toxicology, 66*, 403–407.

Feany, M. B., & Bender, W. W. (2000). A Drosophila model of Parkinson's disease. *Nature, 404*, 394–398.

Felicio, A. C., Shih, M. C., Godeiro-Junior, C., Andrade, L. A., Bressan, R. A., & Ferraz, H. B. (2009). Molecular imaging studies in Parkinson disease: Reducing diagnostic uncertainty. *The Neurologist, 15*, 6–16.

Finkelstein, M. M., & Jerrett, M. (2007). A study of the relationships between Parkinson's disease and markers of traffic-derived and environmental manganese air pollution in two Canadian cities. *Environmental Research, 104*, 420–432.

Finley, J. W., & Davis, C. D. (1999). Manganese deficiency and toxicity: Are high or low dietary amounts of manganese cause for concern? *Biofactors, 10*, 15–24.

Fitsanakis, V. A., Zhang, N., Anderson, J. G., Erikson, K. M., Avison, M. J., Gore, J. C., & Aschner, M. (2008). Measuring brain manganese and iron accumulation in rats following 14 weeks of low-dose manganese treatment using atomic absorption spectroscopy and magnetic resonance imaging. *Toxicological Sciences, 103*, 116–124.

Fleming, M. D., Romano, M. A., Su, M. A., Garrick, L. M., Garrick, M. D., & Andrews, N. C. (1998). Nramp2 is mutated in the anemic Belgrade (b) rat: Evidence of a role for Nramp2 in endosomal iron transport. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 1148–1153.

Freeland-Graves, J. H., & Lin, P. H. (1991). Plasma uptake of manganese as affected by oral loads of manganese, calcium, milk, phosphorus, copper, and zinc. *Journal of the American College of Nutrition, 10*, 38–43.

Garcia, S. J., Gellein, K., Syversen, T., & Aschner, M. (2007). Iron deficient and manganese supplemented diets alter metals and transporters in the developing rat brain. *Toxicological Sciences, 95*, 205–214.

Garcia-Aranda, J. A., Wapnir, R. A., & Lifshitz, F. (1983). In vivo intestinal absorption of manganese in the rat. *Journal of Nutrition, 113*, 2601–2607.

Gavin, C. E., Gunter, K. K., & Gunter, T. E. (1990). Manganese and calcium efflux kinetics in brain mitochondria. Relevance to manganese toxicity. *Biochemical Journal, 266*, 329–334.

Gitler, A. D., Chesi, A., Geddie, M. L., Strathearn, K. E., Hamamichi, S., Hill, K. J., Caldwell, K. A., Caldwell, G. A., Cooper, A. A., Rochet, J. C., & Lindquist, S. (2009). Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nature Genetics, 41*, 308–315.

Gorell, J. M., Johnson, C. C., Rybicki, B. A., Peterson, E. L., Kortsha, G. X., Brown, G. G., & Richardson, R. J. (1999). Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. *Neurotoxicology, 20*, 239–247.

Graumann, R., Paris, I., Martinez-Alvarado, P., Rumanque, P., Perez-Pastene, C., Cardenas, S. P., Marin, P., Diaz-Grez, F., Caviedes, R., Caviedes, P., & Segura-Aguilar, J. (2002). Oxidation of dopamine to aminochrome as a mechanism for neurodegeneration of dopaminergic systems in Parkinson's disease. Possible neuroprotective role of DT-diaphorase. *Polish Journal of Pharmacology, 54*, 573–579.

Green, D. R., & Reed, J. C. (1998). Mitochondria and apoptosis. *Science, 281*, 1309–1312.

Guilarte, T. R. (2010). Manganese and Parkinson's disease: A critical review and new findings. *Environmental Health Perspectives, 118*, 1071–1080.

Gunter, T. E., & Pfeiffer, D. R. (1990). Mechanisms by which mitochondria transport calcium. *American Journal of Physiology, 258*, C755–C786.

Hardy, G. (2009). Manganese in parenteral nutrition: Who, when, and why should we supplement? *Gastroenterology, 137*, S29–S35.

Hazell, A. S., & Butterworth, R. F. (1999). Hepatic encephalopathy: An update of pathophysiologic mechanisms. *Proceedings of the Society for Experimental Biology and Medicine, 222*, 99–112.

Hazelwood, L. A., Free, R. B., Cabrera, D. M., Skinbjerg, M., & Sibley, D. R. (2008). Reciprocal modulation of function between the D1 and D2 dopamine receptors and the Na+, K+−ATPase. *Journal of Biological Chemistry, 283*, 36441–36453.

He, L., Girijashanker, K., Dalton, T. P., Reed, J., Li, H., Soleimani, M., & Nebert, D. W. (2006). ZIP8, member of the solute-carrier-39 (SLC39) metal-transporter family: Characterization of transporter properties. *Molecular Pharmacology, 70*, 171–180.

Huang, C. C., Chu, N. S., Lu, C. S., Wang, J. D., Tsai, J. L., Tzeng, J. L., Wolters, E. C., & Calne, D. B. (1989). Chronic manganese intoxication. *Archives of Neurology, 46*, 1104–1106.

Huang, C. C., Weng, Y. H., Lu, C. S., Chu, N. S., & Yen, T. C. (2003). Dopamine transporter binding in chronic manganese intoxication. *Journal of Neurology, 250*, 1335–1339.

Huang, E., Ong, W. Y., & Connor, J. R. (2004). Distribution of divalent metal transporter-1 in the monkey basal ganglia. *Neuroscience, 128*, 487–496.

Israeli, R., Sculsky, M., & Tiberin, P. (1983). Acute intoxication due to exposure to maneb and zineb. A case with behavioral and central nervous system changes. *Scandinavian Journal of Work, Environment & Health, 9*, 47–51.

Jankovic, J. (2008). Parkinson's disease: Clinical features and diagnosis. *Journal of Neurology, Neurosurgery & Psychiatry, 79*, 368–376.

Kannurpatti, S. S., Joshi, P. G., & Joshi, N. B. (2000). Calcium sequestering ability of mitochondria modulates influx of calcium through glutamate receptor channel. *Neurochemical Research, 25*, 1527–1536.

Keen, C. L., Ensunsa, J. L., Watson, M. H., Baly, D. L., Donovan, S. M., Monaco, M. H., & Clegg, M. S. (1999). Nutritional aspects of manganese from experimental studies. *Neurotoxicology, 20*, 213–223.

Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z., & Zhu, Y. G. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution, 152*, 686–692.

Kim, Y., Kim, J. W., Ito, K., Lim, H. S., Cheong, H. K., Kim, J. Y., Shin, Y. C., Kim, K. S., & Moon, Y. (1999). Idiopathic parkinsonism with superimposed manganese exposure: Utility of positron emission tomography. *Neurotoxicology, 20*, 249–252.

Korsmeyer, S. J., Wei, M. C., Saito, M., Weiler, S., Oh, K. J., & Schlesinger, P. H. (2000). Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. *Cell Death and Differentiation, 7*, 1166–1173.

Kulisevsky, J., Pujol, J., Junque, C., Deus, J., Balanzo, J., & Capdevila, A. (1993). MRI pallidal hyperintensity and brain atrophy in cirrhotic patients: Two different MRI patterns of clinical deterioration? *Neurology, 43*, 2570–2573.

Lees, A. J., Hardy, J., & Revesz, T. (2009). Parkinson's disease. *Lancet, 373*, 2055–2066.

Link, C. D. (2006). *C. elegans* models of age-associated neurodegenerative diseases: Lessons from transgenic worm models of Alzheimer's disease. *Experimental Gerontology, 41*, 1007–1013.

Ljung, K., & Vahter, M. (2007). Time to re-evaluate the guideline value for manganese in drinking water? *Environmental Health Perspectives, 115*, 1533–1538.

Lloyd, R. V. (1995). Mechanism of the manganese-catalyzed autoxidation of dopamine. *Chemical Research in Toxicology, 8*, 111–116.

Lockman, P. R., Roder, K. E., & Allen, D. D. (2001). Inhibition of the rat blood–brain barrier choline transporter by manganese chloride. *Journal of Neurochemistry, 79*, 588–594.

Lucaciu, C. M., Dragu, C., Copaescu, L., & Morariu, V. V. (1997). Manganese transport through human erythrocyte membranes. An EPR study. *Biochimica et Biophysica Acta, 1328*, 90–98.

Lucchini, R. G., Albini, E., Benedetti, L., Borghesi, S., Coccaglio, R., Malara, E. C., Parrinello, G., Garattini, S., Resola, S., & Alessio, L. (2007). High prevalence of Parkinsonian disorders associated to manganese exposure in the vicinities of ferroalloy industries. *American Journal of Industrial Medicine, 50*, 788–800.

Lynam, D. R., Roos, J. W., Pfeifer, G. D., Fort, B. F., & Pullin, T. G. (1999). Environmental effects and exposures to manganese from use of methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline. *Neurotoxicology, 20*, 145–150.

Malecki, E. A. (2001). Manganese toxicity is associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons. *Brain Research Bulletin, 55*, 225–228.

McDougall, S. A., Reichel, C. M., Farley, C. M., Flesher, M. M., Der-Ghazarian, T., Cortez, A. M., Wacan, J. J., Martinez, C. E., Varela, F. A., Butt, A. E., & Crawford, C. A. (2008). Postnatal manganese exposure alters dopamine transporter function in adult rats: Potential impact on nonassociative and associative processes. *Neuroscience, 154*, 848–860.

Meeker, J. D., Susi, P., & Flynn, M. R. (2007). Manganese and welding fume exposure and control in construction. *Journal of Occupational and Environmental Hygiene, 4*, 943–951.

Mena, I., Marin, O., Fuenzalida, S., & Cotzias, G. C. (1967). Chronic manganese poisoning. Clinical picture and manganese turnover. *Neurology, 17*, 128–136.

Mendieta Zeron, H., Rodriguez, M. R., Montes, S., & Castaneda, C. R. (2011). Blood manganese levels in patients with hepatic encephalopathy. *Journal of Trace Elements in Medicine and Biology, 25*, 225.

Mergler, D., Huel, G., Bowler, R., Iregren, A., Belanger, S., Baldwin, M., Tardif, R., Smargiassi, A., & Martin, L. (1994). Nervous system dysfunction among workers with long-term exposure to manganese. *Environmental Research, 64*, 151–180.

Milatovic, D., Yin, Z., Gupta, R. C., Sidoryk, M., Albrecht, J., Aschner, J. L., & Aschner, M. (2007). Manganese induces oxidative impairment in cultured rat astrocytes. *Toxicological Sciences, 98*, 198–205.

Morello, M., Canini, A., Mattioli, P., Sorge, R. P., Alimonti, A., Bocca, B., Forte, G., Martorana, A., Bernardi, G., & Sancesario, G. (2008). Sub-cellular localization of manganese in the basal ganglia of normal and manganese-treated rats An electron spectroscopy imaging and electron energy-loss spectroscopy study. *Neurotoxicology, 29*, 60–72.

Murphy, V. A., Wadhwani, K. C., Smith, Q. R., & Rapoport, S. I. (1991). Saturable transport of manganese(II) across the rat blood–brain barrier. *Journal of Neurochemistry, 57*, 948–954.

Neff, N. H., Barrett, R. E., & Costa, E. (1969). Selective depletion of caudate nucleus dopamine and serotonin during chronic manganese dioxide administration to squirrel monkeys. *Experientia, 25*, 1140–1141.

Negga, R., Rudd, D. A., Davis, N. S., Justice, A. N., Hatfield, H. E., Valente, A. L., Fields, A. S., & Fitsanakis, V. A. (2011a). Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in *Caenorhabditis elegans*. *Neurotoxicology, 32*, 331–341.

Negga, R., Stuart, J. A., Machen, M. L., Salva, J., Lizek, A. J., Richardson, S. J., Osborne, A. S., Mirallas, O., McVey, K. A., & Fitsanakis, V. A. (2011b). Exposure to Glyphosate- and/or Mn/Zn-Ethylene-bis-Dithiocarbamate-containing pesticides leads to degeneration of gamma-aminobutyric acid and dopamine neurons in *Caenorhabditis elegans*. *Neurotoxicity Research, 21*, 281.

Newland, M. C., & Weiss, B. (1992). Persistent effects of manganese on effortful responding and their relationship to manganese accumulation in the primate globus pallidus. *Toxicology and Applied Pharmacology, 113*, 87–97.

Newland, M. C., Cox, C., Hamada, R., Oberdorster, G., & Weiss, B. (1987). The clearance of manganese chloride in the primate. *Fundamental and Applied Toxicology, 9*, 314–328.

Nong, A., Teeguarden, J. G., Clewell, H. J., 3rd, Dorman, D. C., & Andersen, M. E. (2008). Pharmacokinetic modeling of manganese in the rat IV: Assessing factors that contribute to brain accumulation during inhalation exposure. *Journal of Toxicology and Environmental Health. Part A, 71*, 413–426.

Olanow, C. W. (2004). Manganese-induced parkinsonism and Parkinson's disease. *Annals of the New York Academy of Sciences, 1012*, 209–223.

Ono, J., Harada, K., Kodaka, R., Sakurai, K., Tajiri, H., Takagi, Y., Nagai, T., Harada, T., Nihei, A., Okada, A., et al. (1995). Manganese deposition in the brain during long-term total parenteral nutrition. *Journal of Parenteral and Enteral Nutrition (JPEN), 19*, 310–312.

Pal, P. K., Samii, A., & Calne, D. B. (1999). Manganese neurotoxicity: A review of clinical features, imaging and pathology. *Neurotoxicology, 20*, 227–238.

Peneder, T. M., Scholze, P., Berger, M. L., Reither, H., Heinze, G., Bertl, J., Bauer, J., Richfield, E. K., Hornykiewicz, O., & Pifl, C. (2011). Chronic exposure to manganese decreases striatal dopamine turnover in human alpha-synuclein transgenic mice. *Neuroscience, 180*, 280–292.

Pentschew, A., Ebner, F. F., & Kovatch, R. M. (1963). Experimental manganese encephalopathy in monkeys. A preliminary report. *Journal of Neuropathology and Experimental Neurology, 22*, 488–499.

Perl, D. P., & Olanow, C. W. (2007). The neuropathology of manganese-induced Parkinsonism. *Journal of Neuropathology and Experimental Neurology, 66*, 675–682.

Petit, P. X., Susin, S. A., Zamzami, N., Mignotte, B., & Kroemer, G. (1996). Mitochondria and programmed cell death: Back to the future. *FEBS Letters, 396*, 7–13.

Prabhakaran, K., Chapman, G. D., & Gunasekar, P. G. (2011). Alpha-Synuclein overexpression enhances manganese-induced neurotoxicity through the NF-kappaB-mediated pathway. *Toxicology Mechanisms and Methods, 21*, 435–443.

Racette, B. A., McGee-Minnich, L., Moerlein, S. M., Mink, J. W., Videen, T. O., & Perlmutter, J. S. (2001). Welding-related parkinsonism: Clinical features, treatment, and pathophysiology. *Neurology, 56*, 8–13.

Racette, B. A., Antenor, J. A., McGee-Minnich, L., Moerlein, S. M., Videen, T. O., Kotagal, V., & Perlmutter, J. S. (2005). [^{18}F]FDOPA PET and clinical features in parkinsonism due to manganism. *Movement Disorders, 20*, 492–496.

Reaney, S. H., & Smith, D. R. (2005). Manganese oxidation state mediates toxicity in PC12 cells. *Toxicology and Applied Pharmacology, 205*, 271–281.

Reichel, C. M., Wacan, J. J., Farley, C. M., Stanley, B. J., Crawford, C. A., & McDougall, S. A. (2006). Postnatal manganese exposure attenuates cocaine-induced locomotor activity and reduces dopamine transporters in adult male rats. *Neurotoxicology and Teratology, 28*, 323–332.

Riccio, A., Mattei, C., Kelsell, R. E., Medhurst, A. D., Calver, A. R., Randall, A. D., Davis, J. B., Benham, C. D., & Pangalos, M. N. (2002). Cloning and functional expression of human short TRP7, a candidate protein for store-operated Ca^{2+} influx. *Journal of Biological Chemistry, 277*, 12302–12309.

Rivera-Mancia, S., Rios, C., & Montes, S. (2011). Manganese accumulation in the CNS and associated pathologies. *Biometals, 24*, 811–825.

Roth, J. A. (2006). Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. *Biological Research, 39*, 45–57.

Roth, J. A. (2009). Are there common biochemical and molecular mechanisms controlling manganism and parkisonism. *Neuromolecular Medicine, 11*, 281–296.

Salazar, J., Mena, N., Hunot, S., Prigent, A., Alvarez-Fischer, D., Arredondo, M., Duyckaerts, C., Sazdovitch, V., Zhao, L., Garrick, L. M., Nunez, M. T., Garrick, M. D., Raisman-Vozari, R., & Hirsch, E. C. (2008). Divalent metal transporter 1 (DMT1) contributes to neurodegeneration in animal models of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 18578–18583.

Santamaria, A. B., Cushing, C. A., Antonini, J. M., Finley, B. L., & Mowat, F. S. (2007). State-of-the-science review: Does manganese exposure during welding pose a neurological risk? *Journal of Toxicology and Environmental Health. Part B, Critical Reviews, 10*, 417–465.

Schmidt, E., Seifert, M., & Baumeister, R. (2007). Caenorhabditis elegans as a model system for Parkinson's disease. *Neurodegenerative Diseases, 4*, 199–217.

Schneider, J. S., Decamp, E., Koser, A. J., Fritz, S., Gonczi, H., Syversen, T., & Guilarte, T. R. (2006). Effects of chronic manganese exposure on cognitive and motor functioning in non-human primates. *Brain Research, 1118*, 222–231.

Scholte, H. R. (1988). The biochemical basis of mitochondrial diseases. *Journal of Bioenergetics and Biomembranes, 20*, 161–191.

Seth, P. K., & Chandra, S. V. (1984). Neurotransmitters and neurotransmitter receptors in developing and adult rats during manganese poisoning. *Neurotoxicology, 5*, 67–76.

Settivari, R., Levora, J., & Nass, R. (2009). The divalent metal transporter homologues SMF-1/2 mediate dopamine neuron sensitivity in *caenorhabditis elegans* models of manganism and parkinson disease. *Journal of Biological Chemistry, 284*, 35758–35768.

Sikk, K., Haldre, S., Aquilonius, S. M., & Taba, P. (2011). Manganese-induced parkinsonism due to ephedrone abuse. *Parkinsons Disease, 2011*, 865319.

Smith, R. A., Latchney, L. R., & Senior, A. E. (1985). Tight divalent metal binding to Escherichia coli F1-adenosinetriphosphatase. Complete substitution of intrinsic magnesium by manganese or cobalt and studies of metal binding sites. *Biochemistry, 24*, 4490–4494.

Spahr, L., Butterworth, R. F., Fontaine, S., Bui, L., Therrien, G., Milette, P. C., Lebrun, L. H., Zayed, J., Leblanc, A., & Pomier-Layrargues, G. (1996). Increased blood manganese in cirrhotic patients: relationship to pallidal magnetic resonance signal hyperintensity and neurological symptoms. *Hepatology, 24*, 1116–1120.

Sriram, K., Lin, G. X., Jefferson, A. M., Roberts, J. R., Wirth, O., Hayashi, Y., Krajnak, K. M., Soukup, J. M., Ghio, A. J., Reynolds, S. H., Castranova, V., Munson, A. E., & Antonini, J. M. (2010). Mitochondrial dysfunction and loss of Parkinson's disease-linked proteins contribute to neurotoxicity of manganese-containing welding fumes. *The FASEB Journal, 24*, 4989–5002.

Stastny, D., Vogel, R. S., & Picciano, M. F. (1984). Manganese intake and serum manganese concentration of human milk-fed and formula-fed infants. *American Journal of Clinical Nutrition, 39*, 872–878.

Stokes, A. H., Hastings, T. G., & Vrana, K. E. (1999). Cytotoxic and genotoxic potential of dopamine. *Journal of Neuroscience Research, 55*, 659–665.

Takeda, A., Sawashita, J., & Okada, S. (1995). Biological half-lives of zinc and manganese in rat brain. *Brain Research, 695*, 53–58.

Tolosa, E., Wenning, G., & Poewe, W. (2006). The diagnosis of Parkinson's disease. *Lancet Neurology, 5*, 75–86.

Tong, M., Dong, M., & de la Monte, S. M. (2009). Brain insulin-like growth factor and neurotrophin resistance in Parkinson's disease and dementia with Lewy bodies: Potential role of manganese neurotoxicity. *Journal of Alzheimer's Disease, 16*, 585–599.

Ulmer, T. S., & Bax, A. (2005). Comparison of structure and dynamics of micelle-bound human alpha-synuclein and Parkinson disease variants. *Journal of Biological Chemistry, 280*, 43179–43187.

Van Swinderen, B., & Andretic, R. (2011). Dopamine in Drosophila: Setting arousal thresholds in a miniature brain. *Proceedings: Biological Sciences, 278*, 906–913.

Verity, M. A. (1999). Manganese neurotoxicity: A mechanistic hypothesis. *Neurotoxicology, 20*, 489–497.

von Campenhausen, S., Bornschein, B., Wick, R., Botzel, K., Sampaio, C., Poewe, W., Oertel, W., Siebert, U., Berger, K., & Dodel, R. (2005). Prevalence and incidence of Parkinson's disease in Europe. *European Neuropsychopharmacology, 15*, 473–490.

Wang, D., & Xing, X. (2008). Assessment of locomotion behavioral defects induced by acute toxicity from heavy metal exposure in nematode *Caenorhabditis elegans*. *Journal of Environmental Sciences (China), 20*, 1132–1137.

Wasserman, G. A., Liu, X., Parvez, F., Ahsan, H., Levy, D., Factor-Litvak, P., Kline, J., van Geen, A., Slavkovich, V., LoIacono, N. J., Cheng, Z., Zheng, Y., & Graziano, J. H. (2006). Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. *Environmental Health Perspectives, 114*, 124–129.

Weissenborn, K., Ehrenheim, C., Hori, A., Kubicka, S., & Manns, M. P. (1995). Pallidal lesions in patients with liver cirrhosis: Clinical and MRI evaluation. *Metabolic Brain Disease, 10*, 219–231.

Yin, Z., Aschner, J. L., dos Santos, A. P., & Aschner, M. (2008). Mitochondrial-dependent manganese neurotoxicity in rat primary astrocyte cultures. *Brain Research, 1203*, 1–11.

Yin, Z., Jiang, H., Lee, E. S., Ni, M., Erikson, K. M., Milatovic, D., Bowman, A. B., & Aschner, M. (2010). Ferroportin is a manganese-responsive protein that decreases manganese cytotoxicity and accumulation. *Journal of Neurochemistry, 112*, 1190–1198.

Zayed, J., Thibault, C., Gareau, L., & Kennedy, G. (1999). Airborne manganese particulates and methylcyclopentadienyl manganese tricarbonyl (MMT) at selected outdoor sites in Montreal. *Neurotoxicology, 20*, 151–157.

Zhang, S., Fu, J., & Zhou, Z. (2004). In vitro effect of manganese chloride exposure on reactive oxygen species generation and respiratory chain complexes activities of mitochondria isolated from rat brain. *Toxicology In Vitro, 18*, 71–77.

Zlotkin, S. H., Atkinson, S., & Lockitch, G. (1995). Trace elements in nutrition for premature infants. *Clinics in Perinatology, 22*, 223–240.

Zoratti, M., & Szabo, I. (1995). The mitochondrial permeability transition. *Biochimica et Biophysica Acta, 1241*, 139–176.

Mechanisms of Dopamine Oxidation and Parkinson's Disease

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Contents

Abstract

Dopamine's ability to oxidize to aminochrome explains why this molecule is both an essential neurotransmitter for movement control and a neurotoxic compound that induces toxicity and apoptosis in cell lines. Dopamine spontaneously

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oxidizes to aminochrome in the presence of oxygen due to the protons on the dopamine hydroxyl groups that are dissociated when dopamine is in the cytosol (pH 7.4). Dopamine oxidation is prevented by vesicular monoaminergic transporter-2 (VMAT-2) that takes up dopamine into the monoaminergic synaptic vesicles where the low pH prevents dopamine oxidation. Dopamine in the cytosol can also be degraded by monoamine oxidase (MAO) and catechol *ortho*-methyltransferase (COMT) soluble isoform. However, under certain unknown conditions dopamine oxidize to aminochrome, the precursor of neuromelanin, since the neuromelanin pigment is found in the human substantia nigra. Aminochrome participates in two neurotoxic reactions: (i) the one-electron reduction of aminochrome, which is catalyzed by flavoenzymes that use NADH or NADPH as electron donors. This reaction produces leukoaminochrome *o*-semiquinone radical, which is extremely reactive with oxygen that autoxidizes, depleting both NADH and O_2 required for ATP synthesis when the flavoenzymes use NADH; and (ii) aminochrome forms adducts with proteins such as alpha-synuclein-inducing and alpha-synuclein-stabilizing neurotoxic protofibrils. In addition, aminochrome inactivate parkin an E3 ubiquitin ligase of proteasomal system, complexes I and III of electron transport chain, tyrosine hydroxylase, actin, and α- and β-tubulin. Aminochrome is also able to participate in three neuroprotective reactions such as (i) polymerization to neuromelanin, which is a pigment localized in substantia nigra and is present in normal subjects; (ii) aminochrome two-electron reduction to leukoaminochrome catalyzed by DT-diaphorase, which prevents aminochrome neurotoxic reactions; and (iii) glutathione conjugation of aminochrome catalyzed by glutathione *S*-transferase M2-2. The role of aminochrome in the degeneration of dopaminergic neurons in Parkinson's disease is discussed. Aminochrome may induce the focal neurodegeneration of dopaminergic neurons through mechanisms involving mitochondrial dysfunction, protein aggregation, oxidative stress, and protein degradation dysfunction.

Keywords

Alpha synuclein • Aminochrome • Aromatic amino acid decarboxylase • Catechol orto-methyl-transferase • Dopamine • DT-diaphorase (NQO1) • Free radicals • Glutathione-S-transferase M2-2 • Mitochondria complex I • Mono amine oxidase • Oxidation • Parkin • Parkinson's disease • Tyrosine hydroxylase • VMAT-2

List of Abbreviations

AADC	Aromatic amino acid decarboxylase
COMT	Catechol *ortho*-methyltransferase
GST M2-2	Glutathione *S*-transferase M2-2
L-dopa	L-dihydroxyphenylalanine
MAO	Monoamine oxidases
TH	Tyrosine hydroxylase
VMAT-2	Vesicular monoaminergic transporter-2

1 Dopamine

Dopaminergic neurons play a key role in motor activity. Dopamine is an essential neurotransmitter synthesized from the amino acid tyrosine. It is stored and transported in monoaminergic vesicles, released during neurotransmission, and is taken up by dopamine transporters from the intersynaptic space. Dopamine is synthesized in several steps. The first step involves the formation of L-dihydroxyphenylalanine (L-dopa) from tyrosine by the enzyme tyrosine hydroxylase (TH). The L-dopa is then decarboxylated by aromatic amino acid decarboxylase (AADC) to generate dopamine and CO_2. Dopamine has two hydroxyl groups that are dissociated in the cytosol at pH 7.4. This dissociation allows the oxidation of dopamine to *o*-quinones. However, dopamine accumulates in monoaminergic vesicles because TH and AADC are associated with the vesicular monoaminergic transporter-2 (VMAT-2). This transporter is present in the membranes of monoaminergic synaptic vesicles and generates a complex with the enzymes (Cartier et al. 2010). Thus, tyrosine is converted to L-dopa by TH and is immediately decarboxylated by ADDC to dopamine. The dopamine is then taken up by VMAT-2 into the monoaminergic synaptic vesicles. The uptake prevents free dopamine from accumulating in the cytosol and the oxidation of dopamine to *o*-quinone. The dopamine uptake mediated by VAMT-2 is associated with a vesicular ATPase that is translocated into the vessel as ATP is hydrolyzed to ADP, Pi, and one proton (H^+). This process creates a proton gradient (Fig. 1). The VMAT-2 transporter exports two protons as one molecule of dopamine is taken into the cell (Chaudhry et al. 2008; Knoth et al. 1981). The pH inside the monoaminergic synaptic vesicles has been estimated to be 2–2.4 pH units lower than the pH in the cytosol (Guillot and Miller 2009). The dopamine inside the monoaminergic synaptic vesicles is stable. It does not oxidize to *o*-quinone because it is well protonated. The free dopamine in the cytosol participates in three reactions. First, the dopamine can undergo deaminative oxidation to become dihydroxyphenylacetic acid. This reaction is catalyzed by monoamine oxidases. Second, the dopamine catechol structure can be oxidized to become the *o*-quinone aminochrome. Finally, dopamine can be methylated by *ortho* methyltransferases (Fig. 1).

2 Dopamine Degradation

2.1 The Oxidative Deamination of Dopamine

The monoamine oxidases (MAO, E.C. 1.4.3.4) degrade excess dopamine in the cytosol by catalyzing the oxidative deamination of the dopamine amino group to 3,4-dihydroxyphenylacetaldehyde. Ammonia and hydrogen peroxide are also produced in this reaction. 3,4-Dihydroxyphenylacetaldehyde is metabolized by aldehyde dehydrogenase to 3,4-dihydroxyphenylacetic acid (DOPAC) as NAD^+ is simultaneously reduced to NADH (Fig. 2). The MAO enzymes

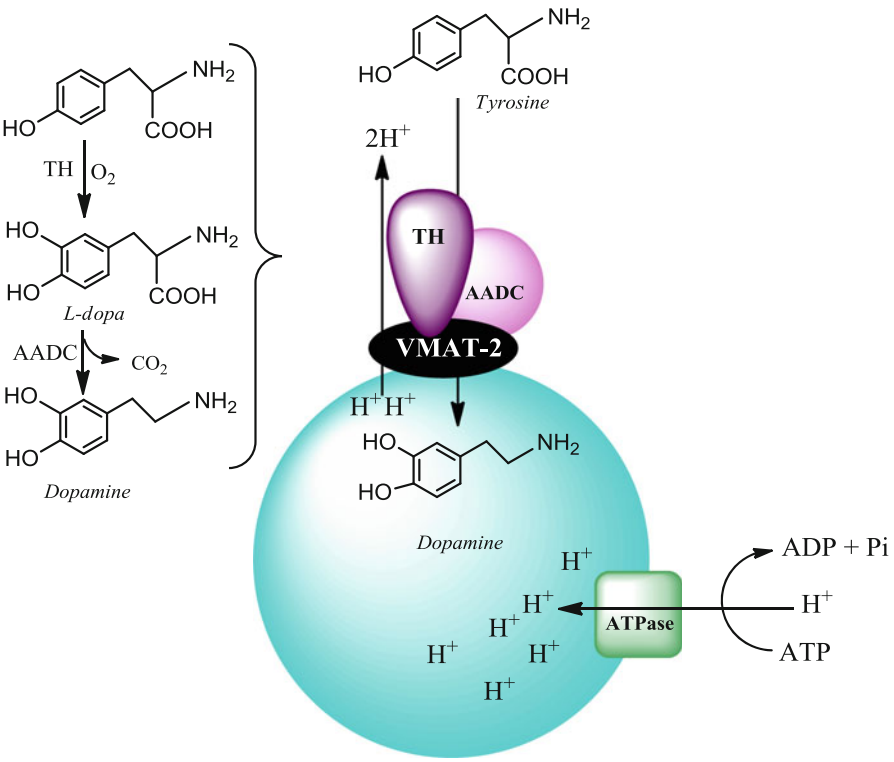

Fig. 1 Dopamine synthesis from tyrosine catalyzed by tyrosine hydroxylase (*TH*) and aromatic amino acid decarboxylase (*AADC*) and uptake into monoaminergic synaptic vesicles mediated by VMAT-2

are flavoenzymes with multiple isoforms. The isozymes are labeled A and B and share 70 % identity. These enzymes carry sequences specific to FAD (Bach et al. 1988) and are localized to the outer membranes of the mitochondria in neurons, glial cells, and other cell types (Weyler et al. 1990; Shih et al. 1997). MAO-A is mainly found in catecholaminergic neurons, whereas MAO-B is found in serotonergic and histaminergic neurons as well as in astrocytes (Westlund et al. 1988; Saura et al. 1994). MAO-A acts on mainly monoaminergic neurotransmitters such as serotonin, dopamine, noradrenaline, and adrenaline. In contrast, the substrates of MAO-B include trace amines such as tyramine and phenylethylamine (Strolin-Benedetti et al. 1992). The nonselective inhibitors of MAO-A and MAO-B include isocarboxazid¸ phenelzine, nialamide, iproniazid, iproclozide, and tranylcypromine. The selective inhibitors of MAO-A include clorgyline, brofaromine, moclobemide, toloxatone, linezolid, befloxatone, and cimoxatone. The selective inhibitors of MAO-B include selegiline and rasagiline (for review, see Bortolato et al. 2008). The MAO in dopaminergic neurons degrades dopamine to maintain low cytosolic concentrations and

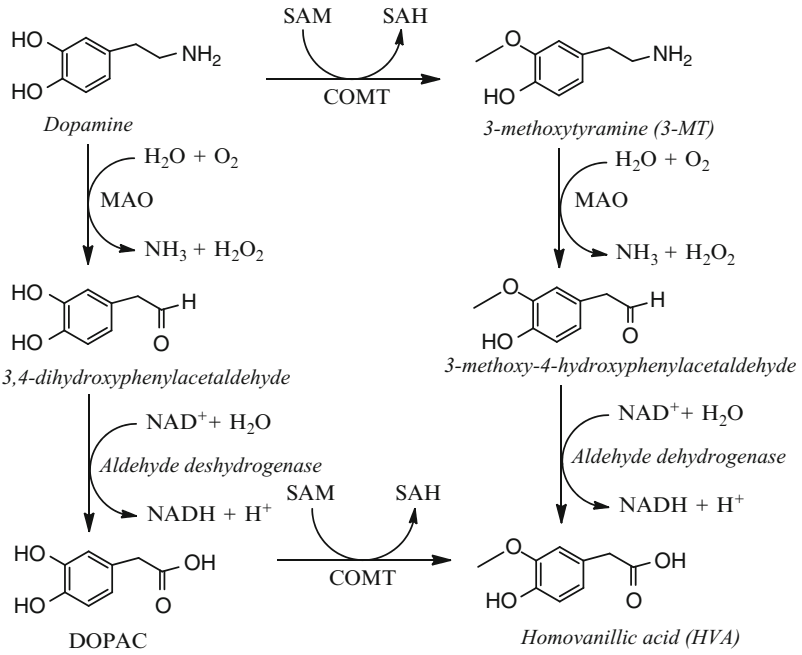

Fig. 2 Dopamine degradation catalyzed by monoamine oxidase (*MAO*) and catechol *ortho*-methyltransferase (*COMT*)

to prevent dopamine oxidation. However, MAO activity is associated with oxidative stress. This process is a result of hydrogen peroxide formation from the oxidative deamination of dopamine. Hydrogen peroxide is a precursor to the hydroxyl radical. Rasagiline may protect the brain in Parkinson's disease model systems by inhibiting the formation of reactive oxygen species derived from the deaminative oxidation of dopamine catalyzed by MAO-B (Weinreb et al. 2011).

2.2 *Ortho*-Methylation of Dopamine

Dopamine is also degraded by catechol *ortho*-methyltransferase (COMT; EC 2.1.1.6). This enzyme converts dopamine to 3-methoxytyramine, which is then metabolized to 3-methoxy-4-hydroxyphenylacetaldehyde by MAO. Finally, homovanillic acid is formed when aldehyde dehydrogenase catalyzes the oxidation of 3-methoxy-4-hydroxyphenylacetaldehyde (Fig. 2). NADH is also formed in this reaction. Homovanillic acid is also formed by COMT-mediated methylation of the hydroxyl group on DOPAC. Two isoforms of COMT have been discovered: The first is the soluble isoform S-COMT, and the second is the membrane-bound isoform MB-COMT. Both the S- and MB-COMT isoforms are found in microglial

and astroglial cells. COMT is present in pyramidal neurons, cerebellar Purkinje and granular cells, and striatal spiny neurons (Myöhänen et al. 2010). MB-COMT is localized to the cell body, axons, and dendrites of rat cortical neurons. The C-terminal catalytic domain of MB-COMT is in the extracellular space. This domain inactivates the synaptic and extrasynaptic dopamine in presynaptic and postsynaptic neurons (Chen et al. 2011). The downregulation of COMT by estrogen most severely affects the prefrontal cortex and kidneys, where COMT is physiologically important for dopamine metabolism (Schendzielorz et al. 2011). Entacapone has been applied to inhibit COMT in Parkinson's disease treatment. This inhibition prolongs the half-life of L-dopa and allows the delivery of continuous stimulation to the neuronal dopaminergic receptors (Marin and Obeso 2010). A single nucleotide polymorphism (1947) that controls COMT activity has been identified (Tan et al. 2005).

3 Dopamine Oxidation to *Ortho*-Quinones

3.1 Dopamine Oxidation to Aminochrome

The protons on the dopamine hydroxyl groups are dissociated when dopamine is in the cytosol (pH 7.4). Dopamine spontaneously oxidizes in the presence of oxygen, even in the absence of metal-ion catalysts (Linert et al. 1996). Dopamine oxidation can produce a dopamine *o*-semiquinone radical and a superoxide radical (reaction 1). Two dopamine *o*-semiquinone radicals can react in a disproportionation reaction to produce one molecule of dopamine *o*-quinone and one molecule of dopamine (reaction 2, Fig. 3). Alternatively, the dopamine *o*-semiquinone radical can be one-electron oxidized by oxygen to produce dopamine *o*-quinone and a superoxide radical (reaction 3, Fig. 3). Dopamine oxidation can also be catalyzed by metals. Manganese (III) oxidizes dopamine under aerobic and anaerobic conditions without forming superoxide radicals because manganese (III) catalyzes the formation of both dopamine *o*-semiquinone and dopamine *o*-quinone (Segura-Aguilar and Lind 1989). Copper sulfate (II) and iron chloride (III) oxidize dopamine by forming a complex with it (Paris et al. 2001, 2005a, b). Sodium metaperiodate also catalyzes dopamine oxidation (Graham et al. 1978). Further, prostaglandin H synthase, lactoperoxidase, cytochrome P450, xanthine oxidase, and dopamine β-monooxygenase catalyze dopamine oxidation through peroxidase activity (Galzigna et al. 2000; Thompson et al. 2000; Segura-Aguilar 1996; Foppoli et al. 1997; Hastings 1995). Tyrosinase can catalyze the two-electron oxidation of dopamine to dopamine *o*-quinone. This process does not form dopamine *o*-semiquinone radicals or consume oxygen (Segura-Aguilar et al. 1998; Jimenez et al. 1984).

Dopamine *o*-quinone is only stable at a pH below 2.0. Thus, this molecule is not stable at physiological pH (Segura-Aguilar and Lind 1989). The amino group of dopamine spontaneously rearranges and undergoes cyclization to become

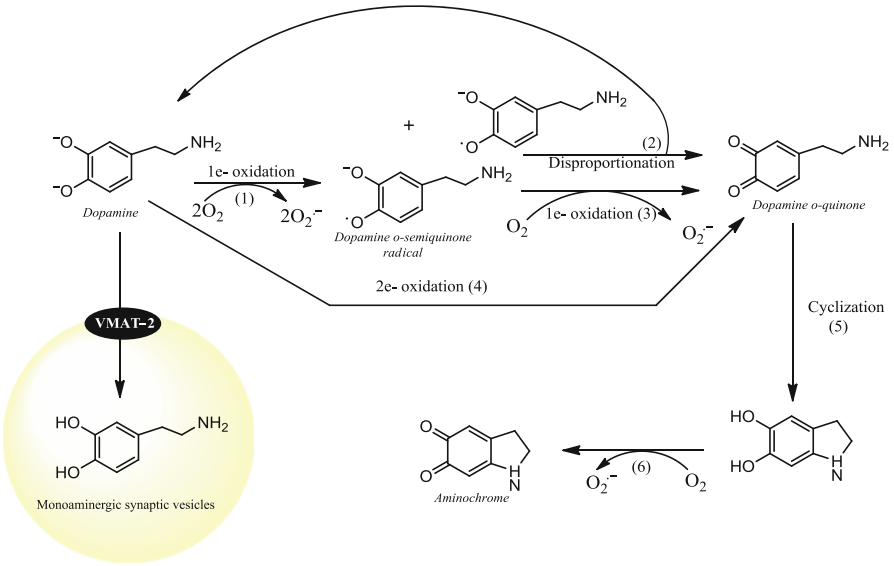

Fig. 3 Dopamine oxidation to aminochrome

leukoaminochrome (reaction 5). Leukoaminochrome undergoes oxidation to become aminochrome (reaction 6). Purified aminochrome (also called dopaminechrome) is stable for approximately 3 h (Paris et al. 2010). This timeframe suggests that aminochrome is the relevant end product of dopamine oxidation in neuromelanin-containing dopaminergic neurons.

Aminochrome formation is dependent on the presence of free cytosolic dopamine that can be oxidized. The efficient uptake of dopamine into monoaminergic vesicles is mediated by VMAT-2, and the degradation of free dopamine by MAO prevents the oxidation of dopamine to aminochrome. However, under certain conditions, free dopamine in the cytosol can oxidize to form aminochrome. The presence of neuromelanin in the substantia nigra of healthy controls suggests that dopamine oxidation occurs in vivo because aminochrome is the precursor of neuromelanin.

3.2 Aminochrome Tautomerization

Aminochrome is an unstable molecule that rearranges to 5,6-dihydroxyindole (reaction 1), which is then oxidized to become 5,6-indolequinone (Fig. 4, reaction 2) (Napolitano et al. 2011). However, tyrosinase-catalyzed dopamine oxidation in the absence of metals produces aminochrome that is stable for 3 h after purification (Paris et al. 2010). From a physiological point of view, 3 h is adequate for a molecule to be one- or two-electron reduced by flavoenzymes or to form adducts with proteins. The 5,6-indolequinone is in equilibrium with the quinone methide even though

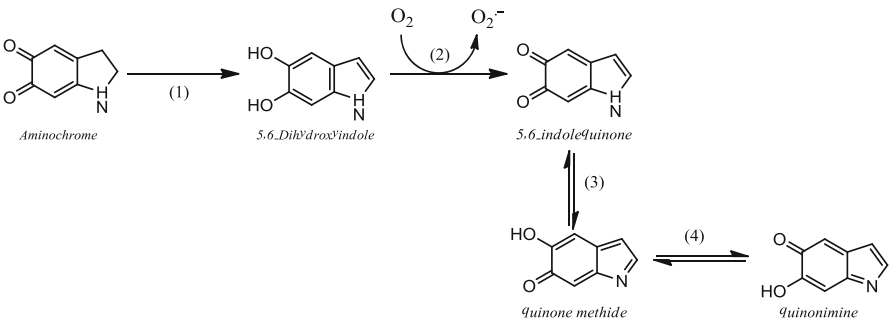

Fig. 4 Aminochrome tautomerization

the presence of transition metal cations favors the formation of the quinone methide (reaction 3). The quinone methide is also in equilibrium with quinonimine (reaction 4, Fig. 4; Pezzella et al. 2007).

4 Aminochrome Metabolism

4.1 Aminochrome Polymerization to Neuromelanin

Aminochrome is not completely stable. At high concentrations, it polymerizes to form a dark pigment called neuromelanin (Fig. 5). Neuromelanin formation appears to be a normal process because it is present in dopaminergic neurons of the substantia nigra in healthy subjects without Parkinson's disease. It accumulates with age (Zecca et al. 2002). Neuromelanin appears to have a neuroprotective function through the chelation of metals (Gerlach et al. 2003; Hong and Simon 2007) and the binding of such proteins as alpha-synuclein (Fasano et al. 2006). However, neuromelanin induces apoptosis in cell cultures (Naoi et al. 2008). Interestingly, extracellular neuromelanin also induces microglial activation in the substantia nigra. As a result of this process, superoxide radicals, nitric oxide, hydrogen peroxide, and pro-inflammatory factors are produced, and tyrosine hydroxylase-positive neurons are lost (Zhang et al. 2011). However, neuromelanin is normally stored in double-membrane vacuoles, and the possible neurotoxic effects described by Zhang are avoided.

4.2 Aminochrome Adducts with Proteins

Aminochrome forms adducts with proteins that may be involved in the pathogenesis of Parkinson's disease. Alpha-synuclein is hypothesized to cause damage through the generation of neurotoxic protofibrils and has been associated with familial Parkinson's disease. The role of alpha-synuclein in sporadic Parkinson's disease remains an open question. However, aminochrome forms adducts with alpha-synuclein (Fig. 6). This process induces the formation and stabilization of

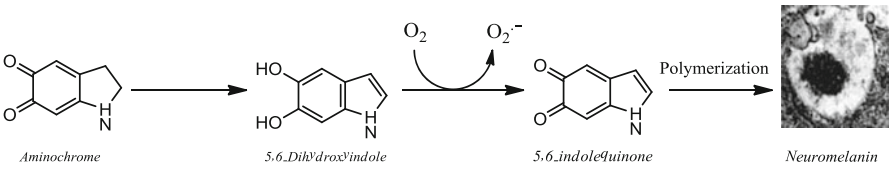

Fig. 5 Aminochrome as precursor of neuromelanin pigment

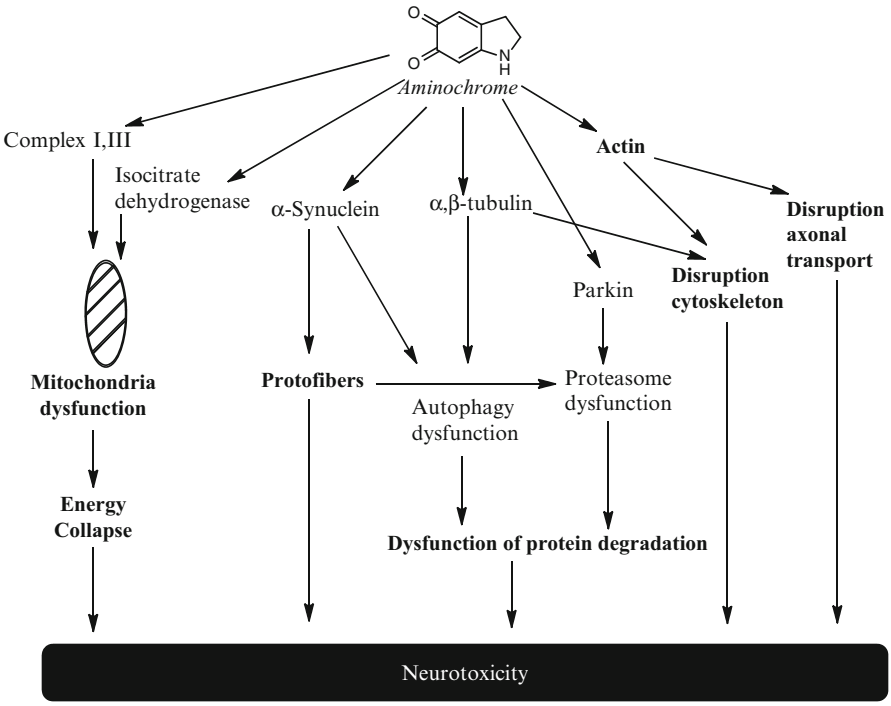

Fig. 6 Formation of aminochrome adducts with proteins

neurotoxic protofibrils that may play a key role in alpha-synuclein neurotoxicity (Conway et al. 2001; Norris et al. 2005). Therefore, aminochrome may be the link between alpha-synuclein and sporadic Parkinson's disease. In familial Parkinson's disease, the formation of neurotoxic protofibrils is dependent on the presence of a specific point mutation that changes alpha-synuclein properties (Polymeropoulos et al. 1997). In sporadic Parkinson's disease, however, the formation of neurotoxic protofibrils is dependent on the formation of aminochrome. The aminochrome forms adduct with other proteins such as actin and α- and β-tubulin and disrupt cytoskeletal architecture (Fig. 6; Paris et al. 2010). Dopamine o-quinone, a transient precursor of aminochrome at physiological pH, forms adducts with and inactivates parkin, a ubiquitin ligase of the proteasomal system (LaVoie et al. 2005).

This result suggests that aminochrome inactivates the proteasomal system, which is consistent with another report (Zafar et al. 2006). Interestingly, a mutation in the parkin gene has also been associated with a familial form of juvenile Parkinson's disease (Kitada et al. 1998). Aminochrome (dopamine o-quinone) forms adduct with the mitochondrial complex I (Van Laar et al. 2009) and induces energy collapse by inactivating mitochondrial electron transport and ATP production. Aminochrome (dopamine *o*-quinone) also forms adducts with and inactivates tyrosine hydroxylase, human dopamine transporter, and tryptophan hydroxylase (Xu et al. 1998; Whitehead et al. 2001; Kuhn and Arthur 1998).

4.3 Aminochrome One-Electron Reduction

The aminochrome quinone can be one-electron reduced to become a leukoaminochrome *o*-semiquinone radical. This process is catalyzed by flavoenzymes that transfer electrons from NADH or NADPH to aminochrome. The leukoaminochrome *o*-semiquinone radical is extremely reactive under aerobic conditions. It catalyzes the reduction of oxygen to superoxide and generates a redox cycle between the leukoaminochrome *o*-semiquinone radical and aminochrome (Baez et al. 1995; Segura-Aguilar et al. 1998).

The redox cycling between the leukoaminochrome *o*-semiquinone radical and aminochrome is important for aminochrome neurotoxicity. This process is catalyzed by flavoenzymes and transfers electrons from NADH or NADPH. NADH and oxygen depletion through redox cycling results in energy collapse because these molecules are no longer available for ATP synthesis in the mitochondria (Fig. 7). This effect is potentiated by the formation of superoxide radicals. These radicals spontaneously or enzymatically generate hydrogen peroxide, the precursor of hydroxyl radicals. NADPH can also be used as the electron donor in the reaction catalyzed by flavoenzymes. NADPH is involved in the reaction catalyzed by glutathione reductase that keeps GSH in the reduced state. As a result of NADPH depletion, GSH is unable to exert its antioxidant action, and oxidative stress is thereby increased. The one-electron reduction of aminochrome is neurotoxic in catecholaminergic cells (Paris et al. 2001, 2005a, b, 2009, 2010, 2011; Arriagada et al. 2004; Fuentes et al. 2007; Díaz-Véliz et al. 2008; Muñoz et al. 2012).

4.4 Aminochrome Two-Electron Reduction

DT-diaphorase (EC.1.6.99.2) is a flavoenzyme composed of two subunits and contains two molecules of FAD. This enzyme is unique among flavoenzymes because it catalyzes the two-electron reduction of quinones to hydroquinones. Both NADH and NADPH can be used as electron donators in this reaction. DT-diaphorase is mainly localized in the cytosol, but approximately 5 % of this enzyme is associated with the mitochondria and endoplasmic reticulum. It is found in

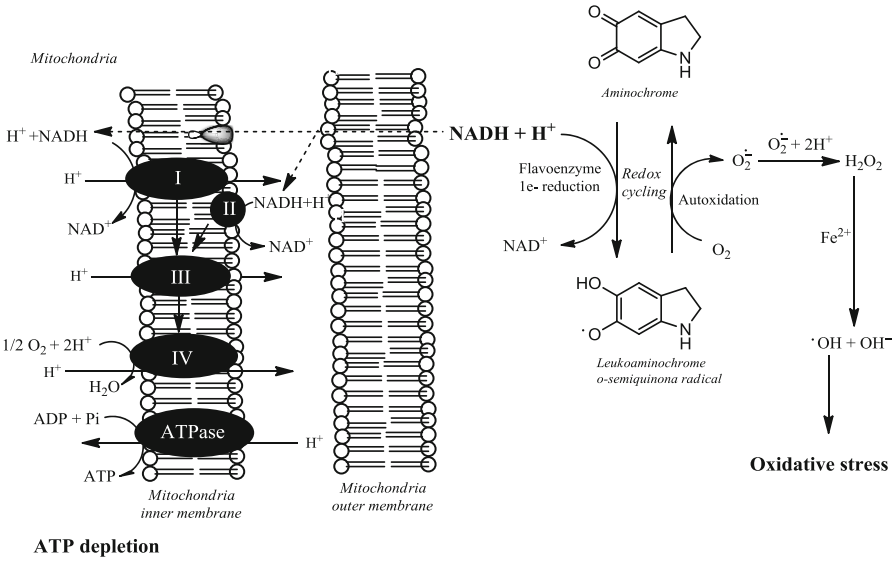

Fig. 7 One-electron reduction of aminochrome catalyzed by flavoenzymes that use NADH as electron donor

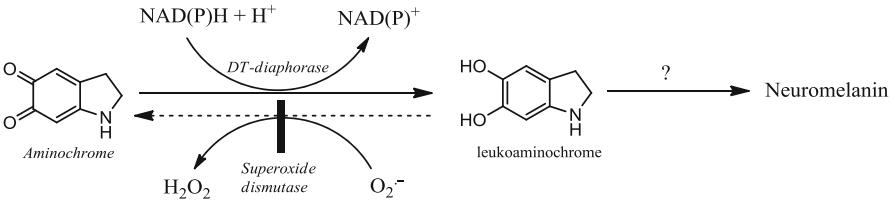

Fig. 8 DT-diaphorase catalyzes the two-electron reduction of aminochrome

different tissues and organs including the brain. DT-diaphorase immunoreactivity co-localizes with tyrosine hydroxylase-like immunoreactivity, suggesting that DT-diaphorase is expressed in the dopaminergic neurons of both the substantia nigra and ventral tegmental area. The dense network of the DT-diaphorase-immunoreactive fibers in the striatum and the dopaminergic innervations were simultaneously lost following a 6-hydroxydopamine lesion. It is important to mention that DT-diaphorase constitutes 97 % of the total cytosolic quinone reductase activity in the rat substantia nigra. DT-diaphorase immunoreactivity has also been found in Bergmann glia, astrocytes, and tanycytes (Schultzberg et al. 1988). DT-diaphorase catalyzes the reduction of aminochrome to leukoaminochrome (Segura-Aguilar and Lind 1989). Leukoaminochrome autoxidizes in the presence of superoxide radicals (Fig. 8). However, the presence of superoxide dismutase

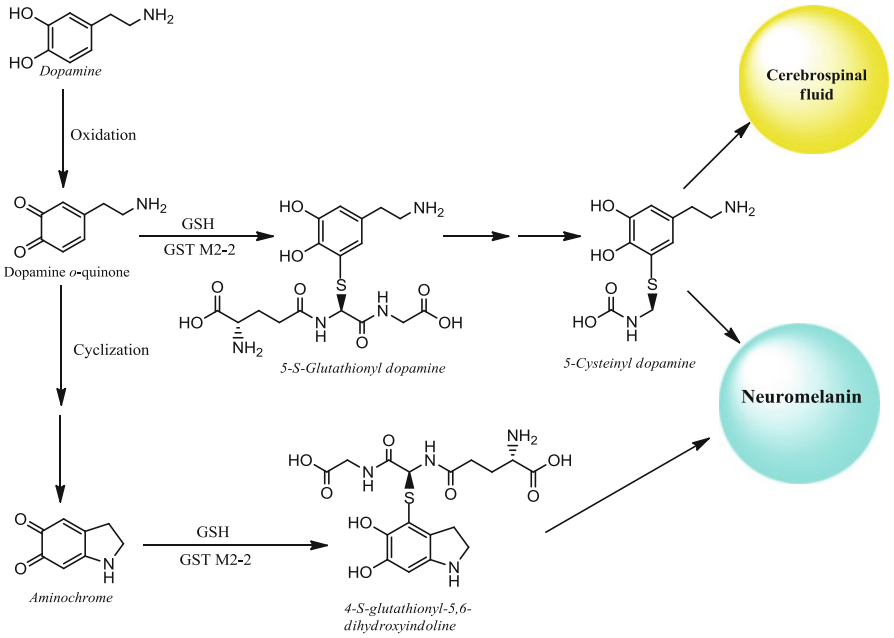

Fig. 9 Glutathione (*GSH*) conjugation of aminochrome and dopamine *o*-quinone catalyzed by glutathione *S*-transferase M2-2 (*GST M2-2*)

in the cytosol prevents leukoaminochrome autoxidation (Baez et al. 1995). These in vitro results suggest that DT-diaphorase may protect against aminochrome neurotoxicity. Cell culture studies support this idea: DT-diaphorase inhibition with dicoumarol or siRNA induces aminochrome neurotoxicity (Arriagada et al. 2004; Lozano et al. 2010; Paris et al. 2011; Muñoz et al. 2012). DT-diaphorase also prevents the formation of alpha-synuclein protofibrils (Segura-Aguilar et al. 2006; Cardenas et al. 2008) and the cytoskeletal disruption arising from adduct formation with actin and α- and β-tubulin (Paris et al. 2010).

4.5 Aminochrome Conjugation with Glutathione

Aminochrome can be conjugated with glutathione by glutathione *S*-transferase M2-2 (GST M2-2) to 4-*S*-glutathionyl-5,6-dihydroxyindoline (Fig. 9). The 4-*S*-glutathionyl-5,6-dihydroxyindoline molecule is stable in the presence of biological oxidizing agents such as oxygen, superoxide radicals, and hydrogen peroxide (Segura-Aguilar et al. 1997; Baez et al. 1997). This stability suggests that the molecule is a final elimination product. Interestingly, the precursor of aminochrome dopamine *o*-quinone is also conjugated by GST M2-2–5-glutathionyl-dopamine

(Fig. 9). This process prevents the formation of aminochrome (Dagnino-Subiabre et al. 2000). The tripeptide γ-L-Glu-L-Cys-Gly of all glutathione conjugates undergoes glutathione degradation to cysteine conjugation. Therefore, 5-glutathionyl-dopamine is ultimately converted to 5-S-cysteinyl-dopamine (Shen et al. 1996). Interestingly, 5-*S*-cysteinyl-dopamine has been detected in the cerebrospinal fluid of PD patients and in dopamine-rich brain regions such as the caudate nucleus, putamen, globus pallidus, and substantia nigra as well as in neuromelanin (Cheng et al. 1996, Rosengren et al. 1985, Carstam et al. 1991). GST M2-2 also catalyzes the conjugation of dopa *o*-quinone to 5-glutathionyl dopa. The tripeptide glutathione on 5-glutathionyl dopa undergoes degradation to generate 5-S-cysteinyl-dopa (Dagnino-Subiabre et al. 2000). Melanoma cells produce and release 5-S-cysteinyl-dopa, which is ultimately excreted through urine (Ito et al. 1984). Therefore, the conjugation of glutathione must be protective against aminochrome neurotoxicity.

4.6 Aminochrome and Parkinson's Disease

The discovery that Parkinson's disease patients show a loss of neuromelanin-containing dopaminergic neurons led to intensive research focused on understanding the mechanisms involved in the loss of dopaminergic neurons, which results in motor symptoms. Recently, the presymptomatic and symptomatic phases of Parkinson's disease were subdivided into six stages (Braak et al. 2004). The progressive loss of nigral neurons with Lewy bodies is considered to be an essential neuropathological feature. However, olfactory disturbances, sleep fragmentation, and depression precede the motor symptoms (Wolters and Braak 2006). The disease may initially affect the olfactory bulb, enteric plexus, and motor component of cranial nerve X (stage 1). It may progress to the locus coeruleus, caudal raphe, and magnocellular reticular formation (stage 2). Approximately 5 years before the manifestation of the motor symptoms, the substantia nigra, amygdala central subnucleus, Meynert's nucleus, and pedunculopontine tegmental nucleus are affected (stage 3). Approximately 4 years after the manifestation of motor symptoms, the transentorhinal cortex, CA2 plexus, and intralaminar thalamic nuclei are affected (stage 4). Approximately 10 years after the manifestation of motor symptoms, the prefrontal cortex and tertiary sensory association areas are affected (stage 5). Finally, the secondary and then the primary motor and sensory areas are affected (stage 6) (Hawkes et al. 2010).

Despite the therapeutic efficacy of L-dopa for Parkinson's disease, the role of substantia nigral neurodegeneration (stage 3) in the motor symptoms of the disease remains unclear. The association of the alpha-synuclein (Polymeropoulos et al. 1997) and parkin (Hattori et al. 1998; Abbas et al. 1999) genes with the familial form of Parkinson's disease was important in understanding possible mechanisms underlying the sporadic form of the disease. Several other genes have also been associated with familial Parkinson's disease

(for review, see Corti et al. 2011). Despite the enormous advances in the field, the neurotoxins and mechanisms involved in Parkinson's-related neurodegeneration still remain unclear. However, the mechanisms of dopaminergic neurodegeneration are generally thought to involve mitochondrial dysfunction, alpha-synuclein aggregation in neurotoxic protofibrils, protein degradation dysfunction, oxidative stress, and neuroinflammation (Schapira 2011; Conway et al. 2001; McNaught et al. 2004; Cuervo et al. 2010; Schapira and Jenner 2011).

The proposed timeline for Parkinson's disease (Hawkes et al. 2010) suggests that approximately 5 years elapse between the stage 3 damage to the substantia nigra and the appearance of motor symptoms (stage I in the Hoehn and Yahr scale). The progression of the disease after the manifestation of the motor symptoms is also very slow. It takes approximately 10 years to progress to stage III of the Hoehn and Yahr scale. These data refute the possibility that an exogenous neurotoxin is involved in the idiopathic degeneration of the dopaminergic neurons in the substantia nigra. This possibility is unlikely because MPTP induced severe parkinsonian motor symptoms in 3 days (Williams 1984). It is also important to note that the neuromelanin-containing dopaminergic neurons in the substantia nigra are lost, suggesting that the dopamine in the degenerative cells oxidizes to aminochrome, the precursor of neuromelanin.

We propose that aminochrome is the endogenous neurotoxin involved in the neurodegeneration of the neuromelanin-containing dopaminergic neurons in Parkinson's disease. This compound induces focal neurotoxic events in the years prior to the development of motor symptoms. The following evidence supports this idea: (i) Aminochrome induces mitochondrial dysfunction by forming adducts with complexes I and III in the electron transport chain as well as isocitrate dehydrogenase (Van Laar et al. 2009). The redox cycling induced by aminochrome one-electron reduction depletes NADH, thereby decreasing ATP production in cell culture (Muñoz et al. 2012). (ii) Aminochrome induces the formation and stabilization of alpha-synuclein protofibrils (Norris et al. 2005). (iii) Aminochrome induces protein degradation dysfunction by inactivating parkin (Xu et al. 1998), a ubiquitin ligase involved in the proteasomal system. In addition, alpha-synuclein protofibrils inhibit chaperone-mediated autophagy (Xilouri et al. 2009) and the 26S proteasomal system (Zhang et al. 2008). Aminochrome also induces the aggregation of α- and β-tubulin through forming adducts with these proteins (Paris et al. 2010). Tubulin aggregation prevents the microtubule formation required for the fusion of autophagocytic vacuoles and lysosomes (Monastyrska et al. 2009). (iv) Finally, aminochrome induces oxidative stress by undergoing one-electron reduction to become the leukoaminochrome *o*-semiquinone radical (Paris et al. 2001, 2005; Arriagada et al. 2004). Interestingly, aminochrome may play a role in four of the five mechanisms that are generally accepted as being involved in the degeneration of neuromelanin-containing dopaminergic neurons in Parkinson's disease.

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References

Abbas, N., Lücking, C. B., Ricard, S., Dürr, A., Bonifati, V., De Michele, G., Bouley, S., Vaughan, J. R., Gasser, T., Marconi, R., Broussolle, E., Brefel-Courbon, C., Harhangi, B. S., Oostra, B. A., Fabrizio, E., Böhme, G. A., Pradier, L., Wood, N. W., Filla, A., Meco, G., Denefle, P., Agid, Y., & Brice, A. (1999). A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's disease. *Human Molecular Genetics, 8*, 567–574.

Arriagada, A., Paris, I., Sanchez de las Matas, M. J., Martinez-Alvarado, P., Cardenas, S., Castañeda, P., Graumann, R., Perez-Pastene, C., Olea-Azar, C., Couve, E., Herrero, M. T., Caviedes, P., & Segura-Aguilar, J. (2004). On the neurotoxicity of leukoaminochrome o-semiquinone radical derived of dopamine oxidation: Mitochondria damage, necrosis and hydroxyl radical formation. *Neurobiology of Disease, 16*, 468–477.

Bach, A. W. J., Lan, N. C., Johnson, D. L., Abell, C. W., Bemkenek, M. E., Kwan, S.-W., Seeburg, P. H., & Shih, J. C. (1988). CDNA cloning of human liver monoamine oxidase A and B: Molecular basis of differences in enzymatic properties. *Proceedings of the National Academy of Sciences, 85*, 4934–4938.

Baez, S., Linderson, Y., & Segura-Aguilar, J. (1995). Superoxide dismutase and catalase enhance autoxidation during one-electron reduction of aminochrome by NADPH-cytochrome P-450 reductase. *Biochemical and Molecular Medicine, 54*, 12–18.

Baez, S., Segura-Aguilar, J., Widersten, M., Johansson, A. S., & Mannervik, B. (1997). Glutathione transferases catalyse the detoxication of oxidized metabolites (o-quinones) of catecholamines and may serve as an antioxidant system preventing degenerative cellular processes. *Biochemistry Journal*, *324*(Pt 1), 25–28.

Bortolato, M., Chen, K., & Shih, J. C. (2008). Monoamine oxidase inactivation: From pathophysiology to therapeutics. *Advanced Drug Delivery Reviews, 60*, 1527–1533.

Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H., & Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell and Tissue Research, 318*, 121–134.

Cardenas, S. P., Perez-Pastene, C., Couve, E., & Segura-Aguilar, J. (2008). The DT-diaphorase prevents the aggregation of α-synuclein induced by aminochrome. *Neurotoxicity Research, 13*, 136.

Cartier, E. A., Parra, L. A., Baust, T. B., Quiroz, M., Salazar, G., Faundez, V., Egaña, L., & Torres, G. E. (2010). A biochemical and functional protein complex involving dopamine synthesis and transport into synaptic vesicles. *Journal of Biological Chemistry, 151*, 1957–1966.

Carstam, R., Brinck, C., Hindemith-Augustsson, A., Rorsman, H., & Rosengren, E. (1991). The neuromelanin of the human substantia nigra. *Biochimica et Biophysica Acta*, *1097*(2), 152–160.

Chaudhry, F. A., Edwards, R. H., & Fonnum, F. (2008). Vesicular neurotransmitter transporters as targets for endogenous and exogenous toxic substances. *Annual Review of Pharmacology and Toxicology*, *48*, 277–301.

Chen, J., Song, J., Yuan, P., Tian, Q., Ji, Y., Ren-Patterson, R., Liu, G., Sei, Y., & Weinberger, D. R. (2011). Orientation and cellular distribution of membrane-bound catechol-*O*-methyltransferase in cortical neurons: Implications for drug development. *Journal of Biological Chemistry, 286*, 34752–34760.

Cheng, F. C., Kuo, J. S., Chia, L. G., & Dryhurst, G. (1996). Elevated 5-S-cysteinyldopamine/homovanillic acid ratio and reduced homovanillic acid in cerebrospinal fluid: Possible markers for and potential insights into the pathoetiology of Parkinson's disease. *Journal of Neural Transmission*, *103*, 433–446.

Conway, K. A., Rochet, J. C., Bieganski, R. M., & Lansbury, P. T., Jr. (2001). Kinetic stabilization of the a-synuclein protofibril by a dopamine- α-synuclein adduct. *Science, 294*, 1346–1349.

Corti, O., Lesage, S., & Brice, A. (2011). What genetics tells us about the causes and mechanisms of Parkinson's disease. *Physiological Reviews, 91*, 1161–1218.

Cuervo, A. M., Wong, E. S., & Martinez-Vicente, M. (2010). Protein degradation, aggregation, and misfolding. *Movement Disorders, 25*(Suppl 1), S49–S54.

Dagnino-Subiabre, A., Cassels, B. K., Baez, S., Johansson, A. S., Mannervik, B., & Segura-Aguilar, J. (2000). Glutathione transferase M2-2 catalyzes conjugation of dopamine and dopa *o*-quinones. *Biochemical and Biophysical Research Communications, 274*, 32–36.

Díaz-Véliz, G., Paris, I., Mora, S., Raisman-Vozari, R., & Segura-Aguilar, J. (2008). Copper neurotoxicity in rat substantia nigra and striatum is dependent on DT-diaphorase inhibition. *Chemical Research in Toxicology, 21*, 1180–1185.

Fasano, M., Bergamasco, B., & Lopiano, L. (2006). Is neuromelanin changed in Parkinson's disease? Investigations by magnetic spectroscopies. *Journal of Neural Transmission, 113*, 769–774.

Foppoli, C., Coccia, R., Cini, C., & Rosei, M. A. (1997). Catecholamines oxidation by xanthine oxidase. *Biochimica et Biophysica Acta, 1334*, 200–206.

Fuentes, P., Paris, I., Nassif, M., Caviedes, P., & Segura-Aguilar, J. (2007). Inhibition of VMAT-2 and DT-diaphorase induce cell death in a substantia nigra-derived cell line – An experimental cell model for dopamine toxicity studies. *Chemical Research in Toxicology, 20*, 776–783.

Galzigna, L., De Iuliis, A., & Zanatta, L. (2000). Enzymatic dopamine peroxidation in substantia nigra of human brain. *Clinica Chimica Acta, 300*, 131–138.

Gerlach, M., Double, K. L., Ben-Shachar, D., Zecca, L., Youdim, M. B., & Riederer, P. (2003). Neuromelanin and its interaction with iron as a potential risk factor for dopaminergic neurodegeneration underlying Parkinson's disease. *Neurotoxicity Research, 5*, 35–44.

Graham, D. G., Tiffany, S. M., Bell, W. R., Jr., & Gutknecht, W. F. (1978). Autoxidation versus covalent binding of autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Molecular Pharmacology, 14*, 644–653.

Guillot, T. S., & Miller, G. W. (2009). Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons. *Molecular Neurobiology*, *39*(2), 149–170. doi:10.1007/s12035-009-8059-y.

Hastings, T. G. (1995). Enzymatic oxidation of dopamine: The role of prostaglandin H synthase. *Journal of Neurochemistry, 64*, 919–924.

Hattori, N., Matsumine, H., Asakawa, S., Kitada, T., Yoshino, H., Elibol, B., Brookes, A. J., Yamamura, Y., Kobayashi, T., Wang, M., Yoritaka, A., Minoshima, S., Shimizu, N., & Mizuno, Y. (1998). Point mutations (Thr240Arg and Gln311Stop) [correction of Thr240Arg and Ala311Stop] in the Parkin gene. *Biochemical and Biophysical Research Communications, 249*, 754–758.

Hawkes, C. H., Del Tredici, K., & Braak, H. (2010). A timeline for Parkinson's disease. *Parkinsonism & Related Disorders, 16*, 79–84.

Hong, L., & Simon, J. D. (2007). Current understanding of the binding sites, capacity, affinity, and biological significance of metals in melanin. *The Journal of Physical Chemistry. Series, B, 111*, 7938–7947.

Ito, S., Kato, T., Maruta, K., Fujita, K., & Kurahashi, T. (1984). Determination of DOPA, dopamine, and 5-*S*-cysteinyl-DOPA in plasma, urine, and tissue samples by high-performance liquid chromatography with electrochemical detection. *Journal of Chromatography, 311*, 154–159.

Jimenez, M., Garcia-Carmona, F., Garcia-Canovas, F., Iborra, J. L., Lozano, J. A., & Martinez, F. (1984). Chemical intermediates in dopamine oxidation by tyrosinase, and kinetic studies of the process. *Archives of Biochemistry and Biophysics, 235*, 438–448.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., & Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature, 392*, 605–608.

Knoth, J., Zallakian, M., & Njus, D. (1981). Stoichiometry of H^+-linked dopamine transport in chromaffin granule ghosts. *Biochemistry*, *20*(23), 6625–6629.

Kuhn, D. M., & Arthur, R., Jr. (1998). Dopamine inactivates tryptophan hydroxylase and forms a redox-cycling quinoprotein: Possible endogenous toxin to serotonin neurons. *Journal of Neuroscience, 18*, 7111–7117.

LaVoie, M. J., Ostaszewski, B. L., Weihofen, A., Schlossmacher, M. G., & Selkoe, D. J. (2005). Dopamine covalently modifies and functionally inactivates parkin. *Nature Medicine, 11*, 1159–1161.

Linert, W., Herlinger, E., Jameson, R. F., Kienzl, E., Jellinger, K., & Youdim, M. B. (1996). Dopamine, 6-hydroxydopamine, iron, and dioxygen – Their mutual interactions and possible implication in the development of Parkinson's disease. *Biochimica et Biophysica Acta, 1316*, 160–168.

Lozano, J., Muñoz, P., Nore, B. F., Ledoux, S., & Segura-Aguilar, J. (2010). Stable expression of short interfering RNA for DT-diaphorase induces neurotoxicity. *Chemical Research in Toxicology, 23*, 1492–1496.

Marin, C., & Obeso, J. A. (2010). Catechol-*O*-methyltransferase inhibitors in preclinical models as adjuncts of L-dopa treatment. *International Review of Neurobiology, 95*, 191–205.

McNaught, K. S., Perl, D. P., Brownell, A. L., & Olanow, C. W. (2004). Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Annals of Neurology, 56*, 149–162.

Monastyrska, I., Rieter, E., Klionsky, D. J., & Reggiori, F. (2009). Multiple roles of the cytoskeleton in autophagy. *Biological Reviews of the Cambridge Philosophical Society, 84*, 431–448.

Muñoz, P., Paris, I., Sanders, L. H., Greenamyre, J. T., & Segura-Aguilar, J. (2012). Overexpression of VMAT-2 and DT-diaphorase protects substantia nigra-derived cells against aminochrome neurotoxicity. *Biochimica et Biophysica Acta*, *1822*(7), 1125–1136. doi:10.1016/j.bbadis.2012.03.010.

Myöhänen, T. T., Schendzielorz, N., & Männistö, P. T. (2010). Distribution of catechol-*O*-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. *Journal of Neurochemistry, 113*, 1632–1643.

Naoi, M., Maruyama, W., Yi, H., Yamaoka, Y., Shamoto-Nagai, M., Akao, Y., Gerlach, M., Tanaka, M., & Riederer, P. (2008). Neuromelanin selectively induces apoptosis in dopaminergic SH-SY5Y cells by deglutathionylation in mitochondria: Involvement of the protein and melanin component. *Journal of Neurochemistry, 105*, 2489–2500.

Napolitano, A., Manini, P., & d'Ischia, M. (2011). Oxidation chemistry of catecholamines and neuronal degeneration: an update. *Current Medicinal Chemistry*, *18*(12), 1832–1845.

Norris, E. H., Giasson, B. I., Hodara, R., Xu, S., Trojanowski, J. Q., Ischiropoulos, H., & Lee, V. M. (2005). Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome – Mediated conformational alterations. *Journal of Biological Chemistry, 280*, 21212–21219.

Paris, I., Dagnino-Subiabre, A., Marcelain, K., Bennett, L. B., Caviedes, P., Caviedes, R., Olea-Azar, C., & Segura-Aguilar, J. (2001). Copper neurotoxicity is dependent on dopamine-mediated copper uptake and one-electron reduction of aminochrome in a rat substantia nigra neuronal cell line. *Journal of Neurochemistry, 77*, 519–529.

Paris, I., Martinez-Alvarado, P., Perez-Pastene, C., Vieira, M. N., Olea-Azar, C., Raisman-Vozari, R., Cardenas, S., Graumann, R., Caviedes, P., & Segura-Aguilar, J. (2005a). Monoamine transporter inhibitors and norepinephrine reduce dopamine-dependent iron dependent iron toxicity in cells derived from the substantia nigra. *Journal of Neurochemistry, 92*, 1021–1032.

Paris, I., Martinez-Alvarado, P., Cardenas, S., Perez-Pastene, C., Graumann, R., Fuentes, P., Olea-Azar, C., Caviedes, P., & Segura-Aguilar, J. (2005b). Dopamine-dependent iron toxicity in cells derived from rat hypothalamus. *Chemical Research in Toxicology, 18*, 415–419.

Paris, I., Perez-Pastene, C., Couve, E., Caviedes, P., Ledoux, S., & Segura-Aguilar, J. (2009). Copper dopamine complex induces mitochondrial autophagy preceding caspase-independent apoptotic cell death. *Journal of Biological Chemistry, 284*, 13306–13315.

Paris, I., Perez-Pastene, C., Cardenas, S., Iturriaga-Vasquez, P., Muñoz, P., Couve, E., Caviedes, P., & Segura-Aguilar, J. (2010). Aminochrome induces disruption of actin, alpha-, and

beta-tubulin cytoskeleton networks in substantia-nigra-derived cell line. *Neurotoxicity Research, 18*, 82–92.

Paris, I., Muñoz, P., Huenchuguala, S., Couve, E., Sanders, L. H., Greenamyre, J. T., Caviedes, P., & Segura-Aguilar, J. (2011). Autophagy protects against aminochrome-induced cell death in substantia nigra-derived cell line. *Toxicological Sciences, 121*, 376–388.

Pezzella, A., Crescenzi, O., Natangelo, A., Panzella, L., Napolitano, A., Navaratnam, S., Edge, R., Land, E. J., Barone, V., & D'Ischia, M. (2007). Chemical, pulse radiolysis and density functional studies of a new, labile 5,6-indolequinone and its semiquinone. *Journal of Organic Chemistry, 72*, 1595–1603.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., & Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science, 276*, 2045–2047.

Rosengren, E., Linder-Eliasson, E., & Carlsson, A. (1985). Detection of 5-S-cysteinyldopamine in human brain. *Journal of Neural Transmission*, *63*, 247–253.

Saura, J., Luque, J. M., Cesura, A. M., Da Prada, M., Chan-Palay, V., Huber, G., Loffler, J., & Richards, J. G. (1994). Increased monoamine oxidase B activity in plaque-associated astrocytes of Alzheimer brains revealed by quantitative enzyme radioautography. *Neuroscience, 62*, 15–30.

Schapira, A. H. (2011). Mitochondrial pathology in Parkinson's disease. *The Mount Sinai Journal of Medicine, 78*, 872–881.

Schapira, A. H., & Jenner, P. (2011). Etiology and pathogenesis of Parkinson's disease. *Movement Disorders, 26*, 1049–1055.

Schendzielorz, N., Rysa, A., Reenila, I., Raasmaja, A., & Mannisto, P. T. (2011). Complex estrogenic regulation of catechol-*O*-methyltransferase (COMT) in rats. *Journal of Physiology and Pharmacology, 62*, 483–490.

Schultzberg, M., Segura-Aguilar, J., & Lind, C. (1988). Distribution of DT diaphorase in the rat brain: Biochemical and immunohistochemical studies. *Neuroscience*, *27*(3), 763–776.

Segura-Aguilar, J. (1996). Peroxidase activity of liver microsomal vitamin D 25-hydroxylase and cytochrome P450 1A2 catalyzes 25-hydroxylation of vitamin D3 and oxidation of dopamine to aminochrome. *Biochemical and Molecular Medicine, 58*, 122–129.

Segura-Aguilar, J., Baez, S., Widersten, M., Welch, C. J., & Mannervik, B. (1997). Human class Mu glutathione transferases, in particular isoenzyme M2-2, catalyze detoxication of the dopamine metabolite aminochrome. *Journal of Biological Chemistry*, *272*(9), 5727–5731.

Segura-Aguilar, J., & Lind, C. (1989). On the mechanism of Mn^{3+} induced neurotoxicity of dopamine: Prevention of quinone derived oxygen toxicity by DT-diaphorase and superoxide dismutase. *Chemico-Biological Interactions, 72*, 309–324.

Segura-Aguilar, J., Metodiewa, D., & Welch, C. (1998). Metabolic activation of dopamine *o*-quinones to *o*-semiquinones by NADPH cytochrome P450 reductase may play an important role in oxidative stress and apoptotic effects. *Biochimica et Biophysica Acta, 1381*, 1–6.

Segura-Aguilar, J., Cardenas, S., Riveros, A., Fuentes-Bravo, P., Lozano, J., Graumann, R., Paris, I., Nassif, M., & Caviedes, P. (2006). DT-diaphorase prevents the formation of alpha-synuclein adducts with aminochrome. *Society for Neuroscience: Abstract Archive, 824*, 17.

Shen, X. M., Xia, B., Wrona, M. Z., & Dryhurst, G. (1996). Synthesis, redox properties, in vivo formation, and neurobehavioral effects of N-acetylcysteinyl conjugates of dopamine: possible metabolites of relevance to Parkinson's disease. *Chemical Research in Toxicology*, *9*, 1117–1126.

Shih, J. C., Grimsby, J., & Chen, K. (1997). Molecular biology of monoamine oxidase A and B: Their role in the degradation of serotonin. In H. G. Baumgarten & M. Gothert (Eds.), *Handbook of experimental pharmacology* (Serotoninergic neurons and 5-HT receptors in the CNS, Vol. 129, pp. 655–670). Berlin: Springer.

Strolin-Benedetti, M., Dostert, P., & Tipton, K. F. (1992). Developmental aspects of the monoamine-degrading enzyme monoamine oxidase. *Developmental Pharmacology and Therapeutics, 18*, 191–200.

Tan, E. K., Cheah, S. Y., Fook-Chong, S., Yew, K., Chandran, V. R., Lum, S. Y., & Yi, Z. (2005). Functional COMT variant predicts response to high dose pyridoxine in Parkinson's disease. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 137B*, 1–4.

Thompson, M., Capdevila, J. H., & Strobel, H. W. (2000). Recombinant cytochrome P450 2D18 metabolism of dopamine and arachidonic acid. *Journal of Pharmacology and Experimental Therapeutics, 294*, 1120–1130.

Van Laar, V. S., Mishizen, A. J., Cascio, M., & Hastings, T. G. (2009). Proteomic identification of dopamine-conjugated proteins from isolated rat brain mitochondria and SH-SY5Y cells. *Neurobiology of Disease, 34*, 487–500.

Weinreb, O., Amit, T., Riederer, P., Youdim, M. B., & Mandel, S. A. (2011). Neuroprotective profile of the multitarget drug rasagiline in Parkinson's disease. *International Review of Neurobiology, 100*, 127–149.

Westlund, K. N., Denney, R. M., Rose, R. M., & Abell, C. W. (1988). Localization of distinct monoamine oxidase A and monoamine oxidase B cell populations in human brainstem. *Neuroscience, 25*, 439–456.

Weyler, W., Hsu, Y. P., & Breakefield, X. O. (1990). Biochemistry and genetics of monoamine oxidase. *Pharmacology and Therapeutics, 47*, 391–417.

Whitehead, R. E., Ferrer, J. V., Javitch, J. A., & Justice, J. B. (2001). Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter. *Journal of Neurochemistry, 76*, 1242–1251.

Williams, A. (1984). MPTP parkinsonism. *British Medical Journal, 289*, 1401–1402.

Wolters, E. C., & Braak, H. (2006). Parkinson's disease: Premotor clinico-pathological correlations. *Journal of Neural Transmission. Supplementum, 70*, 309–319.

Xilouri, M., Vogiatzi, T., Vekrellis, K., Park, D., & Stefanis, L. (2009). Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. *PLoS One, 4*, e5515. doi:10.1371/journal.pone.0005515.

Xu, Y., Stokes, A. H., Roskoski, R., Jr., & Vrana, K. E. (1998). Dopamine, in the presence of tyrosinase, covalently modifies and inactivates tyrosine hydroxylase. *Journal of Neuroscience Research, 54*, 691–697.

Zafar, K. S., Siegel, D., & Ross, D. (2006). A potential role for cyclized quinones derived from dopamine, DOPA, and 3,4-dihydroxyphenylacetic acid in proteasomal inhibition. *Molecular Pharmacology, 70*, 1079–1086.

Zecca, L., Fariello, R., Riederer, P., Sulzer, D., Gatti, A., & Tampellini, D. (2002). The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. *FEBS Letters, 510*, 216–220.

Zhang, N. Y., Tang, Z., & Liu, C. W. (2008). Alpha-synuclein protofibrils inhibit 26S proteasome-mediated protein degradation: Understanding the cytotoxicity of protein protofibrils in neurodegenerative disease pathogenesis. *Journal of Biological Chemistry, 283*, 20288–20298.

Zhang, W., Phillips, K., Wielgus, A. R., Liu, J., Albertini, A., Zucca, F. A., Faust, R., Qian, S. Y., Miller, D. S., Chignell, C. F., Wilson, B., Jackson-Lewis, V., Przedborski, S., Joset, D., Loike, J., Hong, J. S., Sulzer, D., & Zecca, L. (2011). Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: Implications for progression of Parkinson's disease. *Neurotoxicity Research, 19*, 63–72.

Neuroinflammation and Parkinson's Disease

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Abstract

Parkinson's disease (PD) is characterized by a slow and progressive degeneration of dopaminergic neurons in the substantia nigra *pars compacta*. To date, despite an intensive research, the cause of the neuronal loss in PD is poorly understood. It has been postulated that neuroinflammatory mechanisms might contribute to the cascade of events leading to this neuronal degeneration. In this review, we describe the evidence for neuroinflammatory processes from *postmortem*, in vivo and in vitro studies in PD. We further identify the cellular types associated with neuroinflammation that are involved in the degeneration of dopaminergic neurons in animal models and PD patients. Overall, available data support the importance of non-cell-autonomous pathological mechanisms in PD, which are mostly mediated by activated glial and peripheral immune cells. This cellular response to neurodegeneration triggers deleterious events (e.g., oxidative stress and cytokine-receptor-mediated apoptosis), which might eventually lead to dopaminergic cell death and hence disease progression. Novel studies have also demonstrated that genetic markers could be implicated in neuroinflammation and even in the role of glucocorticoids in sustaining activated microglia in inflammatory processes. Finally, based on these evidences, we highlight possible therapeutic strategies aimed at downregulating these inflammatory processes that might be important to slow down the progression of PD.

Keywords

Immunomodulatory therapy • Inflammation • Microglia • Neuroinflammation • Parkinson's disease

1 Introduction to Parkinson's Disease

1.1 Overview

Parkinson's disease (PD) is the second most common neurodegenerative disorder in developed countries and the most common neurodegenerative movement disorder (Calabrese 2007; Dorsey et al. 2007; Rochet et al. 2012; Schapira et al. 2009). It is an age-dependent disease with an average age of onset of 55 years of age, approximately 1 % of 65-year-olds are affected, and this rises to 4–5 % in 85-year-olds, while the risk for developing PD increases 5-fold by the age of 70 (Bauso et al. 2012; de Lau et al. 2004; de Rijk et al. 1997; Fahn 2003; Khedr et al. 2012). Two forms of the disease have been characterized: a sporadic form, which generally affects 95 % of all patients and whose etiology is unknown, and a familiar genetic form, which affects 7–10 % of all cases and is linked to mutations in a restricted number of genes (Dauer and Przedborski 2003; Kumar et al. 2012; Martin et al. 2011; Pastor 2012). However, during the last decades, genome-wide association studies (GWAS) have provided evident gains in interpreting the genetic of PD (Nalls et al. 2011; Satake et al. 2009; Simon-Sanchez et al. 2009), and recently, 9 of

the 11 postulated susceptibility single-nucleotide polymorphisms (SNPs) (Nalls et al. 2011) have been corroborated for PD (Sharma et al. 2012).

PD is characterized by motor symptoms like resting tremor, slowed movement, postural instability, and muscle rigidity (Reichmann 2010); however, there are other symptoms that may include olfactory dysfunctions, urinary problems, constipation, or sleep disruptions and which can halt or even slow down the progression of PD (Erro et al. 2012; Grinberg et al. 2010; Pastor 2012; Peeraully and Tan 2012). The motor symptoms can be treated with dopaminergic drugs; however, the effectiveness diminishes as the severity of the clinical symptoms increases due to progression of the underlying neurodegeneration (Schapira et al. 2009), and moreover, this treatment only improves the symptomatic motor dysfunction.

1.2 Etiology and Molecular Pathways of Neurodegeneration

PD is a neurodegenerative disorder with a complex, multifactorial etiology. In recent years, there has been increasing evidences to support a role for genetic factors in this cause. These evidences have come from twin and familiar studies, where a screening of the most potential susceptibility genes was done. Together with genetic factors, environmental factors may play a role in the disease progress; however, the most accepted theory for PD etiology is that there is a complex interplay between susceptible genes and environmental influences in the causation of PD (Kwok 2010; Soreq et al. 2012).

For non-familiar forms of PD, the dominate view is that multiple causes exist that are decisive in disease initiation and progression; among all of them the age, the genetic predisposition, and the exposition to environmental toxins are especially critical (Nagatsu and Sawada 2006). At the cellular level, three types of cellular alterations may be distinguished in the pathogenesis of PD: the production of reactive oxygen species, dysfunctions in mitochondrial respiratory chain, and an aberrant protein aggregation (Banerjee et al. 2009). Presently, mitochondrial complex I toxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-1'-dimethyl-4-4'-bipyridinium (paraquat), and rotenone, are used for inducing nigrostriatal neuronal degeneration due to dopaminergic (DA) neurons that are specially susceptible to this substances (Meredith et al. 2008).

Following a histological point of view, within the brain the most striking pathological features are the severe loss of dopaminergic neurons in the substantia nigra (SN), the presence of proteinaceous inclusions principally composed of fibrillar α-synuclein, and ubiquitinated proteins within some remaining nigral neurons called Lewy bodies (LBs) or abnormal neurites called Lewy neurites (LNs) (Lees et al. 2009; Lewy 1912; Trétiakoff 1919). However, nowadays LBs and LNs stained with antibodies to ubiquitin, α-synuclein, and other biomarkers can be detected in other brain regions apart from the SN, like the locus coeruleus, amygdala, dorsal raphe nucleus, thalamus, and cerebral cortex (Braak et al. 2003; Halliday et al. 2011; McLean et al. 2012). This would explicate why PD debut in patients is associated not with motor dysfunctions but rather with a variety of

non-motor symptoms including those concerning the autonomic nervous system, a subset of which are prodromal and can antecede the motor symptoms by 10 years (Dubow 2007; Grinberg et al. 2010; Kaufmann et al. 2004; Poewe 2007; Tolosa and Pont-Sunyer 2011). According to Braak and Braak's *postmortem* anatomopathological studies, the first areas that are affected by the illness are the dorsal IX/X motor nucleus, medulla oblongata, and pontine tegmentum, and over time, LBs and LNs will affect midbrain regions as SN and finally neocortical regions (Braak et al. 2003). It is not until after reaching specifically the SN *pars compacta* (SNpc) (stage 3 of Braak and Braak classification) that clinical symptoms appear due to the deficits in dopamine release at the level of the striatum (Del Tredici et al. 2002; Picconi et al. 2012).

1.3 Pathogenesis of Parkinson's Disease

Among the environmental toxins, which have been postulated with the development and progression of PD, it has been suggested that pesticides (e.g., paraquat or rotenone), infectious agents (e.g., virus), and heavy metals could have a crucial role (Mizuno et al. 2001; Rohn and Catlin 2011). However, it is not important what insult initially provokes neurodegeneration, since parkinsonism animal models and the function of genes implicated in familial PD form suggest that the main culprits that trigger degeneration in SNpc dopaminergic neurons are the ubiquitin-proteasome pathway, mitochondrial dysfunction, and oxidative stress (Elstner et al. 2011; Kanthasamy et al. 2010). While the pathogenic mechanisms that ultimately cause PD are still unclear, because the key molecular and cellular events underlying development of PD are divergent, it is believed that the progressive nature of PD is characterized by the activation of neuroinflammatory pathways that over time trigger neurodegeneration (Dauer and Przedborski 2003; Gao and Hong 2008; Tansey and Goldberg 2010).

2 Neuroinflammation in the Pathogenesis of PD

Glial activation and inflammatory reactions play important roles in DA neuronal death in PD patients and animal models inducing microglial activation, disruption of the blood–brain barrier (BBB), and infiltration of peripheral immune cells (Block and Hong 2005; Choi et al. 2005; Panaro and Cianciulli 2012). However, all these changes are not exclusive of PD, since neuroinflammatory processes are also present in other neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (Cunningham 2012; Hirsch and Hunot 2009). The purpose of this chapter is to describe the evidences of neuroinflammatory processes associated with the nigrostriatal DA neuronal degeneration not only in the brains of PD patients even more in animal models of parkinsonism. Finally, we will discuss the effectiveness of using specific targets on inflammatory pathways that could relent the progression of PD.

2.1 Hallmarks of Neuroinflammation

Neuroinflammation is the brain response to infections and injuries associated with most acute or chronic neurodegenerative diseases (Nencini et al. 2003; Schmidt et al. 2005). Neuropathological and neuroradiological studies have shown that prior to significant loss of neurons, a neuroinflammatory process characterized by an increase in glial activity often occurs (Frank-Cannon et al. 2009; Fuhrmann et al. 2010; Ratai et al. 2011). The dogma that the nervous and immune systems were isolated with litter interaction except during disease and/or trauma was based on the evidences that immune responses were blunted in the central nervous system (CNS) and that the blood–brain barrier (BBB) prevented infiltration of immune cells and molecules into the CNS (Prendergast and Anderton 2009). However, recently, the convergence of unexpected results from diverse fields has demonstrated a bidirectional communication between these systems. Firstly, it has been postulated for many years that the BBB was an impermeable barrier to immune cells and most diffusible factors produced in the periphery. However, last studies have clarified that, in response to insult, the permeability of BBB may increase or become dysregulated and also that cytokines produced by glial cells in the brain can cross the compromised BBB and activate immune cells (Deverman and Patterson 2009; Prat et al. 2001). In response to cytokine activity, blood-derived monocytes will migrate through the compromised BBB into the CNS and aid microglia in causing neural inflammation, degeneration, and cell death (Hakimi et al. 2011). Moreover, immune cell infiltration into the CNS is more common than previously believed. Indeed, many neurological diseases, including Alzheimer's and Parkinson's disease, are accompanied by neuroinflammation, and anti-inflammatory drugs reduce the risk for Alzheimer's and Parkinson's disease (Lucin and Wyss-Coray 2009). Secondly, there is now growing evidence that neural-immune cross talk may even occur in non-disease conditions, including in the healthy brain, where the permeability of the BBB can be regulated under normal conditions (Chung et al. 2010).

The terms neuroinflammation and neurodegeneration are intimately linked since neuroinflammation consists of an innate immune response which involves the activation of microglial cells which produces several products like cytokines, chemokines, complement cascade proteins, prostaglandins, and reactive oxygen species in response to a peripheral or central insult (Chung et al. 2010), and the term "neurodegenerative" disease, as PD and AD are, is based on the feature that it is primarily the death and dysfunction of neurons that cause disability, being, the activation of various components of the innate immune system, recognized as a feature of these "neurodegenerative" diseases (Tansey and Goldberg 2010). This association is very important under the clinical point of view because if the immune system, as it is supposed, plays any active role in a neurodegenerative disease, for better or for worse, then modifying it – enhancing favorable behaviors or suppressing harmful ones – may be capable of slowing the progression of the disease. The role of neuroinflammation in neurodegenerative diseases is still controversial, since neuroinflammatory response may exert protective but also

detrimental effects, the reason why it is suggested to be a "double-edged sword" (McGeer and McGeer 2004; Wyss-Coray and Mucke 2002).

2.2 Cell Types Involved in Neuroinflammation

In innate immunity, peripheral effectors are myeloid-derived blood monocytes that additionally give rise to macrophages and a specific type of antigen-presenting cells (APC) that are dendritic cells (DC) (Kurant 2011). The role of these macrophages/APC is performed by the brain microglia in the CNS, being the primary effectors of immune surveillance and self-renewing and arising from fetal myeloid progenitors (Eglitis and Mezey 1997). Microglia play a homeostatic role in the CNS and respond to environmental stresses and immunologic challenges by removing dying cells (Nakamura 2002), entering a state of chronic activation in which they release toxic factors resulting on ongoing damage to surrounding tissue (Ransohoff and Perry 2009). Although the key molecular and cellular events underlying development of neurodegenerative diseases are clearly divergent among them, one common way in which these divergent molecular or cellular events (e.g., aberrant protein misfoldings, peroxidations, mutations, aggregation) may all contribute over time to neuronal death is activating resident microglial populations in specific brain areas. In the case of genetic mutations or prolonged/repeated environmental exposures, where the initial stimulus that triggered microglial activation is not silenced/eliminated, a self-sustaining cycle of neuroinflammation can ensue, and such a chronic inflammatory environment is presumptive to cause neuronal dysfunctions and death of compromised neuronal populations.

Astrocytes, another member of the glial cell family, contribute to virtually every aspect of brain function, including ionic homeostasis, energy metabolism, and synaptic signalling, and play an important role in the stabilization of the permeability of the BBB (Zlokovic 2008). The varied and important roles of astrocytes have evolved to allow increasingly complex nervous systems to operate efficiently and with high fidelity. For example, astrocytes figure prominently in glutamatergic synaptic transmission, a basic event of brain function: high-affinity glutamate uptake into astrocytes meliorates the temporal and spatial fidelity of glutamatergic signalling, and astrocytes afterward move glutamine back to neurons for the synthesis of more glutamate (Hertz and Zielke 2004; Uwechue et al. 2012). Recent studies have demonstrated that the important and dynamic contributions of astrocytes to normal brain function demand that neuron and astrocyte relationship must be seen as an equal partner relation where this harmonious collaboration will produce a desired function (Ransom and Ransom 2012). So, it has to be discarded as the historical view of astrocytes as simple "scaffolding" cells; nowadays the most accurate view is to denominate astrocytes as "partner cells" (Ransom and Ransom 2012).

During past decades the role of peripheral immune cells in neuroinflammation was very controversial. Besides this controversy, increased accumulation of activated microglia and astrocytes and leukocytes adhering to postcapillary venules are

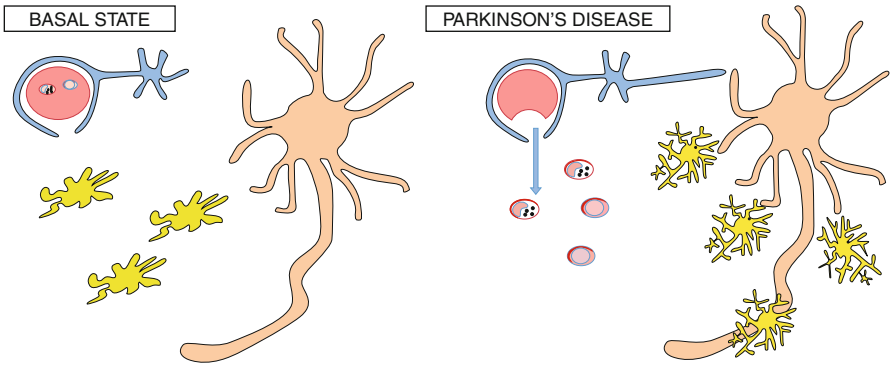

Fig. 1 Neuroinflammation triggers the activation of the peripheral immune system. Under neuroinflammatory conditions, there is a modification of the local microenvironment through early microglial activation that allows peripheral immune cell infiltration. Contributing to neuroinflammation, the presence of newly created vessels that have not likely developed the restrictive properties of the blood–brain barrier, failing to protect the parenchyma from peripheral immune cells and inflammatory factors in the peripheral circulation, has been shown

observed in the affected brain areas in neurodegenerative diseases, suggesting the presence of an ongoing inflammatory process (Casoli et al. 2010; Chung et al. 2010). As neuroinflammation triggers the activation of peripheral immune system, several studies implicated subsets of T cells taking part of the neurodegenerative processes. The mechanism related with this specific recruitment of T cells is still unknown, but it might involve a modification of the local microenvironment through early microglial activation and innate neuroinflammatory processes (Chung et al. 2010) (Fig. 1).

2.3 Evidence for a Role of Neuroinflammation in PD

2.3.1 Role of Microglia in PD

Data from postmortem studies provided the first evidence for a role of neuroinflammation in PD, since a massive microglial activity was detected in the brain of PD patients (McGeer et al. 1988b). Moreover, in postmortem investigations of humans exposed to MPTP, activated microglia have been detected 16 years after the last drug exposure (Langston et al. 1999). Since McGeer's initial observation (McGeer et al. 1988b), activated microglia have been identified in the putamen, hippocampus, transentorhinal cortex, cingulated cortex, and temporal cortex of PD brains (Imamura et al. 2003). However, since these are end-stage cases, whether this activation was a general consequence of neuronal death or the reflection of the active participation of microglia in the neurodegenerative process remains unclear (Graeber and Streit 2010).

In order to elucidate the role of microglia activation in PD, several groups have studied several animal models using MPTP, rotenone, and 6-OHDA that exhibit levels of microglial activation similar to what is found in PD (Arai et al. 1999;

Barcia et al. 2003; Carvey et al. 2005; Cicchetti et al. 2002; Gao et al. 2002). However, recent studies have demonstrated that in the case of paraquat and rotenone, microglial activation are likely non-cell autonomous and may occur as a result of dopaminergic neuron damage or by factors released by neurons and/or other cells (Klintworth et al. 2009). Similar to what has been described in MPTP-intoxicated humans, an activated microglia and dopaminergic cell loss was also found in the SN of primates years after they were treated with MPTP (Arai et al. 1999; McGeer et al. 2003), even in those parkinsonian animals never treated neither with levodopa nor with dopaminergic agonists (Barcia et al. 2004), suggesting that microglia plays an active role in the pathology of PD and may indeed perpetuate the degeneration of dopaminergic neurons once activated.

Moreover, the presence of microglial activation participating in neuroinflammatory processes has also been confirmed on a molecular basis. Levels of proinflammatory mediators, including TNFα, IL-1β, IL-6, reactive oxygen species (ROS), and eicosanoids, are elevated in the brains and peripheral blood mononuclear cells (PBMC) of PD patients (McGeer et al. 1988b; Mogi et al. 1994; Nagatsu et al. 2000; Nagatsu and Sawada 2005).

Oxidative stress is caused by an imbalance between the production and destruction of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The production of ROS/RNS under neuropathological conditions would be modulated by microglial NADPH oxidase and nitric oxidase synthase (NOS) (Paramo et al. 2010). Nitrite (as an indicator for nitric oxide free radicals) in the cerebrospinal fluid, as well as increased expression of inducible nitric oxide synthase (iNOS) within the SN, was also found in PD patients (Qureshi et al. 1995). Moreover, an association between Parkinson's disease and polymorphisms in the nNOS and iNOS genes has been described (Levecque et al. 2003). In the MPTP model of parkinsonism, NADPH oxidase and inducible nitric oxidase synthase (iNOS) are known to be major sources of ROS/RNS production where they mediate DA neuronal death in SN (Liberatore et al. 1999; Wu et al. 2002, 2003). Moreover, other in vivo and in vitro studies have demonstrated that microglia-derived NADPH oxidase/iNOS play a crucial role in the production of ROS/RNS and conduce to DA neuronal death following the oxidative stress pathway (Qin et al. 2005; Rodriguez-Pallares et al. 2007). Consequently, the vast majority of therapeutic attention directed at inflammation within the CNS in patients with PD has been directed toward microglial cell activity.

Numerous investigations have proposed a deleterious role of microglial activation in PD based on the vulnerability of dopaminergic neurons to various microglia-derived proinflammatory cytokines (De Lella Ezcurra et al. 2010; Ferrari et al. 2006; Stone et al. 2009), while α-synuclein can directly induce activation of microglia and release of proinflammatory cytokines, leading to dopaminergic neurodegeneration (Hirsch and Hunot 2009; Zhang et al. 2005). Using animal models, it has been shown that α-synuclein overexpression triggers an early microglial activation in the SN as well as elicits an increased expression of proinflammatory molecules such as IL-1β and TNFα, prior to dopaminergic neurodegeneration (Kachroo and Schwarzschild 2012; Mosharov et al. 2009;

Thiruchelvam et al. 2004). In addition, α-synuclein is released from cells in vitro and is capable of directly activating microglia (Jin et al. 2007; Maguire-Zeiss 2008; Zhang et al. 2005). Taken together, these observations place α-synuclein and microglial activation together as early players contributing to neuroinflammation that may mediate ulterior dopaminergic neurodegeneration (Gao et al. 2011).

However, it seems that the plasticity of microglia must be considered with regard to their contribution in PD, and their role (whether beneficial or detrimental) may depend on the stimuli present and the stage of disease (Li et al. 2007; Michelucci et al. 2009; Sanchez-Guajardo et al. 2010).

One approach that can connect microglial activation with PD pathogenesis involves in vivo imaging. Positron emission tomography (PET) imaging in combination with the isoquinoline ^{11}C-PK11195 BP_{ND}, which is a ligand of the peripheral binding site of benzodiazepine, is used to track microglial activation (Gerhard et al. 2006a; Ouchi et al. 2005). Although there was a controversy about the efficiency of this marker as a microglial detector, based on discrepancies between in vitro and in vivo experiments, nowadays it is accepted that PK11195 binds to the peripheral binding site of benzodiazepine on activated microglia and macrophages. A significant increase in this PK1195 marker, reflecting microglial activation, was reported in the midbrain and putamen of PD patients when compared to controls, and this activation positively correlated with the motor severity of PD (Bartels et al. 2010; Ouchi et al. 2005). These findings demonstrated the crucial pathogenic importance of early microglial activation for the progression of the disease which suggested that an early introduction of neuroprotective drug to slow down or inhibit microglial activation could be a suitable therapeutic target in PD. Additionally, PD patients exhibited significantly increased ^{11}C-PK11195 BP_{ND} in the basal ganglia, pons, and frontal and temporal cortical regions (Gerhard et al. 2006a). Gerhard and colleagues demonstrated that the increased microglial activation remained unchanged for 2 years, while the patients deteriorated clinically during this period (Gerhard et al. 2006a), similar to what had been described in MPTP-induced parkinsonian monkeys (Arai et al. 1999; Barcia et al. 2004). In relation with this sustained microglial activation, it has been recently described that IFN-γ signalling, associated with TNF-α expression, has a crucial and cell-specific role in stimulating and maintaining glial cell activation in parkinsonism (Barcia et al. 2011).

In sum, PET imaging and in vivo and in vitro studies support the hypothesis that microglial activation occurs early in PD where they remain activated for longer periods and possibly drive progression of the disease (Gerhard et al. 2006a, b) (Fig. 2).

2.3.2 Role of Astroglia and Oligodendroglia in PD

Astrocytes, the major glial components of the CNS, constitute up to 20–50 % of brain volume and provide the optimal microenvironment for neuronal function by exerting active control on the cerebral blood flow (Gordon et al. 2008; Mulligan and MacVicar 2004). Although the presence of reactive astrocytes is evident in the most affected brain areas of PD patients having a role in the progression of the disease (Forno et al. 1992; Teismann and Schulz 2004), the specific role of this cell type is still unclear. It was previously described that one of the main functions of astrocytes is to secrete neurotrophic factors in SN for inducing the survival of dopaminergic

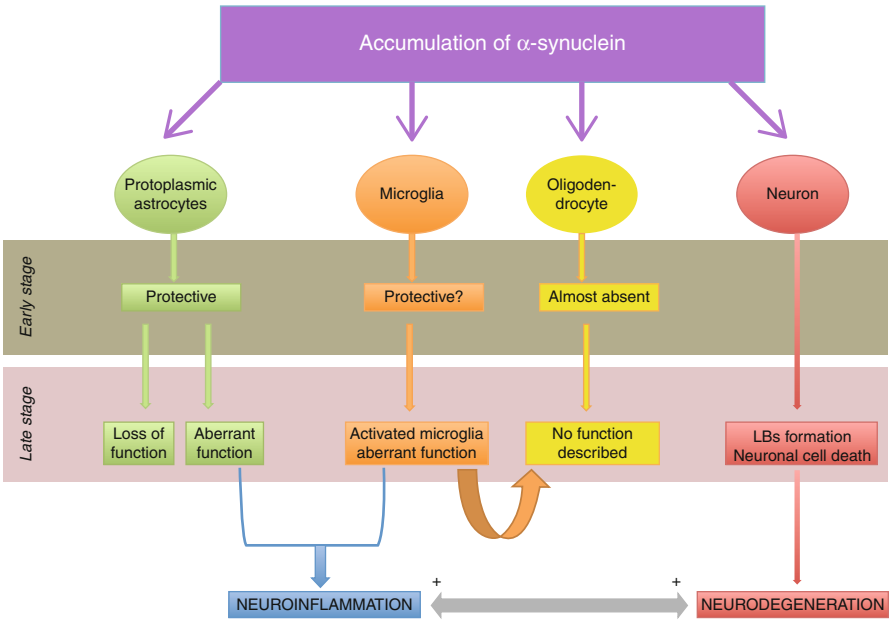

Fig. 2 Effect of α-synuclein accumulation on neuroinflammation associated with PD. Associated with PD, there is an accumulation of α-synuclein at different levels. During the early stages of neurodegeneration, astrocytes may quickly respond to α-synuclein released from degenerating neurons to protect against neurodegeneration. During the long time course of neurodegeneration, the activated astrocytes may lose their protective properties or might be aberrantly activated, leading to stimulation of neuroinflammation. On the other hand, microglia may be as well protective during the early stages of neurodegeneration, but at late stages of the disease, it would be aberrantly activated, contributing to neuroinflammation and perpetuate neurodegeneration. Concerning oligodendrocytes, the presence of α-synuclein deposits in oligodendroglial cells has been described, but its function is still not clear

neurons (McGeer and McGeer 2008). Although the relationship between reactive astrocytes and the release of neurotrophic factors is ambiguous, based on previous studies it was concluded that the decreased levels of astrocyte-derived neurotrophic factors are directly responsible for dopaminergic neurodegeneration in PD (Knott et al. 2002; Saavedra et al. 2006; Sandhu et al. 2009).

As it was previously described in this chapter, Lewy pathology follows a progressive pattern beginning in the anterior olfactory structures, dorsal motor nucleus of the vagal nerve, and portions of the enteric nervous system and evolving in caudo-rostral manner within the CNS (Braak and Del Tredici 2008). Unexpectedly, as PD progressed, α-synuclein-positive inclusions were also detected in astrocytes that were not known to produce α-synuclein (Wakabayashi et al. 2000). However, recent works have demonstrated, in cultured cells and α-synuclein overexpressed animal models, that α-synuclein proteins released from neuronal cells are taken up by astrocytes through endocytosis and form inclusion bodies, and this abnormal inclusions incite changes in astrocytic gene expression profiles reflective of

a proinflammatory response (Lee et al. 2010; Tousi et al. 2012). Finally, it was recently described that of the two main types of astrocytes, fibrous and protoplasmic, only protoplasmic astrocytes are involved in PD, where they become nonreactive and accumulate α-synuclein (Halliday and Stevens 2011). In sum, glial cells are responsible for the progression of PD and play an important role in initiating the early tissue response. In particular, early dysfunction and α-synuclein accumulation in astrocytes causes recruitment of phagocytic microglia that attack selected neurons in restricted brain regions causing the clinical symptoms of PD (Halliday and Stevens 2011) (Fig. 2).

In relation with oligodendrocytes, although neuroinflammation associated with PD has been attributed to activated microglia and astrocytes in both SN and striatum, the role of oligodendrocytes is unknown. Despite the presence of α-synuclein-positive filamentous inclusions in oligodendrocytes in the midbrain of PD patients, which was almost absent in PD patients with mild nigral degeneration (Khedr et al. 2012; Wakabayashi et al. 2000), the correlation between oligodendrocytes and parkinsonism has been barely explored (McGeer and McGeer 2008; Takagi et al. 2007). Recently our group has described in the MPTP model of parkinsonism that the loss of dopaminergic neurons and of their axons projecting to the striatum, in MPTP-induced parkinsonism in both mice and macaques, is accompanied by a prominent oligodendrogliosis along the nigrostriatal pathway, which significantly presents a negative correlation with striatal dopaminergic innervation (Annese et al. 2012). This exacerbation in oligodendroglial response could be induced by the cytokines released by both microglia and astrocytes involved in neuroinflammation (Arai et al. 1999; McGeer and McGeer 2008) (Fig. 3). However, the function and mechanisms triggered by oligodendrocytes and the importance of these α-synuclein deposits must be further investigated in both experimental models of parkinsonism and PD.

2.3.3 Role of T Cells in Mediating Neuroinflammation-Induced PD

A role of T cells in neuroinflammation associated with neurodegenerative diseases had been several times denied based on the idea that the CNS has traditionally been considered "immune privileged" due to the presence of innate microglia and the protection of the BBB, but recently numerous evidences have discarded this hypothesis. In relation with PD, a role for an adaptive immune response in the etiology of PD has been also demonstrated. For example, the presence of T lymphocytes in the midbrain of PD patients suggests that the potential role of infiltrated peripheral cells is related to PD pathogenesis (McGeer et al. 1988a). Even more, T cell infiltration has been found in CNS tissues of PD patients (Brochard et al. 2009; Miklossy et al. 2006), and a markedly increase in the density of IFN-γ–positive cells has been detected in brains of PD patients (Hunot et al. 1999). Moreover, there are reports indicating that nitrated α-synuclein activates peripheral leukocytes in draining lymphoid tissue and mediated adaptive immune responses in potentiating microglial activation and exacerbating neuronal death (Benner et al. 2008). However, whether this inflammation is the consequence or the cause of neuronal injury is still unclear. Recently, it was reported that $CD4^+$ T cells are cytotoxic in a MPTP parkinsonism model; therefore, invading $CD4^+$

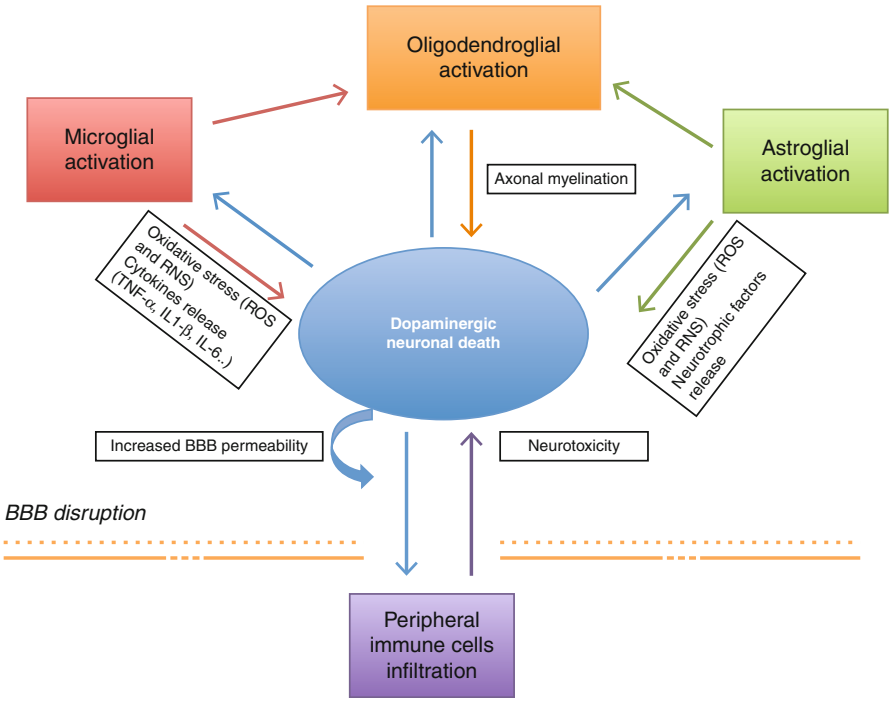

Fig. 3 Processes implicated in neuroinflammatory pathway associated with PD. Dopaminergic cell death induces microglial activation, a reactive astrocytic response, and also blood–brain barrier (BBB) disruption. Through the "hole" in the BBB, peripheral immune cells are allowed to infiltrate. This exacerbated neuroinflammation inducing increased neuronal death through the release of ROS/RNS oxidative products, cytokines, and neurotrophic factors

T cell-mediated immune response contributes to DA neurodegeneration through the Fas/FasL pathway (Brochard et al. 2009). These studies innovatively implicate the adaptive immune system, similar to the innate immune system, not only in the response to but also in an active participation in the pathogenesis of PD. However, more work needs to be done to determine if and how they will serve as a potential target for therapy in PD (Fig. 3).

2.4 Genetic Markers in PD

Numerous monogenic genes have been associated with familial forms of PD (Satake et al. 2009; Simon-Sanchez et al. 2009; Tan and Skipper 2007; Tan et al. 2007), but this accounts for only 10 % of the PD population, a reason why the relative contribution of genes and environmental factors in these cases of sporadic PD is still unclear (Tan and Skipper 2007). In the last years, a few common genetic risk variants could be associated with PD, but this is far to explain the etiology of all the cases (Hill-Burns et al. 2011; Tan et al. 2007). However, recent genome-wide

association studies have identified genetic markers linked to neuroinflammation, providing an open window to understand the pathophysiologic mechanism of PD (Hamza et al. 2010; Nalls et al. 2011; Satake et al. 2009; Simon-Sanchez et al. 2009). In the last decade, various studies suggested a possible influence of HLA alleles (HLA-DR and HLA-DQ) in PD (Hill-Burns et al. 2011; Montgomery et al. 2010; Stranger et al. 2007).

This hypothesis was demonstrated in anatomopathological studies, where PD brains exhibited upregulation of DR antigens and presence of DR-positive reactive microglia (McGeer and McGeer 2008), and in a genome-wide association study, which demonstrated a specially significant correlation between HLA-DR allele and late-onset PD (Hamza et al. 2010; Hill-Burns et al. 2011; Kumar et al. 2012). On the other hand, other studies have analyzed the relationship between a given genetic polymorphism in neuroinflammation-associated genes and the risk of PD. The most consistent genetic evidence implicates TNF in the initiation and progression of PD; a single polymorphism in the TNF promoter resulted in a higher TNF production especially overrepresented in early-onset sporadic PD (Nishimura et al. 2001; Wahner et al. 2007; Wu et al. 2007). On the contrary, TNF polymorphisms affecting its receptor 1 have been found to be significantly decreased in PD patients (Kruger et al. 2000). Other studies on single-nucleotide polymorphisms in the IL-1α and IL-1β families also corroborate the genetic role in the PD-associated neuroinflammation (Hirsch and Hunot 2009).

This genetic association between inflammatory genetic markers and PD suggests a strong influence of genes on the neuroinflammation process linked to PD and offers new targets for drug development and pharmacogenetics.

2.5 Glucocorticoids and PD

The human being is born with the ability to respond to stress, and the physiological stress response begins when the brain detects a homeostatic challenge and activates the sympathetic nervous system, which releases catecholamines, followed by the slower activation of the hypothalamic-pituitary-adrenal (HPA) axis: hypothalamic secretion of corticotropin-releasing hormone into the pituitary portal circulation activates pituitary secretion of adrenocorticotropic hormone, which then induces the glucocorticoids (GCs) that are secreted by the adrenals (Papadimitriou and Priftis 2009; Sorrells et al. 2009). Interestingly, the functioning of the HPA axis exhibits an enormous individual variability that aids to justify the considerable individual differences in vulnerability to stress-related disease, including psychiatric disorders and neurodegenerative diseases.

GC-GR responses to neuronal injury are complex, for instance, an excess of GCs (such as occurs in chronic stress) was found to aggravate neuronal injury in experimental ischemia (Sorrells et al. 2009), while an important neuronal survival effect of GR was presented in an acute lipopolysaccharide (LPS)-induced inflammatory lesion model (Nadeau and Rivest 2003). Several reports have proposed that the GC-GR responses might be crucially linked to PD pathogenesis. Thus, chronically high levels

of GCs were shown to exacerbate motor deficits in 6-hydroxy-DA-treated rats (Smith et al. 2008). This experimental observation corroborates clinical data showing that stress can trigger or worsen motor symptoms in PD patients (Smith et al. 2002). The involvement of GC-GR in DN survival after MPTP intoxication was suggested previously in adrenalectomized mice (Sugama et al. 2009) as well as in transgenic mice harboring antisense GR (Morale et al. 2004).

Recent studies by our group, linking neuroinflammation in PD pathogenesis and the mechanistic actions of GR, exposed that GR is associated with the p65 subunit of NF-κB in microglia nuclei and modulates the transactivation potential of NF-κB (Ros-Bernal et al. 2011). GC-GR system is altered in PD and this may adversely affect DN survival (Barcia et al. 2009). GR dysfunction in PD may result in a chronic inflammatory reaction, and further in-depth work on its glial actions might open innovative therapeutic perspectives.

3 Anti-inflammatory Therapy in PD

The hypothesis that neuroinflammation is extremely implicated in PD progression has become more and more convincing due to the accumulation of preclinical studies, imaging studies, and *postmortem* analysis studies that strongly implicate inflammatory processes in the progressive degeneration of the nigrostriatal pathway. Up to now, it has to be determined if anti-inflammatory therapy in humans could induce any beneficial alteration in PD progression.

Previous clinical trials with anti-inflammatory compounds have failed probably because of many reasons such as the advance stage of the patients enrolled, the dose regimen, or the wrong chosen compound. According to this, minocycline, which was successful in a phase II clinical trial, attenuating nigrostriatal DA neurodegeneration in an animal model (Du et al. 2001; Peng et al. 2006; Ravina et al. 2006), has been proved in a phase II clinical trial with controversial effects (Dodel et al. 2010). Therefore, we need more clinical studies before dismissing the effectiveness of long-term anti-inflammatory compounds. However, taking into account that the innate inflammatory response is not always detrimental, even beneficial (Wyss-Coray 2006), maybe the long-term inhibition of inflammatory response is detrimental for PD treatment (Kanthasamy et al. 2010; Wyss-Coray and Mucke 2002). Instead, more selective targets of inflammatory mediators and participants in cell-death pathways could be considered in future therapies to prevent neuronal loss in PD.

Recent studies have demonstrated that specific drug treatment does appear to reduce susceptibility to PD and immunomodulatory therapies are currently a major area in PD research (Appel et al. 2010; Li et al. 2011). Based on peripheral immune cell activation, immunomodulatory therapeutic strategies may develop vaccines for antigens to trigger cell-mediated anti-inflammatory responses (Benner et al. 2008; Brochard et al. 2009) as well as, once it is clear that BBB is altered in PD, to hamper the migration of immune cells across this BBB (Tan and Skipper 2007). Moreover, these immunomodulatory strategies may also be of

interest for cell transplantation therapies in order to induce a longer survival of grafted DNs which are affected by such an immune-related hostile microenvironment (Satake et al. 2009).

4 Conclusion

Neuroinflammation is widely considered to participate in the progression of PD (Table 1). Microglia-derived inflammatory factors such as TNF-α and IL-1β trigger harmful downstream signalling pathways and accelerate neuronal death in the SN. Activated microglia also increase the production of ROS that impose oxidative stress on the intra-neuronal antioxidant system, consequently exacerbating neurodegeneration in PD. But not only microglia, even astroglia and maybe

Table 1 Most relevant articles correlating neuroinflammation and Parkinson's disease

Evidence of inflammation in Parkinson's disease	
Human data	
Increased levels of cytokines in the CSF and striatum in PD brains	Blum-Degen et al. 1995
	Mogi et al. 1994a, b
	Muller et al. 1998
Increased number of activated microglia in PD brain	Banati et al. 1998
	Knott et al. 1999
	Imamura et al. 2003
	McGeer et al. 1988b
	Ouchi et al. 2005
	Sawada et al. 2008
Sustained microglial activity in humans exposed to MPTP years	Langston et al. 1999
Increased cytokine levels	Hunot et al. 1996
	Knott et al. 2000
	Mogi et al. 2000
	Mogi et al. 2007
	Reale et al. 2009
	Ros-Bernal et al. 2011
Parkinsonism models data	
Microglial activation in PD models	Barcia et al. 2004
	Carvey et al. 2005
	Cicchetti et al. 2002
	Gao et al. 2002
	Klintworth et al. 2009
	Greenamyre et al. 2003
LPS-induced microglial activation leads to dopaminergic degeneration	Chung et al. 2012
	Sui et al. 2009
	Zhang et al. 2010
	Zhang et al. 2012

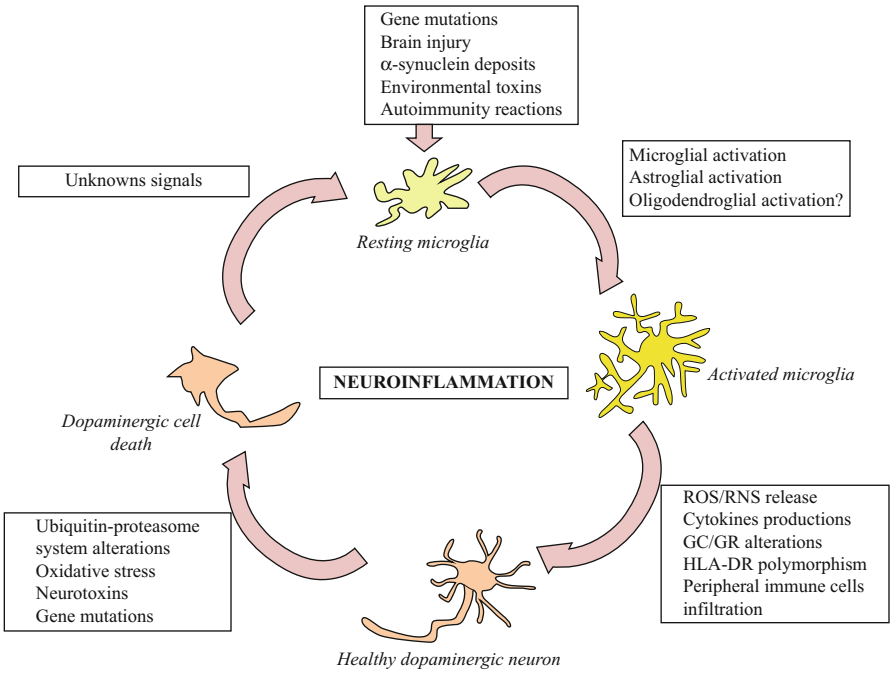

Fig. 4 Neuroinflammatory steps associated with dopaminergic cell death. Diverse initiation processes including gene mutations, brain injury, and alpha-synuclein deposits will activate immune cells in the CNS inducing microglial and astroglial activation, the production of cytokines (TNF-α, IFN-γ, etc.), and the release of oxidative species such as ROS and RNS. This neuroinflammatory response may also include the involvement of components of the peripheral immune system and the alteration of the GC/GR pathway. As a result of this neuroinflammatory process, an environment of sustained increase of inflammatory and oxidative stress in the SNpc would be created. Alternatively, a healthy neuron may become dysfunctional as a result of alterations in the ubiquitin-proteasome system, direct exposition to neurotoxins, and the general oxidative stress inducing the dopaminergic cell death. Finally, this dying/dead dopaminergic neuron would secrete unknown signals that would induce phagocytosis by activated microglia (Adapted from Tansey et al. 2007)

oligodendrocytes have a main role in controlling neuroinflammatory processes in PD. In relation to the sustained microglia activation, GC-GR system is misbalanced in PD and contributes to the neuroinflammatory processes, where some genetic markers such as HLA-DR could be the new targets for drug development and pharmacogenetics against neurodegeneration (Fig. 4).

In sum, the inflammatory component of PD is having significant attention among researchers, and it will probably be assumed in the clinical scenario in the coming years. To date, there are numerous preclinical trials testing the protective effects of anti-inflammatory drugs in animal models of parkinsonism, and we expect that hopefully some of them will soon be brought into phase I trials. However, further researches on novel targets are needed to understand the specific aspects of neuroinflammation in PD.

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References

Annese, V., Barcia, C., Ros-Bernal, F., Gomez, A., Ros, C. M., De Pablos, V., & Herrero, M. T. (2012). Evidence of oligodendrogliosis in MPTP-induced parkinsonism. *Neuropathology and Applied Neurobiology*. doi:10.1111/j.1365-2990.2012.01271.x.

Appel, S. H., Beers, D. R., & Henkel, J. S. (2010). T cell-microglial dialogue in Parkinson's disease and amyotrophic lateral sclerosis: are we listening? *Trends in Immunology, 31*(1), 7–17. doi:10.1016/j.it.2009.09.003 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review].

Arai, T., Ueda, K., Ikeda, K., Akiyama, H., Haga, C., Kondo, H., & Tsuchiya, K. (1999). Argyrophilic glial inclusions in the midbrain of patients with Parkinson's disease and diffuse lewy body disease are immunopositive for NACP/alpha-synuclein. *Neuroscience Letters, 259*(2), 83–86.

Banerjee, R., Starkov, A. A., Beal, M. F., & Thomas, B. (2009). Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis. *Biochimica et Biophysica Acta, 1792*(7), 651–663. doi:10.1016/j.bbadis.2008.11.007. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Review].

Banati, R. B., Daniel, S. E., & Blunt, S. B. (1998). Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease. [Research Support, Non-U.S. Gov't]. *Movement Disorders*, *13*(2), 221–227. doi: 10.1002/mds.870130205.

Barcia, C., Fernandez Barreiro, A., Poza, M., & Herrero, M. T. (2003). Parkinson's disease and inflammatory changes. *Neurotoxicity Research, 5*(6), 411–418. [Research Support, Non-U.S. Gov't Review].

Barcia, C., Sanchez Bahillo, A., Fernandez-Villalba, E., Bautista, V., Poza, Y. P. M., Fernandez-Barreiro, A., & Herrero, M. T. (2004). Evidence of active microglia in substantia nigra pars compacta of parkinsonian monkeys 1 year after MPTP exposure. *GLIA, 46*(4), 402–409. doi:10.1002/glia.20015.

Barcia, C., Ros, F., Carrillo, M. A., Aguado-Llera, D., Ros, C. M., Gomez, A., Nombela, C., de Pablos, V., Fernández-Villalba, E., & Herrero, M. T. (2009). Inflammatory response in parkinsonism. *Journal of Neural Transmission. Supplementum, 73*, 245–252. [Historical Article Research Support, Non-U.S. Gov't Review].

Barcia, C., Ros, C. M., Annese, V., Gomez, A., Ros-Bernal, F., Aguado-Yera, D., Martínez-Pagán, M. E., de Pablos, V., Fernandez-Villalba, E., & Herrero, M. T. (2011). IFN-gamma signaling, with the synergistic contribution of TNF-alpha, mediates cell specific microglial and astroglial activation in experimental models of Parkinson's disease. *Cell Death and Disease, 2*, e142. doi:10.1038/cddis.2011.17 [Research Support, Non-U.S. Gov't].

Bartels, A. L., Willemsen, A. T., Doorduin, J., de Vries, E. F., Dierckx, R. A., & Leenders, K. L. (2010). [11C]-PK11195 PET: Quantification of neuroinflammation and a monitor of anti-inflammatory treatment in Parkinson's disease? *Parkinsonism & Related Disorders, 16*(1), 57–59. doi:10.1016/j.parkreldis.2009.05.005 [Clinical Trial Research Support, Non-U.S. Gov't].

Bauso, D. J., Tartari, J. P., Stefani, C. V., Rojas, J. I., Giunta, D. H., & Cristiano, E. (2012). Incidence and prevalence of Parkinson's disease in Buenos Aires city, Argentina. *European Journal of Neurology, 19*(8), 1108–1113. doi:10.1111/j.1468-1331.2012.03683.x.

Benner, E. J., Banerjee, R., Reynolds, A. D., Sherman, S., Pisarev, V. M., Tsiperson, V., & Gendelman, H. E. (2008). Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PloS One, 3*(1), e1376. doi:10.1371/journal.pone.0001376 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

Block, M. L., & Hong, J. S. (2005). Microglia and inflammation-mediated neurodegeneration: Multiple triggers with a common mechanism. *Progress in Neurobiology, 76*(2), 77–98. doi:10.1016/j.pneurobio.2005.06.004. [Review].

Blum-Degen, D., Muller, T., Kuhn, W., Gerlach, M., Przuntek, H., & Riederer, P. (1995). Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. *Neuroscience Letters*, *202*(1-2), 17–20.

Braak, H., & Del Tredici, K. (2008). Invited article: Nervous system pathology in sporadic Parkinson disease. *Neurology, 70*(20), 1916–1925. doi:10.1212/01.wnl.0000312279.49272.9f. [Research Support, Non-U.S. Gov't Review].

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging, 24*(2), 197–211. [Research Support, Non-U.S. Gov't].

Brochard, V., Combadiere, B., Prigent, A., Laouar, Y., Perrin, A., Beray-Berthat, V., & Hunot, S. (2009). Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *The Journal of Clinical Investigation, 119*(1), 182–192. doi:10.1172/JCI36470. [Research Support, Non-U.S. Gov't].

Calabrese, V. P. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology, 69*(2), 223–224. doi:10.1212/01.wnl.0000271777.50910.73. [Comment Letter]; author reply 224.

Carvey, P. M., Zhao, C. H., Hendey, B., Lum, H., Trachtenberg, J., Desai, B. S., & Ling, Z. D. (2005). 6-Hydroxydopamine-induced alterations in blood–brain barrier permeability. *European Journal of Neuroscience, 22*(5), 1158–1168. doi:10.1111/j.1460-9568.2005.04281.x. [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.].

Casoli, T., Di Stefano, G., Balietti, M., Solazzi, M., Giorgetti, B., & Fattoretti, P. (2010). Peripheral inflammatory biomarkers of Alzheimer's disease: The role of platelets. *Biogerontology, 11*(5), 627–633. doi:10.1007/s10522-010-9281-8. [Review].

Choi, D. K., Pennathur, S., Perier, C., Tieu, K., Teismann, P., Wu, D. C., & Przedborski, S. (2005). Ablation of the inflammatory enzyme myeloperoxidase mitigates features of Parkinson's disease in mice. *Journal of Neuroscience, 25*(28), 6594–6600. doi:10.1523/JNEUROSCI.0970-05.2005. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.].

Chung, Y. C., Ko, H. W., Bok, E., Park, E. S., Huh, S. H., Nam, J. H., & Jin, B. K. (2010). The role of neuroinflammation on the pathogenesis of Parkinson's disease. *BMB Reports, 43*(4), 225–232. [Research Support, Non-U.S. Gov't Review].

Chung, E. S., Bok, E., Chung, Y. C., Baik, H. H., & Jin, B. K. (2012). Cannabinoids prevent lipopolysaccharide-induced neurodegeneration in the rat substantia nigra in vivo through inhibition of microglial activation and NADPH oxidase. [Research Support, Non-U.S. Gov't]. *Brain and Research*, *1451*, 110–116. doi: 10.1016/j.brainres.2012.02.058.

Cicchetti, F., Brownell, A. L., Williams, K., Chen, Y. I., Livni, E., & Isacson, O. (2002). Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. *European Journal of Neuroscience, 15*(6), 991–998. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.].

Cunningham, C. (2012). Microglia and neurodegeneration: The role of systemic inflammation. *GLIA*. doi:10.1002/glia.22350.

Dauer, W., & Przedborski, S. (2003). Parkinson's disease: Mechanisms and models. *Neuron, 39*(6), 889–909. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S. Review].

De Lau, L. M., Giesbergen, P. C., de Rijk, M. C., Hofman, A., Koudstaal, P. J., & Breteler, M. M. (2004). Incidence of parkinsonism and Parkinson disease in a general population: The Rotterdam study. *Neurology, 63*(7), 1240–1244.

De Lella Ezcurra, A. L., Chertoff, M., Ferrari, C., Graciarena, M., & Pitossi, F. (2010). Chronic expression of low levels of tumor necrosis factor-alpha in the substantia nigra elicits progressive neurodegeneration, delayed motor symptoms and microglia/macrophage activation. *Neurobiology of Disease, 37*(3), 630–640. doi:10.1016/j.nbd.2009.11.018. [Research Support, Non-U.S. Gov't].

De Rijk, M. C., Tzourio, C., Breteler, M. M., Dartigues, J. F., Amaducci, L., Lopez-Pousa, S., & Rocca, W. A. (1997). Prevalence of parkinsonism and Parkinson's disease in Europe: The EUROPARKINSON collaborative study. European community concerted action on the epidemiology of Parkinson's disease. *Journal of Neurology, Neurosurgery & Psychiatry, 62*(1), 10–15.

Del Tredici, K., Rub, U., De Vos, R. A., Bohl, J. R., & Braak, H. (2002). Where does Parkinson disease pathology begin in the brain? *Journal of Neuropathology and Experimental Neurology, 61*(5), 413–426 [Research Support, Non-U.S. Gov't].

Deverman, B. E., & Patterson, P. H. (2009). Cytokines and CNS development. *Neuron, 64*(1), 61–78. doi:10.1016/j.neuron.2009.09.002. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review].

Dodel, R., Spottke, A., Gerhard, A., Reuss, A., Reinecker, S., Schimke, N., & Eggert, K. (2010). Minocycline 1-year therapy in multiple-system-atrophy: Effect on clinical symptoms and [(11) C] (R)-PK11195 PET (MEMSA-trial). *Movement Disorders, 25*(1), 97–107. doi:10.1002/mds.22732. [Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't].

Dorsey, E. R., Constantinescu, R., Thompson, J. P., Biglan, K. M., Holloway, R. G., Kieburtz, K., & Tanner, C. M. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology, 68*(5), 384–386. doi:10.1212/01.wnl.0000247740.47667.03. [Meta-Analysis].

Du, Y., Ma, Z., Lin, S., Dodel, R. C., Gao, F., Bales, K. R., & Paul, S. M. (2001). Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 98*(25), 14669–14674. doi:10.1073/pnas.251341998.

Dubow, J. S. (2007). Autonomic dysfunction in Parkinson's disease. *Disease-a-Month, 53*(5), 265–274. doi:10.1016/j.disamonth.2007.02.004. [Review].

Eglitis, M. A., & Mezey, E. (1997). Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proceedings of the National Academy of Sciences of the United States of America, 94*(8), 4080–4085.

Elstner, M., Morris, C. M., Heim, K., Bender, A., Mehta, D., Jaros, E., & Prokisch, H. (2011). Expression analysis of dopaminergic neurons in Parkinson's disease and aging links transcriptional dysregulation of energy metabolism to cell death. *Acta Neuropathologica, 122*(1), 75–86. doi:10.1007/s00401-011-0828-9. [Research Support, Non-U.S. Gov't].

Erro, R., Santangelo, G., Picillo, M., Vitale, C., Amboni, M., Longo, K., & Barone, P. (2012). Link between non-motor symptoms and cognitive dysfunctions in de novo, drug-naive PD patients. *Journal of Neurology*. doi:10.1007/s00415-011-6407-0.

Fahn, S. (2003). Description of Parkinson's disease as a clinical syndrome. *Annals of the New York Academy of Sciences, 991*, 1–14. [Historical Article Review].

Ferrari, C. C., Pott Godoy, M. C., Tarelli, R., Chertoff, M., Depino, A. M., & Pitossi, F. J. (2006). Progressive neurodegeneration and motor disabilities induced by chronic expression of IL-1beta in the substantia nigra. *Neurobiology of Disease, 24*(1), 183–193. doi:10.1016/j.nbd.2006.06.013. [Research Support, Non-U.S. Gov't].

Forno, L. S., DeLanney, L. E., Irwin, I., Di Monte, D., & Langston, J. W. (1992). Astrocytes and Parkinson's disease. *Progress in Brain Research, 94*, 429–436. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Review].

Frank-Cannon, T. C., Alto, L. T., McAlpine, F. E., & Tansey, M. G. (2009). Does neuroinflammation fan the flame in neurodegenerative diseases? *Molecular Neurodegeneration, 4*, 47. doi:10.1186/1750-1326-4-47.

Fuhrmann, M., Bittner, T., Jung, C. K., Burgold, S., Page, R. M., Mitteregger, G., & Herms, J. (2010). Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. *Nature Neuroscience, 13*(4), 411–413. doi:10.1038/nn.2511. [Comparative Study Research Support, Non-U.S. Gov't].

Gao, H. M., & Hong, J. S. (2008). Why neurodegenerative diseases are progressive: Uncontrolled inflammation drives disease progression. *Trends in Immunology, 29*(8), 357–365. doi:10.1016/j.it.2008.05.002. [Research Support, N.I.H., Intramural Review].

Gao, H. M., Hong, J. S., Zhang, W., & Liu, B. (2002). Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. *Journal of Neuroscience, 22*(3), 782–790.

Gao, H. M., Zhang, F., Zhou, H., Kam, W., Wilson, B., & Hong, J. S. (2011). Neuroinflammation and alpha-synuclein dysfunction potentiate each other, driving chronic progression of neurodegeneration in a mouse model of Parkinson's disease. *Environmental Health Perspectives, 119*(6), 807–814. doi:10.1289/ehp.1003013. [Research Support, N.I.H., Intramural].

Gerhard, A., Pavese, N., Hotton, G., Turkheimer, F., Es, M., Hammers, A., & Brooks, D. J. (2006a). In vivo imaging of microglial activation with [^{11}C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiology of Disease, 21*(2), 404–412. doi:10.1016/j.nbd.2005.08.002. [Research Support, Non-U.S. Gov't].

Gerhard, A., Trender-Gerhard, I., Turkheimer, F., Quinn, N. P., Bhatia, K. P., & Brooks, D. J. (2006b). In vivo imaging of microglial activation with [11C](R)-PK11195 PET in progressive supranuclear palsy. *Movement Disorders, 21*(1), 89–93. doi:10.1002/mds.20668. [Research Support, Non-U.S. Gov't].

Gordon, G. R., Choi, H. B., Rungta, R. L., Ellis-Davies, G. C., & MacVicar, B. A. (2008). Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature, 456*(7223), 745–749. doi:10.1038/nature07525. [Research Support, Non-U.S. Gov't].

Graeber, M. B., & Streit, W. J. (2010). Microglia: Biology and pathology. *Acta Neuropathologica, 119*(1), 89–105. doi:10.1007/s00401-009-0622-0. [Review].

Greenamyre, J. T., Betarbet, R., & Sherer, T. B. (2003). The rotenone model of Parkinson's disease: Genes, environment and mitochondria. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. *Parkinsonism & Related Disorders*, *9*(Suppl. 2), S59–S64.

Grinberg, L. T., Rueb, U., Alho, A. T., & Heinsen, H. (2010). Brainstem pathology and non-motor symptoms in PD. *Journal of Neurological Sciences, 289*(1–2), 81–88. doi:10.1016/j.jns.2009.08.021. [Research Support, Non-U.S. Gov't Review].

Hakimi, M., Selvanantham, T., Swinton, E., Padmore, R. F., Tong, Y., Kabbach, G., & Schlossmacher, M. G. (2011). Parkinson's disease-linked LRRK2 is expressed in circulating and tissue immune cells and upregulated following recognition of microbial structures. *Journal of Neural Transmission, 118*(5), 795–808. doi:10.1007/s00702-011-0653-2. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

Halliday, G. M., & Stevens, C. H. (2011). Glia: Initiators and progressors of pathology in Parkinson's disease. *Movement Disorders, 26*(1), 6–17. doi:10.1002/mds.23455. [Research Support, Non-U.S. Gov't Review].

Halliday, G., Lees, A., & Stern, M. (2011). Milestones in Parkinson's disease–clinical and pathologic features. *Movement Disorders, 26*(6), 1015–1021. doi:10.1002/mds.23669. [Historical Article Review].

Hamza, T. H., Zabetian, C. P., Tenesa, A., Laederach, A., Montimurro, J., Yearout, D., & Payami, H. (2010). Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nature Genetics, 42*(9), 781–785. doi:10.1038/ng.642. [Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.Validation Studies].

Hertz, L., & Zielke, H. R. (2004). Astrocytic control of glutamatergic activity: Astrocytes as stars of the show. *Trends in Neurosciences, 27*(12), 735–743. doi:10.1016/j.tins.2004.10.008. [Research Support, U.S. Gov't, P.H.S.Review].

Hill-Burns, E. M., Factor, S. A., Zabetian, C. P., Thomson, G., & Payami, H. (2011). Evidence for more than one Parkinson's disease-associated variant within the HLA region. *PloS One, 6*(11), e27109. doi:10.1371/journal.pone.0027109 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.].

Hirsch, E. C., & Hunot, S. (2009). Neuroinflammation in Parkinson's disease: A target for neuroprotection? *Lancet Neurology, 8*(4), 382–397. doi:10.1016/S1474-4422(09)70062-6 [Review].

Hunot, S., Boissiere, F., Faucheux, B., Brugg, B., Mouatt-Prigent, A., Agid, Y., & Hirsch, E. C. (1996). Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. [In Vitro]. *Neuroscience*, *72*(2), 355–363.

Hunot, S., Dugas, N., Faucheux, B., Hartmann, A., Tardieu, M., Debre, P., & Hirsch, E. C. (1999). FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-alpha in glial cells. *Journal of Neuroscience, 19*(9), 3440–3447. [Research Support, Non-U.S. Gov't].

Imamura, K., Hishikawa, N., Sawada, M., Nagatsu, T., Yoshida, M., & Hashizume, Y. (2003). Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathologica, 106*(6), 518–526. doi:10.1007/s00401-003-0766-2.

Jin, J., Shie, F. S., Liu, J., Wang, Y., Davis, J., Schantz, A. M., Montine, K. S., Montine, T. J., & Zhang, J. (2007). Prostaglandin E2 receptor subtype 2 (EP2) regulates microglial activation and associated neurotoxicity induced by aggregated alpha-synuclein. *Journal of Neuroinflammation, 4*, 2. doi:10.1186/1742-2094-4-2 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

Kachroo, A., & Schwarzschild, M. A. (2012). Adenosine A_{2A} receptor gene disruption protects in an alpha-synuclein model of Parkinson's disease. *Annals of Neurology, 71*(2), 278–282. doi:10.1002/ana.22630. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.].

Kanthasamy, A., Jin, H., Mehrotra, S., Mishra, R., Kanthasamy, A., & Rana, A. (2010). Novel cell death signaling pathways in neurotoxicity models of dopaminergic degeneration: Relevance to oxidative stress and neuroinflammation in Parkinson's disease. *Neurotoxicology, 31*(5), 555–561. doi:10.1016/j.neuro.2009.12.003. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review].

Kaufmann, H., Nahm, K., Purohit, D., & Wolfe, D. (2004). Autonomic failure as the initial presentation of Parkinson disease and dementia with Lewy bodies. *Neurology, 63*(6), 1093–1095. [Case Reports].

Khedr, E. M., Al Attar, G. S., Kandil, M. R., Kamel, N. F., Abo Elfetoh, N., & Ahmed, M. A. (2012). Epidemiological study and clinical profile of Parkinson's disease in the assiut governorate, Egypt: A community-based study. *Neuroepidemiology, 38*(3), 154–163. doi:10.1159/000335701.

Klintworth, H., Garden, G., & Xia, Z. (2009). Rotenone and paraquat do not directly activate microglia or induce inflammatory cytokine release. *Neuroscience Letters, 462*(1), 1–5. doi:10.1016/j.neulet.2009.06.065. [Research Support, N.I.H., Extramural].

Knott, C., Stern, G., & Wilkin, G. P. (2000). Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. [Research Support, Non-U.S. Gov't]. *Molecular and Cellular Neuroscience*, *16*(6), 724–739. doi: 10.1006/mcne.2000.0914.

Knott, C., Wilkin, G. P., & Stern, G. (1999). Astrocytes and microglia in the substantia nigra and caudate-putamen in Parkinson's disease. *Parkinsonism Related Disorders*, *5*(3), 115–122.

Knott, C., Stern, G., Kingsbury, A., Welcher, A. A., & Wilkin, G. P. (2002). Elevated glial brain-derived neurotrophic factor in Parkinson's diseased nigra. *Parkinsonism & Related Disorders, 8*(5), 329–341. [Research Support, Non-U.S. Gov't].

Kruger, R., Hardt, C., Tschentscher, F., Jackel, S., Kuhn, W., Muller, T., & Riess, O. (2000). Genetic analysis of immunomodulating factors in sporadic Parkinson's disease. *Journal of Neural Transmission, 107*(5), 553–562.

Kumar, K. R., Lohmann, K., & Klein, C. (2012). Genetics of Parkinson disease and other movement disorders. *Current Opinion in Neurology, 25*(4), 466–474. doi:10.1097/WCO.0b013e3283547627.

Kurant, E. (2011). Keeping the CNS clear: Glial phagocytic functions in drosophila. *GLIA, 59*(9), 1304–1311. doi:10.1002/glia.21098. [Review].

Kwok, J. B. (2010). Role of epigenetics in Alzheimer's and Parkinson's disease. *Epigenomics, 2*(5), 671–682. doi:10.2217/epi.10.43. [Review].

Langston, J. W., Forno, L. S., Tetrud, J., Reeves, A. G., Kaplan, J. A., & Karluk, D. (1999). Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. *Annals of Neurology, 46*(4), 598–605 [Case Reports Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.].

Lee, H. J., Suk, J. E., Patrick, C., Bae, E. J., Cho, J. H., Rho, S., & Lee, S. J. (2010). Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. *Journal of Biological Chemistry, 285*(12), 9262–9272. doi:10.1074/jbc.M109.081125. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

Lees, A. J., Hardy, J., & Revesz, T. (2009). Parkinson's disease. *The Lancet, 373*(9680), 2055–2066. doi:10.1016/S0140-6736(09)60492-X. [Review].

Levecque, C., Elbaz, A., Clavel, J., Richard, F., Vidal, J. S., Amouyel, P., & Chartier-Harlin, M. C. (2003). Association between Parkinson's disease and polymorphisms in the nNOS and iNOS genes in a community-based case–control study. *Human Molecular Genetics, 12*(1), 79–86. [Research Support, Non-U.S. Gov't].

Lewy, F. H. (1912). Paralysis agitans. I. Pathologische Anatomie. Lewandowsky's. *in Handbuch der Neurologie, 3*, 920–933

Li, L., Lu, J., Tay, S. S., Moochhala, S. M., & He, B. P. (2007). The function of microglia, either neuroprotection or neurotoxicity, is determined by the equilibrium among factors released from activated microglia in vitro. *Brain Research, 1159*, 8–17. doi:10.1016/j.brainres.2007.04.066. [Research Support, Non-U.S. Gov't].

Li, F., Zhu, S., Wu, C., Yan, C., Liu, Y., & Shugan, L. (2011). Neuroinflammation and cell therapy for Parkinson's disease. *Frontiers in Bioscience (Scholar Edition), 3*, 1407–1420 [Research Support, Non-U.S. Gov't Review].

Liberatore, G. T., Jackson-Lewis, V., Vukosavic, S., Mandir, A. S., Vila, M., McAuliffe, W. G., & Przedborski, S. (1999). Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. *Nature Medicine, 5*(12), 1403–1409. doi:10.1038/70978. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.].

Lucin, K. M., & Wyss-Coray, T. (2009). Immune activation in brain aging and neurodegeneration: too much or too little. *Neuron, 64*(1), 110–122. doi:10.1016/j.neuron.2009.08.039.? [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Review].

Maguire-Zeiss, K. A. (2008). alpha-Synuclein: a therapeutic target for Parkinson's disease. *Pharmacological Research, 58*(5–6), 271–280. doi:10.1016/j.phrs.2008.09.006.? [Research Support, N.I.H., Extramural Review].

Martin, I., Dawson, V. L., & Dawson, T. M. (2011). Recent advances in the genetics of Parkinson's disease. *Annual Review of Genomics and Human Genetics, 12*, 301–325. doi:10.1146/annurev-genom-082410-101440. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review].

McGeer, P. L., & McGeer, E. G. (2004). Inflammation and the degenerative diseases of aging. *Annals of the New York Academy of Sciences, 1035*, 104–116. doi:10.1196/annals.1332.007. [Research Support, Non-U.S. Gov't Review].

McGeer, P. L., & McGeer, E. G. (2008). Glial reactions in Parkinson's disease. *Movement Disorders, 23*(4), 474–483. doi:10.1002/mds.21751. [Research Support, Non-U.S. Gov't Review].

McGeer, P. L., Itagaki, S., Akiyama, H., & McGeer, E. G. (1988a). Rate of cell death in parkinsonism indicates active neuropathological process. *Annals of Neurology, 24*(4), 574–576. doi:10.1002/ana.410240415. [Research Support, Non-U.S. Gov't].

McGeer, P. L., Itagaki, S., Boyes, B. E., & McGeer, E. G. (1988b). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology, 38*(8), 1285–1291. [Research Support, Non-U.S. Gov't].

McGeer, P. L., Schwab, C., Parent, A., & Doudet, D. (2003). Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. *Annals of Neurology, 54*(5), 599–604. doi:10.1002/ana.10728. [Comparative Study Research Support, Non-U.S. Gov't].

McLean, J. R., Hallett, P. J., Cooper, O., Stanley, M., & Isacson, O. (2012). Transcript expression levels of full-length alpha-synuclein and its three alternatively spliced variants in Parkinson's disease brain regions and in a transgenic mouse model of alpha-synuclein overexpression. *Molecular and Cellular Neurosciences, 49*(2), 230–239. doi:10.1016/j.mcn.2011.11.006. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.].

Meredith, G. E., Sonsalla, P. K., & Chesselet, M. F. (2008). Animal models of Parkinson's disease progression. *Acta Neuropathologica, 115*(4), 385–398. doi:10.1007/s00401-008-0350-x. [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S. Review].

Michelucci, A., Heurtaux, T., Grandbarbe, L., Morga, E., & Heuschling, P. (2009). Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-beta. *Journal of Neuroimmunology, 210*(1–2), 3–12. doi:10.1016/j.jneuroim.2009.02.003. [Research Support, Non-U.S. Gov't].

Miklossy, J., Doudet, D. D., Schwab, C., Yu, S., McGeer, E. G., & McGeer, P. L. (2006). Role of ICAM-1 in persisting inflammation in Parkinson disease and MPTP monkeys. *Experimental Neurology, 197*(2), 275–283. doi:10.1016/j.expneurol.2005.10.034. [Comparative Study Research Support, Non-U.S. Gov't].

Mizuno, Y., Hattori, N., Kitada, T., Matsumine, H., Mori, H., Shimura, H., & Shimizu, N. (2001). Familial Parkinson's disease. Alpha-synuclein and parkin. *Advances in Neurology, 86*, 13–21. [Research Support, Non-U.S. Gov't Review].

Mogi, M., Harada, M., Kondo, T., Riederer, P., Inagaki, H., Minami, M., & Nagatsu, T. (1994). Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. [Comparative Study Research Support, Non-U.S. Gov't]. *Neuroscience Letters*, *180*(2), 147–150.

Mogi, M., Harada, M., Riederer, P., Narabayashi, H., Fujita, K., & Nagatsu, T. (1994). Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. [Research Support, Non-U.S. Gov't]. *Neuroscience Letters*, *165*(1–2), 208–210.

Mogi, M., Kondo, T., Mizuno, Y., & Nagatsu, T. (2007). p53 protein, interferon-gamma, and NF-kappaB levels are elevated in the parkinsonian brain. *Neuroscience Letters*, *414*(1), 94–97. doi: 10.1016/j.neulet.2006.12.003.

Montgomery, S. B., Sammeth, M., Gutierrez-Arcelus, M., Lach, R. P., Ingle, C., Nisbett, J., & Dermitzakis, E. T. (2010). Transcriptome genetics using second generation sequencing in a Caucasian population. *Nature, 464*(7289), 773–777. doi:10.1038/nature08903. [Research Support, Non-U.S. Gov't].

Morale, M. C., Serra, P. A., Delogu, M. R., Migheli, R., Rocchitta, G., Tirolo, C., & Marchetti, B. (2004). Glucocorticoid receptor deficiency increases vulnerability of the nigrostriatal dopaminergic system: Critical role of glial nitric oxide. *The FASEB Journal, 18*(1), 164–166. doi:10.1096/fj.03-0501fje.

Mosharov, E. V., Larsen, K. E., Kanter, E., Phillips, K. A., Wilson, K., Schmitz, Y., & Sulzer, D. (2009). Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. *Neuron, 62*(2), 218–229. doi:10.1016/j.neuron.2009.01.033. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

Mulligan, S. J., & MacVicar, B. A. (2004). Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature, 431*(7005), 195–199. doi:10.1038/nature02827. [In Vitro Research Support, Non-U.S. Gov't].

Nadeau, S., & Rivest, S. (2003). Glucocorticoids play a fundamental role in protecting the brain during innate immune response. *Journal of Neuroscience, 23*(13), 5536–5544. [Research Support, Non-U.S. Gov't].

Nagatsu, T., & Sawada, M. (2005). Inflammatory process in Parkinson's disease: Role for cytokines. *Current Pharmaceutical Design, 11*(8), 999–1016. [Review].

Nagatsu, T., & Sawada, M. (2006). Cellular and molecular mechanisms of Parkinson's disease: Neurotoxins, causative genes, and inflammatory cytokines. *Cellular and Molecular Neurobiology, 26*(4–6), 781–802. doi:10.1007/s10571-006-9061-9. [Research Support, Non-U.S. Gov't Review].

Nagatsu, T., Mogi, M., Ichinose, H., & Togari, A. (2000). Changes in cytokines and neurotrophins in Parkinson's disease. Journal of Neural Transmission, *60*(Suppl. 1): 277–290.

Nakamura, Y. (2002). Regulating factors for microglial activation. *Biological and Pharmaceutical Bulletin, 25*(8), 945–953. [Review].

Nalls, M. A., Plagnol, V., Hernandez, D. G., Sharma, M., Sheerin, U. M., Saad, M., & Wood, N. W. (2011). Imputation of sequence variants for identification of genetic risks for Parkinson's disease: A meta-analysis of genome-wide association studies. *The Lancet, 377*(9766), 641–649. doi:10.1016/S0140-6736(10)62345-8. [Meta-Analysis Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.].

Nencini, P., Sarti, C., Innocenti, R., Pracucci, G., & Inzitari, D. (2003). Acute inflammatory events and ischemic stroke subtypes. *Cerebrovascular Diseases, 15*(3), 215–221. doi:10.1159/000068831. [Clinical Trial Randomized Controlled Trial].

Nishimura, M., Mizuta, I., Mizuta, E., Yamasaki, S., Ohta, M., Kaji, R., & Kuno, S. (2001). Tumor necrosis factor gene polymorphisms in patients with sporadic Parkinson's disease. *Neuroscience Letters, 311*(1), 1–4. [Research Support, Non-U.S. Gov't].

Ouchi, Y., Yoshikawa, E., Sekine, Y., Futatsubashi, M., Kanno, T., Ogusu, T., & Torizuka, T. (2005). Microglial activation and dopamine terminal loss in early Parkinson's disease. *Annals of Neurology, 57*(2), 168–175. doi:10.1002/ana.20338.

Panaro, M. A., & Cianciulli, A. (2012). Current opinions and perspectives on the role of immune system in the pathogenesis of Parkinson's disease. *Current Pharmaceutical Design, 18*(2), 200–208. [Review].

Papadimitriou, A., & Priftis, K. N. (2009). Regulation of the hypothalamic-pituitary-adrenal axis. *Neuroimmunomodulation, 16*(5), 265–271. doi:10.1159/000216184. [Review].

Paramo, B., Hernandez-Fonseca, K., Estrada-Sanchez, A. M., Jimenez, N., Hernandez-Cruz, A., & Massieu, L. (2010). Pathways involved in the generation of reactive oxygen and nitrogen species during glucose deprivation and its role on the death of cultured hippocampal neurons. *Neuroscience, 167*(4), 1057–1069. doi:10.1016/j.neuroscience.2010.02.074. [Research Support, Non-U.S. Gov't].

Pastor, P. (2012). Genetic heterogeneity in Parkinson disease: The meaning of GWAS and replication studies. *Neurology*. doi:10.1212/WNL.0b013e318264e3d2.

Peeraully, T., & Tan, E. K. (2012). Genetic variants in sporadic Parkinson's disease: East vs west. *Parkinsonism & Related Disorders, 18*(Suppl 1), S63–S65. doi:10.1016/S1353-8020(11)70021-9. [Research Support, Non-U.S. Gov't].

Peng, J., Xie, L., Stevenson, F. F., Melov, S., Di Monte, D. A., & Andersen, J. K. (2006). Nigrostriatal dopaminergic neurodegeneration in the weaver mouse is mediated via

neuroinflammation and alleviated by minocycline administration. *Journal of Neuroscience, 26*(45), 11644–11651. doi:10.1523/JNEUROSCI.3447-06.2006. [Comparative Study Research Support, N.I.H., Extramural].

Picconi, B., Piccoli, G., & Calabresi, P. (2012). Synaptic dysfunction in Parkinson's disease. *Advances in Experimental Medicine and Biology, 970*, 553–572. doi:10.1007/978-3-7091-0932-8_24. [Review].

Poewe, W. (2007). Dysautonomia and cognitive dysfunction in Parkinson's disease. *Movement Disorders, 22*(Suppl 17), S374–S378. doi:10.1002/mds.21681. [Review].

Prat, A., Biernacki, K., Wosik, K., & Antel, J. P. (2001). Glial cell influence on the human blood–brain barrier. *GLIA, 36*(2), 145–155. [Review].

Prendergast, C. T., & Anderton, S. M. (2009). Immune cell entry to central nervous system–current understanding and prospective therapeutic targets. *Endocrine, Metabolic & Immune Disorders Drug Targets, 9*(4), 315–327. [Research Support, Non-U.S. Gov't Review].

Qin, L., Block, M. L., Liu, Y., Bienstock, R. J., Pei, Z., Zhang, W., & Hong, J. S. (2005). Microglial NADPH oxidase is a novel target for femtomolar neuroprotection against oxidative stress. *The FASEB Journal, 19*(6), 550–557. doi:10.1096/fj.04-2857com. [Comparative Study].

Qureshi, G. A., Baig, S., Bednar, I., Sodersten, P., Forsberg, G., & Siden, A. (1995). Increased cerebrospinal fluid concentration of nitrite in Parkinson's disease. *Neuroreport, 6*(12), 1642–1644. [Research Support, Non-U.S. Gov't].

Ransohoff, R. M., & Perry, V. H. (2009). Microglial physiology: Unique stimuli, specialized responses. *Annual Review of Immunology, 27*, 119–145. doi:10.1146/annurev.immunol.021908.132528. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review].

Ransom, B. R., & Ransom, C. B. (2012). Astrocytes: Multitalented stars of the central nervous system. *Methods in Molecular Biology, 814*, 3–7. doi:10.1007/978-1-61779-452-0_1. [Review].

Ratai, E. M., Annamalai, L., Burdo, T., Joo, C. G., Bombardier, J. P., Fell, R., & Gonzalez, R. G. (2011). Brain creatine elevation and N-acetylaspartate reduction indicates neuronal dysfunction in the setting of enhanced glial energy metabolism in a macaque model of neuroAIDS. *Magnetic Resonance in Medicine, 66*(3), 625–634. doi:10.1002/mrm.22821. [Research Support, N.I.H., Extramural].

Ravina, B., Kieburtz, K., & Tilley, B. (2006). A randomized, double-blind, futility clinical trial of creatine and minocycline in early Parkinson disease. *Neurology, 66*(5), 664–671. doi:10.1212/01.wnl.0000201252.57661.e1. [Clinical Trial, Phase II Multicenter Study Randomized Controlled Trial Research Support, N.I.H., Extramural].

Reichmann, H. (2010). Clinical criteria for the diagnosis of Parkinson's disease. *Neuro-Degenerative Diseases, 7*(5), 284–290. doi:10.1159/000314478. [Review].

Rochet, J. C., Hay, B. A., & Guo, M. (2012). Molecular insights into Parkinson's disease. *Progress in Molecular Biology and Translational Science, 107*, 125–188. doi:10.1016/B978-0-12-385883-2.00011-4. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review].

Rodriguez-Pallares, J., Parga, J. A., Munoz, A., Rey, P., Guerra, M. J., & Labandeira-Garcia, J. L. (2007). Mechanism of 6-hydroxydopamine neurotoxicity: The role of NADPH oxidase and microglial activation in 6-hydroxydopamine-induced degeneration of dopaminergic neurons. *Journal of Neurochemistry, 103*(1), 145–156. doi:10.1111/j.1471-4159.2007.04699.x. [Research Support, Non-U.S. Gov't].

Rohn, T. T., & Catlin, L. W. (2011). Immunolocalization of influenza a virus and markers of inflammation in the human Parkinson's disease brain. *PloS One, 6*(5), e20495. doi:10.1371/journal.pone.0020495. [In Vitro Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.].

Ros-Bernal, F., Hunot, S., Herrero, M. T., Parnadeau, S., Corvol, J. C., Lu, L., & Vyas, S. (2011). Microglial glucocorticoid receptors play a pivotal role in regulating dopaminergic neurodegeneration in parkinsonism. *Proceedings of the National Academy of Sciences of the*

United States of America, 108(16), 6632–6637. doi:10.1073/pnas.1017820108. [Research Support, Non-U.S. Gov't].

Saavedra, A., Baltazar, G., Santos, P., Carvalho, C. M., & Duarte, E. P. (2006). Selective injury to dopaminergic neurons up-regulates GDNF in substantia nigra postnatal cell cultures: Role of neuron-glia crosstalk. *Neurobiology of Disease, 23*(3), 533–542. doi:10.1016/j.nbd.2006.04.008. [Research Support, Non-U.S. Gov't].

Sanchez-Guajardo, V., Febbraro, F., Kirik, D., & Romero-Ramos, M. (2010). Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. *PloS One, 5*(1), e8784. doi:10.1371/journal.pone.0008784. [Research Support, Non-U.S. Gov't].

Sandhu, J. K., Gardaneh, M., Iwasiow, R., Lanthier, P., Gangaraju, S., Ribecco-Lutkiewicz, M., & Sikorska, M. (2009). Astrocyte-secreted GDNF and glutathione antioxidant system protect neurons against 6OHDA cytotoxicity. *Neurobiology of Disease, 33*(3), 405–414. doi:10.1016/j.nbd.2008.11.016.

Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M., & Toda, T. (2009). Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nature Genetics, 41*(12), 1303–1307. doi:10.1038/ng.485. [Research Support, Non-U.S. Gov't].

Schapira, A. H., Agid, Y., Barone, P., Jenner, P., Lemke, M. R., Poewe, W., & Tolosa, E. (2009). Perspectives on recent advances in the understanding and treatment of Parkinson's disease. *European Journal of Neurology, 16*(10), 1090–1099. doi:10.1111/j.1468-1331.2009.02793.x. [Research Support, Non-U.S. Gov't Review].

Schmidt, O. I., Heyde, C. E., Ertel, W., & Stahel, P. F. (2005). Closed head injury–an inflammatory disease? *Brain Research. Brain Research Reviews, 48*(2), 388–399. doi:10.1016/j.brainresrev.2004.12.028. [Research Support, Non-U.S. Gov't Review].

Sharma, M., Ioannidis, J. P., Aasly, J. O., Annesi, G., Brice, A., Van Broeckhoven, C., & Kruger, R. (2012). Large-scale replication and heterogeneity in Parkinson disease genetic loci. *Neurology*. doi:10.1212/WNL.0b013e318264e353.

Simon-Sanchez, J., Schulte, C., Bras, J. M., Sharma, M., Gibbs, J. R., Berg, D., & Gasser, T. (2009). Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nature Genetics, 41*(12), 1308–1312. doi:10.1038/ng.487. [Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't].

Smith, A. D., Castro, S. L., & Zigmond, M. J. (2002). Stress-induced Parkinson's disease: A working hypothesis. *Physiology and Behavior, 77*(4–5), 527–531. [Research Support, U.S. Gov't, P.H.S. Review].

Smith, L. K., Jadavji, N. M., Colwell, K. L., Katrina Perehudoff, S., & Metz, G. A. (2008). Stress accelerates neural degeneration and exaggerates motor symptoms in a rat model of Parkinson's disease. *European Journal of Neuroscience, 27*(8), 2133–2146. doi:10.1111/j.1460-9568.2008.06177.x [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

Soreq, L., Ben-Shaul, Y., Israel, Z., Bergman, H., & Soreq, H. (2012). Meta-analysis of genetic and environmental Parkinson's disease models reveals a common role of mitochondrial protection pathways. *Neurobiology of Disease, 45*(3), 1018–1030. doi:10.1016/j.nbd.2011.12.021. [Meta-Analysis Research Support, Non-U.S. Gov't].

Sorrells, S. F., Caso, J. R., Munhoz, C. D., & Sapolsky, R. M. (2009). The stressed CNS: When glucocorticoids aggravate inflammation. *Neuron, 64*(1), 33–39. doi:10.1016/j.neuron.2009.09.032. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review].

Stone, D. K., Reynolds, A. D., Mosley, R. L., & Gendelman, H. E. (2009). Innate and adaptive immunity for the pathobiology of Parkinson's disease. *Antioxidants & Redox Signaling, 11*(9), 2151–2166. doi:10.1089/ARS.2009.2460. [Review].

Stranger, B. E., Nica, A. C., Forrest, M. S., Dimas, A., Bird, C. P., Beazley, C., & Dermitzakis, E. T. (2007). Population genomics of human gene expression. *Nature Genetics, 39*(10), 1217–1224. doi:10.1038/ng2142. [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

Sugama, S., Takenouchi, T., Kitani, H., Fujita, M., & Hashimoto, M. (2009). Microglial activation is inhibited by corticosterone in dopaminergic neurodegeneration. *Journal of Neuroimmunology, 208*(1–2), 104–114. doi:10.1016/j.jneuroim.2009.01.016. [Comparative Study Research Support, Non-U.S. Gov't].

Takagi, S., Hayakawa, N., Kimoto, H., Kato, H., & Araki, T. (2007). Damage to oligodendrocytes in the striatum after MPTP neurotoxicity in mice. *Journal of Neural Transmission, 114*(12), 1553–1557. doi:10.1007/s00702-007-0790-9. [Research Support, Non-U.S. Gov't].

Tan, E. K., & Skipper, L. M. (2007). Pathogenic mutations in Parkinson disease. *Human Mutation, 28*(7), 641–653. doi:10.1002/humu.20507. [Research Support, Non-U.S. Gov't Review].

Tan, E. K., Zhao, Y., Skipper, L., Tan, M. G., Di Fonzo, A., Sun, L., & Liu, J. J. (2007). The LRRK2 Gly2385Arg variant is associated with Parkinson's disease: Genetic and functional evidence. *Human Genetics, 120*(6), 857–863. doi:10.1007/s00439-006-0268-0. [Research Support, Non-U.S. Gov't].

Tansey, M. G., & Goldberg, M. S. (2010). Neuroinflammation in Parkinson's disease: Its role in neuronal death and implications for therapeutic intervention. *Neurobiology of Disease, 37*(3), 510–518. doi:10.1016/j.nbd.2009.11.004. [Review].

Teismann, P., & Schulz, J. B. (2004). Cellular pathology of Parkinson's disease: Astrocytes, microglia and inflammation. *Cell and Tissue Research, 318*(1), 149–161. doi:10.1007/s00441-004-0944-0. [Review].

Thiruchelvam, M. J., Powers, J. M., Cory-Slechta, D. A., & Richfield, E. K. (2004). Risk factors for dopaminergic neuron loss in human alpha-synuclein transgenic mice. *European Journal of Neuroscience, 19*(4), 845–854. [Comparative Study Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.].

Tolosa, E., & Pont-Sunyer, C. (2011). Progress in defining the premotor phase of Parkinson's disease. *Journal of Neurological Sciences, 310*(1–2), 4–8. doi:10.1016/j.jns.2011.05.027. [Research Support, Non-U.S. Gov't Review].

Tousi, N. S., Buck, D. J., Curtis, J. T., & Davis, R. L. (2012). Alpha-synuclein potentiates interleukin-1beta-induced CXCL10 expression in human A172 astrocytoma cells. *Neuroscience Letters, 507*(2), 133–136. doi:10.1016/j.neulet.2011.12.001. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

Trétiakoff, C. (1919). Contribution à l'étude de l'anatomie pathologique du locus niger de Sœmmering avec quelques déductions relatives à la pathogénie des troubles du tonus musculaire et de la maladie de Parkinson. *Thésis*

Uwechue, N. M., Marx, M. C., Chevy, Q., & Billups, B. (2012). Activation of glutamate transport evokes rapid glutamine release from perisynaptic astrocytes. *The Journal of Physiology, 590*(Pt 10), 2317–2331. doi:10.1113/jphysiol.2011.226605. [Research Support, Non-U.S. Gov't].

Wahner, A. D., Sinsheimer, J. S., Bronstein, J. M., & Ritz, B. (2007). Inflammatory cytokine gene polymorphisms and increased risk of Parkinson disease. *Archives of Neurology, 64*(6), 836–840. doi:10.1001/archneur.64.6.836. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

Wakabayashi, K., Hayashi, S., Yoshimoto, M., Kudo, H., & Takahashi, H. (2000). NACP/alpha-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. *Acta Neuropathologica, 99*(1), 14–20. [Research Support, Non-U.S. Gov't].

Wu, D. C., Jackson-Lewis, V., Vila, M., Tieu, K., Teismann, P., Vadseth, C., & Przedborski, S. (2002). Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *Journal of Neuroscience, 22*(5), 1763–1771. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.].

Wu, D. C., Teismann, P., Tieu, K., Vila, M., Jackson-Lewis, V., Ischiropoulos, H., & Przedborski, S. (2003). NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *Proceedings of the National Academy*

of Sciences of the United States of America, 100(10), 6145–6150. doi:10.1073/pnas.0937239100. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.].

Wu, Y. R., Feng, I. H., Lyu, R. K., Chang, K. H., Lin, Y. Y., Chan, H., & Chen, C. M. (2007). Tumor necrosis factor-alpha promoter polymorphism is associated with the risk of Parkinson's disease. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 144B*(3), 300–304. doi:10.1002/ajmg.b.30435. [Research Support, Non-U.S. Gov't].

Wyss-Coray, T. (2006). Inflammation in Alzheimer disease: Driving force, bystander or beneficial response? *Nature Medicine, 12*(9), 1005–1015. doi:10.1038/nm1484 [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Review].

Wyss-Coray, T., & Mucke, L. (2002). Inflammation in neurodegenerative disease–a double-edged sword. *Neuron, 35*(3), 419–432. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review].

Zhang, W., Wang, T., Pei, Z., Miller, D. S., Wu, X., Block, M. L., & Zhang, J. (2005). Aggregated alpha-synuclein activates microglia: A process leading to disease progression in Parkinson's disease. *The FASEB Journal, 19*(6), 533–542. doi:10.1096/fj.04-2751com.

Zlokovic, B. V. (2008). The blood–brain barrier in health and chronic neurodegenerative disorders. *Neuron, 57*(2), 178–201. doi:10.1016/j.neuron.2008.01.003. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review].

Neuromelanin and Parkinson's Disease

Giorgia Greco

Contents

Abstract

Dark pigmented organelles, present in catecholaminergic neurons in specific brain regions, are indicated with the term neuromelanin (NM). They are complex structures, mainly comprised by granules of melanin polymer, closely associated with peptide and lipid components. Although NM is present in several animals, it is considered as a unique feature of the man due to the extremely higher degree of pigmentation, even in comparison to other primates. For a long time NM was considered an inert cellular waste product of poor interest that in the absence of mechanism of removal accumulates during the entire lifespan. Just recently, NM has received renewed attention for its role in Parkinson's disease (PD), where a

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selective death of the NM-containing neurons of the substantia nigra (SN) pars compacta is observed, while nonpigmented neurons are mostly spared. A physiological accumulation of NM seems to be a protective phenomenon, which prevents several neurotoxic processes. In particular, in dopaminergic neurons of SN, where no ferritin has been detected, NM appears to function as an iron storage system. However, in PD patients NM released by dying neurons can trigger a vicious circle of neuroinflammation and ensuing neuronal death. This chapter presents the structure, the production, and the development of NM, as well as the recent hypotheses about physiological NM role and its behavior in pathological conditions.

Keywords

5,6-dihydroxyindole • 5-*S*-cyteinyldopamine • Benzothiazine • Casing model • Catecholaminergic neurons • Dolichol • Dopaminequinone • Dopaminochrome • Eumelanin • Ferritin • Melanin, peripheral • Microglia activation • Mixed melanogenesis • Neuromelanin • NM, age • NM casing model • NM, degradation products • NM, electron density • NM, enzymatic control • NM, iron binding sites • NM, iron level • NM, iron storage system • NM, lipid component • NM membrane • NM, metal chelator • NM, neuron selective death • NM organelle • NM, organic molecule interaction • NM, oxidation potential • NM, peptide component • NM pigment • Pheomelanin • Pigmented neurons

List of Abbreviations

4-AHPEA	4-amino-3-hydroxyphenylethylamine
AFM	Atomic force microscopy
DA	Dopamine
DAQ	Dopaminoquinone
DOPA	Dihydroxyphenylalanine
DQ	DOPA-quinone
FEL	Free-electron laser
NM	Neuromelanin
PD	Parkinson's disease
PDCA	Pyrrole-2,3-dicarboxylic acid
PEEM	Photoelectron emission microscopy
PTCA	Pyrrole-2,3,5-tricarboxylic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
SN	Substantia nigra
TDCA	Thiazole-4,5-dicarboxylic acid
TTCA	Thiazole-2,4,5-tricarboxylic acid

1 Introduction

Neuromelanin (NM) is a natural brown-black polymeric pigment present in the highest concentration in the catecholaminergic neurons of substantia nigra (SN) and locus coeruleus of human brain.

This pigment belongs to the melanin family, which members include the peripheral melanins, responsible in the mammalian for the coloration of hair, skin, and iris (Prota 1992).

NM has stimulated the interest of numerous scientists for almost one century. Pigmented neurons were observed in man since the 1930s, and it was long assumed that the intense pigmentation of selected brain regions was a unique feature of the human being and did not occur even in other primates (Adler 1939; Foley and Baxter 1958). Some years later, the pigment was observed in SN of the adult gorilla (Adler 1942), chimpanzee, orangutan, gibbon, certain old-world monkeys and lemurs (Scherer 1939), cat and dog (Brown 1943), and horse (Gillilan 1943). On the basis of these results, Marsden (1961) investigated systematically the distribution of the pigment in SN of mammals in 49 species from 11 mammalian orders, founding that the dark granules were present in the nigral cells of the members of the Primates, the Rodentia, the Carnivora, the Perissodactyla, the Artiodactyla, the Xenarthra, and the Marsupialia. The pigmented cells varied from animal to animal only in regard to their number and intensity of the pigmentation. Interestingly, the SN cells of all the examined 16 species of the Primate order were pigmented and more intensely respect to the members of the other orders, reaching the highest degree of pigmentation in man. However, the presence of the pigment in such a wide variety of mammalian orders did not lead to any indication to its function in the brain.

Therefore, for a long time NM was considered an inert cellular waste product of poor interest. Just in recent years, NM has received renewed attention for its role in Parkinson's disease (PD), one of the most common neurodegenerative disorders with clinical features among which bradykinesia, rigidity, tremor, and postural instability (Calne et al. 1992; Gelb et al. 1999). These classic motor symptoms result from the progressive and selective death of the NM-containing neurons (NM, neuron selective death) of the SN pars compacta, which form an integral part of the basal ganglia motor circuit (Double 2012). The loss of the NM-containing neurons of SN is the main pathological process observed in PD, while nonpigmented neurons are mostly spared (Bush et al. 2006), and this is considered a criterion required for a confirmed diagnosis of PD (Braak et al. 2003).

Many questions about the significance of NM are still awaiting answers, despite it may be of primary importance for providing information to the etiology of PD. Until now no unambiguous physiological role has been defined for NM, as well as the relationship between the presence of NM and the neuronal susceptibility has not yet been clarified. In addition, NM biosynthesis, whether it is enzymatically controlled or results from an autoxidation pathway, is still object of investigation.

In this chapter, the distribution, the structure, the biosynthesis, and the development of NM in the human brain are presented. Moreover, the recent hypotheses about NM physiological role, also in relation to its architecture, and NM pathogenic action in the selective degeneration of pigmented neurons during PD are discussed.

2 Distribution and Appearance of Neuromelanin (NM)

In man NM is mainly found in the cytoplasm of the catecholaminergic neurons of the SN pars compacta of the midbrain and in the neurons of the locus coeruleus within the pons, where almost all the neurons are pigmented and a darkened area can be seen macroscopically (Fedorow et al. 2005a). Some pigmented neurons are also found in other regions of the pons and the midbrain, in the hypothalamus, and medulla oblongata (Bazelon et al. 1967; Rosengren et al. 1985), where, however, the number of NM-containing cells is around tenfold lower than those in SN (Bogerts 1981).

Only dopamine (DA) and noradrenaline neurons are reported to contain NM, while no NM is preset in adrenaline neurons. Interestingly, within the brain not all the DA and noradrenaline neurons produce NM. The pigment is found in 95 % of the DA neurons of SN, only in 50 % of the neurons of the ventral tegmental area, and other DA neurons, as, for example, those of the olfactory bulb do not produce the pigment at all (Double et al. 2008). This suggests that even though DA or noradrenaline is required for NM biosynthesis, their presence within the neurons does not ensure NM production.

Due to the number of pigmented neurons in human SN pars compacta and the high concentration of pigment inside the cells, NM isolated from SN is the most studied pigment. It has been evaluated that NM levels in a middle-aged man without any neurological disorders are around 2 μg/mg wet weight of SN (Zecca et al. 2002a).

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) images of NM have revealed that the dark pigment is composed by granules with an average diameter of ∼350 nm, comprised of small spherical particles of ∼30 nm diameter (Bush et al. 2006). Similar morphological organization with substructures of spherical constituents with diameters on the order of 30 nm has been observed also for other members of the melanin family, among which the natural melanin from the ink sac of the cuttlefish *Sepia officinalis* and the melanin isolated from human hair and from bovine and human eyes (Liu and Simon 2003). Besides the natural occurring pigments, also SEM images of bioinspired synthetic melanin have revealed tightly aggregated globe-like nanosized particles with an average diameter of 39 ± 9 nm, resembling the architecture of the natural melanins (Greco et al. 2011).

Differently from the other members of the melanin family, NM pigment is naturally found as organelles (NM organelle) comprising three different electron-dense constituents (NM, electron density). The dark electron-dense component, identified as the melanin polymer itself, is closely associated with a component of intermediate electron density and an electron-lucent component, suggested to be

lipid (Duffy and Tennyson 1965; Moses et al. 1966). The question of whether NM is surrounded by a membrane or not is still debated. Early studies clearly showed that the organelles are membrane bound (Moses et al. 1966; Hirosawa 1968), whereas other works did not confirm this observation (Duffy and Tennyson 1965). Recently, NM synthesis was induced in rat SN neurons and cell cultures by exposure to DOPA (dihydroxyphenylalanine). These model organelles were surrounded by a double membrane separated by ~15 nm. NM isolated from human SN showed similar features, but no strong evidence for the existence of a double membrane was provided (Sulzer et al. 2000). The controversial information in several papers about NM membrane may be the consequence of different purification methodologies, which could alter the aspect of the granule. It has also been proposed that a lack of a clear evidence for a membrane enclosing the NM organelle may be due to the difficulties of preserving membranes in the human brain postmortem (Fedorow et al. 2005a). Further studies are necessary to clarify the presence of a membrane that may play an important role in moderating or even preventing the interaction of the pigment with other neuronal constituents.

A recent investigation has revealed the presence of dark pigmented areas in noncatecholaminergic neurons and in brain regions other than SN and locus coeruleus (Zecca et al. 2008). The organelles observed in neurons of the putamen, premotor cortex, and cerebellum exhibited three components of different electron density surrounded by a double membrane, resembling the NM of SN. The electron-dense component was comprised of substructures of 30 nm diameter and was identified as a member of the melanin family on the basis of its physical-chemical properties.

The pigment was termed NM too, even if it derives biosynthetically from the different precursor DOPA, instead of DA or noradrenaline. The study has shown that pigmented aggregates with common structure, behavior in aging, and interaction with metal ions and lipids, besides NM of SN and locus coeruleus, are ubiquitous within the human brain (Zecca et al. 2008). This result opens new and interesting questions about the function of melanin pigments in the human central nervous system. In particular, the peculiar NM architecture involving different constituents suggests that NM is not likely to be a waste product without significance, as considered until some years ago.

3 Structure of NM

The present information about NM structure derives prevalently from studies performed by several independent research groups on NM isolated from human SN. In addition to the dark melanin pigment, peptide and lipid components concur to form the NM structures of different electron density observed in electron micrographs. The peptide component is covalently bound to the melanin structure and corresponds to ~15 % of the NM weight, while lipids are adsorbed on the surface of the pigment and account for ~20 % of the NM weight (Zecca et al. 2000). In the following paragraphs, the melanin pigment, the peptide, and the lipid components of NM granule are discussed in detail.

3.1 The Melanin Pigment

NM pigment is considered a member of the melanin family, as similarly to the pigment of skin, eyes, and hair, it is a heterogeneous low soluble aromatic polymer, with a stable free radical and high affinity for transition metals (Bridelli et al. 1999).

Several experimental evidences support the common view that in vivo NM derives from oxidation of DA (Double et al. 2008). Two complementary strategies, also used for the coetaneous melanin, have been employed to study the structure of the pigment. Naturally occurring NM has been investigated by different chemical degradation methods and following analysis of the liberated products, while the polymer formation has been mimicked in vitro by oxidation of DA and isolation of intermediate compounds (Wakamatsu et al. 2003; Tse et al. 1976; Young and Babbitt 1983; Zhang and Dryhurst 1994). At the present, in vitro oxidation of DA is commonly used for the preparation of synthetic pigments, as models of NM (Double et al. 2000; Zareba et al. 1995). Both degradative and biosynthetic strategies have shown that NM comprises two distinct classes of polymers: eumelanin and pheomelanin. The pathway for NM biosynthesis has been suggested to be similar to that for the synthesis of peripheral melanins (Ito and Wakamatsu 2006). The first step is DA oxidation to dopamine quinone (DAQ) that for intramolecular cyclization and further oxidation is converted into dopaminochrome. This a reactive specie that can rearrange to 5,6-dihydroxyindole, which for polymeric oxidation leads to the eumelanin. Alternatively, DAQ conjugates to L-cysteine, with ensuing formation of isomeric cisteinly dopamine adducts, mainly 5-*S*-cyteinyldopamine. This adduct for oxidation, followed by intramolecular cyclization of the cysteine chain, gives rise to benzothiazine units, which polymerize leading to the pheomelanin (Zhang and Dryhurst 1994) (Fig. 1).

Peripheral eumelanin and pheomelanin pigmentation (Melanin, peripheral) has been more extensively studied. Both pigments derive from a common precursor, the DOPA-quinone (DQ). Eumelanin appears as a dark pigment responsible for the brown-black coloration of skin and hair, while pheomelanin determines the carroty to fire-red color of hair, commonly found in Celtic populations. Over the past 30 years, chemical analyses have been performed that enable the quantification of the amount of eumelanin and pheomelanin present in a naturally occurring sample of melanin. These works collectively reveal that more often than not, natural melanin pigments consist of both eumelanin and pheomelanin in varying ratios (Ito and Wakamatsu 2003; Thody et al. 1991). Recently, by the use of modern surface techniques, in particular photoelectron emission microscopy (PEEM) coupled to a free-electron laser (FEL), it has been shown that human black hair melanin has a surface oxidation potential of −0.2 V versus the normal hydrogen electrode, attributed to the eumelanin, while melanin form red hair has two surface oxidation potentials of −0.2 and +0.5 V, respectively. The negative potential has been ascribed to eumelanin pigment, whereas the positive to the pheomelanin one. This indicated that melanin from red hair has both types of melanin on or near their surface, and on the basis of the oxidation potential values, eumelanin is an antioxidant, while pheomelanin exhibits a prooxidant behavior (Ito 2006; Simon et al. 2009).

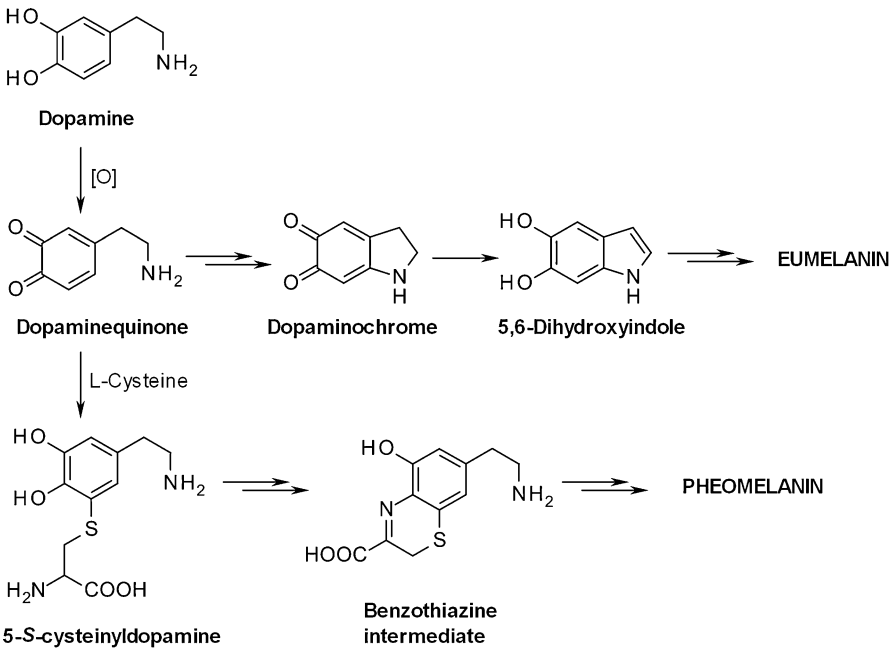

Fig. 1 Formation of indole and benzothiazine units from DA oxidation and their polymerization to eumelanin and pheomelanin pigment of NM granule

Supported by biochemical evidence, a casing model (NM casing model) with a pheomelanin core encased in a eumelanin shell was proposed in 1982 and since then it has been sustained by different other studies (Agrup et al. 1982; Simon and Peles 2010). On the basis of this model and on the eumelanin/pheomelanin ratios, the melanin granule from red hair results to have a tinier eumelanic coat, exposing the pheomelanin core. In the last years epidemiological and clinical data support the hypothesis that eumelanin is photoprotective, defending the tissues from UV-induced damages, whereas pheomelanin may be the main cause of the abnormal susceptibility to erythema and skin cancer, observed in red hair people (Bliss et al. 1995).

Two microanalytical methods, initially developed to quantify the amount of eumelanin and pheomelanin in cutaneous tissues and hair, have been applied to NM isolated from SN (Wakamatsu et al. 2003). The first method consists in the alkaline hydrogen peroxide oxidation of the sample, followed by quantification of pyrrole-2,3-dicarboxylic acid (PDCA) and pyrrole-2,3,5-tricarboxylic acid (PTCA), degradation products of eumelanin (NM, degradation products), and thiazole-4,5-dicarboxylic acid (TDCA) and thiazole-2,4,5-tricarboxylic acid (TTCA), degradation products of pheomelanin. The second method is specific for pheomelanin and it is based on the quantification of 4-amino-3-hydroxyphenylethylamine (4-AHPEA), arising from reductive hydrolysis of the pigment with hydriodic acid (Fig. 2).

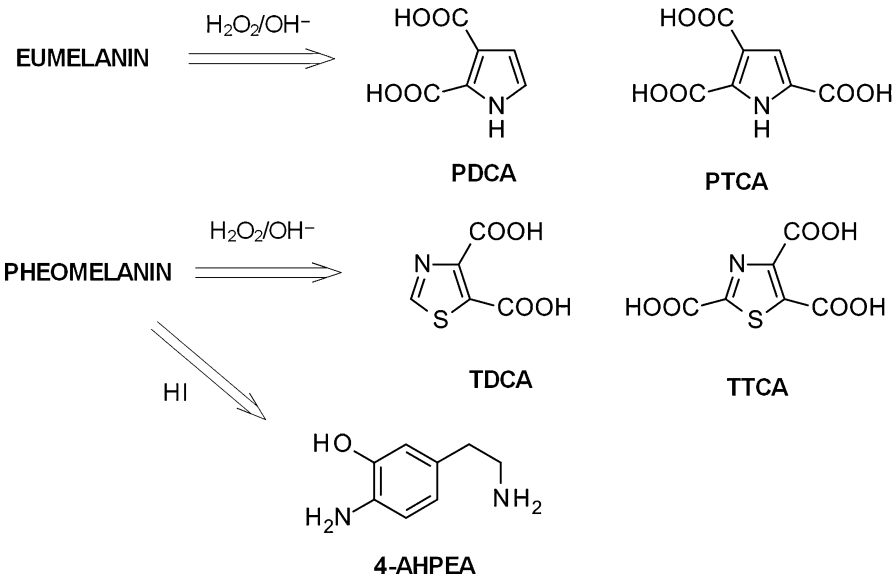

Fig. 2 Degradation products of eumelanin and pheomelanin pigment by treatment of NM granule with alkaline hydrogen peroxide or hydriodic acid

These methods showed in NM isolated from SN the presence of eumelanin and pheomelanin in a ratio of 3:4 (Wakamatsu et al. 2003). FEL–PEEM analysis has revealed for NM granules a single oxidation potential (−0.1 V) (NM, oxidation potential), indicating the presence of only eumelanin on the surface; no superficial pheomelanin have been detected (Bush et al. 2006). Therefore, the eumelanin surface is built up on the pheomelanin core according to the casing model (Fig. 3).

X-ray diffraction studies have shown that NM granule of SN, as other natural peripheral melanin, has a multilayer three-dimensional structure, composed by planar overlapped sheets at about 4.7 Å of distance (Zecca et al. 2008). Just recently the pheomelanin core is receiving attention. By X-ray absorption spectroscopy, the atomic structure around the atom of S in natural and synthetic NM has been investigated, revealing for the first time in a direct and nondegradative way the presence in human NM of heterocyclic S of the benzothiazine type (Crippa et al. 2010).

3.2 Peptide Component in NM

Whereas several efforts have been directed to the comprehension of the pigment structure, only recently the presence of other components in NM granule has been investigated. Zecca et al. (2000) showed a peptide component in NM (NM, peptide component), after treatment with different strong agents for protein removal.

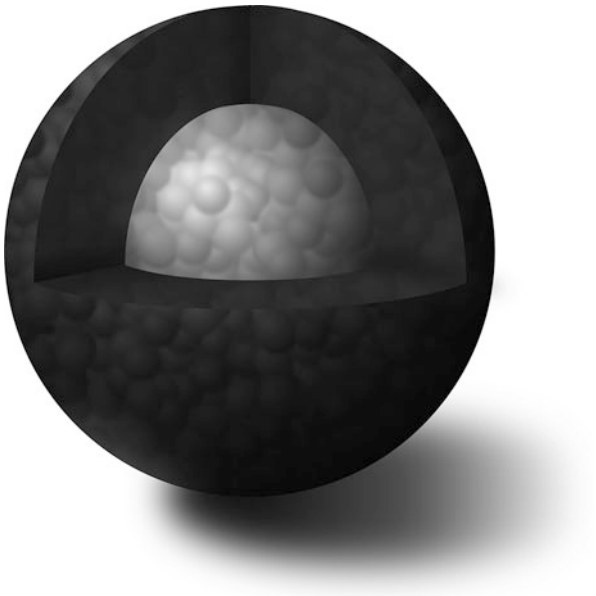

Fig. 3 Representation of NM pigment according to the casing model. A portion of the dark eumelanin surface is cut away to reveal the lighter inner pheomelanin core. Both melanin components are comprised of small spherical particles

The peptides resulted to be covalently bound to the melanin structure, as shown by comparison with a treatment for eliminating only noncovalently bound proteins. The peptide residues were present even after digestion with proteolytic enzymes, indicating that the peptide chains are in some way sterically protected by the melanin pigment. Furthermore, synthetic melanin incubated in brain homogenate showed the ability to bind peptides chemically.

The cysteine units in the intact bound peptide chain represent ~3 % of the entire amino acid content, and their amount is almost unchanged in the strongly bound peptide residues, suggesting that they are localized in proximity of the pigment. The association of melanin to peptide component may result from a direct reaction of catechol moieties of the indole units on the surface of NM granule with the cysteine residues of peptides. At support to this interpretation, a model study has shown the formation of protein-bound cysteinylcatechols by incubation of DA or norepinephrine with bovine serum albumin (Ito et al. 1988). Proteomics of NM granules (NM, proteomic) has allowed the identification of more than 70 proteins, occurring commonly in human brain tissues, mainly lysosomal proteins, sustaining the hypothesis of a genetic program for the formation of NM granules (Tribl et al. 2005).

3.3 Lipid Component in NM

The electron-lucent component comprises 35 % of the NM granule volume (Fedorow et al. 2006) and contains neutral lipids (NM, lipid component). A two-step washing of the granules with methanol and hexane has allowed a completed

removal of the lipid component, proving that they are adsorbed onto the surface of the granule and not chemically bound (Zecca et al. 2000). Interestingly the major lipid component of NM is the polyisoprenoid dolichol, which contains 17–23 isoprenoid units and accounts for approximately 12 % of NM weight (Fedorow et al. 2005b; Ward et al. 2007). Other lipids have been also found, as cholesterol, ubiquinone-10, α-tocopherol, and dolichyl esters, but they are present only in small quantity (Fedorow et al. 2005b).

Small amounts of dolichol are ubiquitously distributed in the body, generally located in cellular membranes, where it is supposed to have a role in the stabilization of the membrane itself. While the phosphorylated form of dolichol is involved in the formation of glycoproteins in the endoplasmatic reticulum (Varki et al. 1999), the function of free dolichol remains to be clarified.

Accumulation of dolichol is associated with neurodegeneration in disorders such as the neuronal ceroid lipofuscinoses (Kin et al. 1983). In NM granule, the presence of large dolichol deposits represents until now the first example of dolichol concentrated in intracellular inclusions in healthy tissue (Fedorow et al. 2006). The association of dolichol with NM results to be specific. Indeed cholesterol, which in all the other brain regions is typically 200-fold higher than dolichol on a mass basis (Andersson et al. 1987), is present at considerably lower concentration in NM lipid. Dolichol may play a structural function, as it can alter membrane lamellar bilayer into nonlamellar or hexagonal structure (Zhou and Troy 2004).

Recently, the influence of NM lipids in the aggregation of the granule has been investigated (Dedov et al. 2007). The removal of all NM lipids resulted in significantly larger granules than those present prior to lipid removal. Interestingly, selective removal of cholesterol by methanol extraction resulted in an eight- to tenfold enhancement in granule size, whereas the pro-aggregation effect of dolichol extraction using hexane was significantly smaller. These data showed that NM lipids, preventing the formation of large pigment aggregates, may be a factor regulating NM granule size in vivo (Dedov et al. 2007).

Under oxidative stress condition, lipid peroxidation appears to contribute to neurodegeneration in PD (Dexter et al. 1989). In this contest, dolichol may be a substrate of the lipid peroxidation, as in vitro studies have shown a time-dependent degradation of dolichol when incubated with a radical initiator (Dedov et al. 2007). These observations are just preliminary and further investigations are required for clarifying the structural and physiological role of NM lipids in normal and pathological conditions.

4 Biosynthesis of NM

The study of neuromelanogenesis is made more complicated than that of peripheral melanin biosynthesis, because NM is absent from all of the commonly used laboratory animals including rodents. A general understanding of the first steps of NM biosynthesis has been provided by investigation of the products arising from in vitro biomimetic oxidation of NM precursors and comparison with what is

known about peripheral melanins (Fig. 1). It is extensively debated whether the NM synthesis is enzymatically controlled (NM, enzymatic control), like all melanins in the periphery, or whether NM arises from a simple autoxidation of DA. Although DA cells in the midbrain express tyrosine hydroxylase, the rate-limiting enzyme for DA synthesis, there is no correlation between the degree of pigmentation and tyrosine hydroxylase immunoreactivity (Gaspar et al. 1983). The enzyme tyrosinase is a key enzyme in the synthesis of peripheral melanin, since it catalyzes the conversion of the amino acid tyrosine to DQ, the precursor of eu- and pheomelanin (Korner and Pawelek 1982). However, immunochemistry studies have no detected tyrosinase in human midbrain (Ikemoto et al. 1998). At support to that, albinos, who are characterized by absence of peripheral pigmentation due to a lack of functional tyrosinase, have normally pigmented neurons in SN (Foley and Baxter 1958). Also the enzyme peroxidase and macrophage migration inhibitory factor have been proposed for NM synthesis. However, their distribution in the brain does not correlate to NM distribution (Okun 1997; Matsunaga et al. 1999). Several other enzymes have been suggested to be associated with NM biosynthesis, but to date no likely candidate has been demonstrated to be involved in the neuromelanogenesis. In the absence of a convincing enzymatic route, for a long period NM has been regarded as a waste product of catecholamine metabolism (Bogerts 1981; Graham 1979; Mann and Yates 1983; Mann et al. 1977). Indeed, NM is not present at birth and increases over the lifespan, supporting the idea that the pigment is a cellular by-product of DA metabolism formed via autoxidation and, in the absence of any mechanism to remove it, accumulates intracellularly with age. However, several evidences are in contrast with this view. First of all, NM and DA do not have the same cellular distribution in human brain and the pigment seems to be a peculiar feature of the man, lacking or at least present in small amount in the midbrain of many other species (Fedorow et al. 2005a). In addition, PD patients treated with DOPA, which it is rapidly converted in DA by the enzyme aromatic acid decarboxylase, do not exhibit increased quantities of NM within their surviving SN neurons as might be expected to be the case if NM represents a product of pure DA autoxidation (Zecca et al. 2001a). Finally, the complex casing structure proposed for the NM pigment and its aggregation with specific protein and lipid components support the existence of a controlled process.

The casing model may be the consequence of a mixed melanogenesis regulated in some way by cysteine levels. In vitro studies on biosynthesis of peripheral melanin have clearly indicated that DQ conjugates to cysteine leading to pheomelanin production until cysteine concentration is above 1 μM. At lower cysteine levels DQ polymerizes in eumelanin (Ito 2003; Land et al. 2003). This favored production of pheomelanin over eumelanin suggests that the first step is the formation of the pheomelanin core, and then, when the cysteine is depleted, eumelanin production starts. In NM production, this casing process is further favored by the fact that DAQ cyclizes much more slowly than DQ, leading to a preferential production of cysteinyldopamines (Ito 2003). The construction of NM granule seems to be a regulated process in such a manner that DAQ is conjugated first with cysteine to form cysteinyldopamine isomers, which are further

oxidized to form a pheomelanic core. This step is followed by the production of a eumelanic surface (Ito 2006). Whereas it is not well understood how initially DA and later the cysteinyldopamines are oxidized for producing the pheomelanin pigment, the synthesis and the following deposition of eumelanin pigment onto the preformed pheomelanic core may be a spontaneous process, in part catalyzed by the prooxidant pheomelanin core, as observed in biomimetic studies (Greco et al. 2011).

5 Developmental Stages of NM

NM concentration in SN pars compacta increases similarly in men and women over the entire lifespan. In the first year of life, NM is in general not detectable. Between 10 and 20 years, the NM levels are 0.3–0.8 μg/mg of SN, between 20 and 50 years are 0.8–2.3 μg/mg SN, and between 50 and 90 are 2.3–3.7 μg/mg of SN (Zecca et al. 2002a) (NM, age).

In accordance with these quantitative analyses, light microscopy images have revealed that no NM is visible in SN neurons in the prenatal or infant brain. The pigment first appears within the cytoplasm of the DA neurons at around the age of 3 years as small granules, whose size and number increase until the age of 20. At this time NM fills, on average, 47 % of the cytoplasm. After the age of 20, the proportion of the cell occupied by the pigment remains stable, but the amount of pigment increases with age (Halliday et al. 2006). Over time the coloration and the appearance of NM change too. Initially, NM occurs as dusty yellow-brown granules, which progressively form dark denser clusters (Halliday et al. 2006). These data may represent a direct observation of the construction of the NM granule in vivo according to the casing model, where the fine yellow-brown pheomelanin is form first and then it is encased by the dark eumelanin shell. However, at the present no evidence of the pigment in the on-growing granule is available. In this contest, further works, leading to clarify the variation of eumelanin/pheomelanin levels with aging, will be a fundamental step in the study of neuromelanogenesis.

Interestingly, also brain dolichol levels increase over the human lifespan. At the age of 6 years, dolichol in human brain gray matter is around 18 μg/g wet weight, exceeding the 200 μg/g after the sixth decade of life (Pullarkat and Reha 1982).

6 Dual Role of NM

In contrast to peripheral melanin, whose main function in the body is to protect underlying tissues from harmful UV radiation (Ito 2006), much less is known about the role of NM.

No physiological function has been defined for NM and two different roles have been suggested, either protective or toxic with respect to the degeneration of catecholaminergic neurons depending on the cellular context.

An increasing number of new studies concord with the view that NM is a cellular protecting system in the normal brain. NM synthesis protects the neurons in the SN, because the melanin component is generated from cytosolic catechols which are not accumulated in synaptic vesicles (Zucca et al. 2004). Therefore, this mechanism ensures the removal of reactive/toxic quinones arising from DA oxidation that otherwise would cause neurotoxicity (Paris et al. 2010). Furthermore, NM can trap a variety of organic molecules, as drugs (e.g., chlorpromazine, haloperidol, imipramine), pesticides (e.g., paraquat), and toxic compounds (e.g., 1-methyl-4-phenylpyridinium (MPP^+), beta-carbolines), and immobilize them as stable adducts, thus protecting against toxicity (Salazar et al. 1978; Lindquist et al. 1988; Ostergren et al. 2004; D'Amato et al. 1986) (NM, organic molecule interaction).

Similar to peripheral melanin, NM is a strong chelator of metal cations such as iron, zinc, copper, manganese, chromium, cobalt, mercury, lead, and cadmium (Liu et al. 2004; Zecca and Swartz 1993; Zecca et al. 2002b) (NM, metal chelator). In NM isolated from SN, iron is the metal occurring at the highest concentration, with levels above 10 μg/mg NM (NM, iron level). In DA neurons of SN ferritin are not present, whereas it has been found in glial cells (Zecca et al. 2004; Moos 2000). Therefore, nowadays NM is the only well-documented storage system of iron in SN neurons (Zucca et al. 2004) (NM, iron storage system). In normal condition, NM can sequester iron ions, playing an important role in the protection the neurons from iron-induced oxidative stress (Zareba et al. 1995). NM contains both high- and low-affinity iron-binding sites, where the higher-affinity binding sites (NM, iron-binding sites) are particularly better able to sequester iron (Double et al. 2003). The pigment in vivo is suggested to be only partially saturated with iron, thus maintaining a residual chelating capacity to protect SN against iron toxicity (Shima et al. 1997). In the presence of high iron levels, NM accumulates iron in low-affinity binding sites, arranged in a ferritin-like oxyhydroxy iron clusters, where it can be redox active, producing free radicals and thus leading to neurotoxic consequences (Gerlach et al. 1995; Double et al. 2003; Zecca et al. 2001b). In parkinsonian subjects, where an abnormal loss of NM-containing neurons is observed, the NM levels are 1.2–1.5 μg/mg of SN pars compacta, which is less than 50 % with respect to the age-matched controls (Zecca et al. 2002a). At support to NM protective role, the levels of redox activity are significantly higher in PD patients (+69 %) and increase in patients with the most severe neuronal loss (Faucheux et al. 2003).

On the other hand, NM can be also toxic in PD patients when it is released by dying neurons of SN. Insoluble extracellular NM is phagocytosed by microglia with consequent induction of neurotoxicity by overactivation of microglia (microglia activation) and ensuing production of reactive oxygen species (ROS) and nitrogen species (RNS), and proinflammatory factors (Zhang et al. 2011). Recent epidemiological studies have suggested that neuroinflammation, in particular in a chronic state, is recognized to contribute to the pathogenesis of PD (Chen et al. 2003; Gao et al. 2011; Wu et al. 2002). An initial damage of pigmented neurons, independently if the factors are genetic or environmental, causes the release of NM, which could trigger a vicious circle of neuronal death that enhances the inflammation. Moreover, as ROS can degrade NM, the consequence may be that the neurons not only lose

a protective agent but also release iron and other cytotoxic metals and compounds, accelerating the neuronal death (Zucca et al. 2004; Shima et al. 1997).

In addition to these studies, there are strong evidences for an alteration of NM structure since the first stages of PD. In particular, it has been observed during the disease a decrease of the lipid component and an increase of the density of the pigment (Halliday et al. 2005). Investigations by magnetic spectroscopies of the pigment structure have revealed that the melanin component undergoes modification (Fasano et al. 2006). Other studies have also showed that α-synuclein protein, a primary component of Lewy bodies, aggregates on NM in SN in parkinsonian, but not in healthy control, brains (Halliday et al. 2005). Therefore, this interaction may explain the higher susceptibility during the disease process of highly pigmented brain regions, such as SN (Double 2012).

7 Implication of NM Architecture in Parkinson's Disease

The casing model reported for NM pigment is particularly intriguing and growing efforts are directed to the study of its formation and function in the brain.

In healthy conditions, the pheomelanin core of NM pigment may be the result of a detoxification process of DAQ through conjugation with cysteine. In fact, SN is the brain region with the highest levels of cysteinyldopamine isomers, the pheomelanin precursors (Spencer et al. 1998). The pheomelanin core may function as scaffold for the eumelanin pigment. Moreover, recent evidences suggest that it could also catalyze eumelanin formation (Greco et al. 2011). The eumelanin surface of NM granule is responsible for neuronal protection. Indeed, it is much more efficient than pheomelanin in binding drugs and metals ions (Mårs and Larsson 1999). In addition, the surface oxidation potential found for the eumelanin is not sufficiently to generate a high level of oxidative stress (Bush et al. 2006). On the other hand, any damage of the protective eumelanin surface by ROS, process that likely occurs in the human brain during PD, could expose the pheomelanin core. Pheomelanin, differently from eumelanin, has well-documented prooxidant property (Ye et al. 2006; Bush et al. 2006); therefore, the exposure of pheomelanin core may induce of a cascade of neurodegenerative events (Ito 2006).

At support to the fact that NM architecture may be involved in some way in the pathogenesis of PD, Gao et al. (2009) have recently reported an interesting correlation between the risk of PD and the hair color. They have shown that individuals with red hair have approximately a twofold higher risk for PD relative to those with black hair. It is known that the peripheral pigment granule of red hair individuals is characterized by a tiny eumelanin coat with consequent permanent exposure of the prooxidant pheomelanin core and this seems to be related to the higher incidence of melanoma in comparison to individuals with black hair (Samokhvalov et al. 2005; Vincensi et al. 1998). These findings should stimulate further researches directed to study of the NM architecture and the changing of melanin composition, in particular the eumelanin/pheomelanin ratio, with age and in pathological conditions.

8 Conclusion

Despites the recent progresses in the comprehension of NM biology, there are still many open questions. The future challenges are the clarification of the NM biosynthesis, its mechanism of turnover, and the role in heath individuals. Because of the complexity of NM granules, major insights into the chemical structure of the pigment, the peptide, and the lipid components would elucidate the possible interactions with other neuronal constituents. Moreover, a detailed understanding of NM structural and physiological changes in pathological conditions, in particular the contribution of NM to the oxidative stress and to neuronal vulnerability, would advance our knowledge of the underlying mechanisms that govern the progression of PD.

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References

Adler, A. (1939). Melanin pigment in the central nervous system of vertebrates. *The Journal of Comparative Neurology, 70*, 315–329.

Adler, A. (1942). Melanin pigment in the brain of the gorilla. *The Journal of Comparative Neurology, 76*, 501–507.

Agrup, G., Hansson, C., Rorsman, H., & Rosengren, E. (1982). The effect of cysteine on oxidation of tyrosine, dopa, and cysteinyldopas. *Archives of Dermatological Research, 272*, 103–115.

Andersson, M., Appelkvist, E. L., Kristensson, K., & Dallner, G. (1987). Distribution of dolichol and dolichyl phosphate in human brain. *Journal of Neurochemistry, 49*, 685–691.

Bazelon, M., Fenichel, G. M., & Randall, J. (1967). Studies on Neuromelanin. I. A melanin system in the human adult brainstem. *Neurology, 17*, 512–519.

Bliss, J. M., Ford, D., Swerdlow, A. J., Armstrong, B. K., Cristofolini, M., Elwood, J. M., Green, A., Holly, E. A., Mack, T., Mackie, R. M., Osterlind, A., Walter, S. D., Peto, J., & Easton, D. F. (1995). Risk of cutaneous melanoma associated with pigmentation characteristics and freckling: Systematic overview of 10 case–control studies. *International Journal of Cancer, 62*, 367–376.

Bogerts, B. (1981). A brainstem atlas of catecholaminergic neurons in man, using melanin as a natural marker. *The Journal of Comparative Neurology, 197*, 63–80.

Braak, H., Del Tredici, K., Rüb, U., de Vos, R. A. I., Jansen Steur, E. N. H., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging, 24*, 197–211.

Bridelli, M. G., Tampellini, D., & Zecca, L. (1999). The structure of neuromelanin and its iron binding site studied by infrared spectroscopy. *FEBS Letters, 457*, 8–22.

Brown, J. O. (1943). Pigmentation in the substantia nigra and locus coeruleus in certain carnivores. *The Journal of Comparative Neurology, 79*, 393–405.

Bush, W. D., Garguilo, J., Zucca, F. A., Albertini, A., Zecca, L., Edwards, G. S., Nemanich, R. J., & Simon, J. D. (2006). The surface oxidation potential of human neuromelanin reveals a spherical architecture with a pheomelanin core and a eumelanin surface. *Proceedings of the National Academy of Sciences USA, 103*, 14785–14789.

Calne, D. B., Snow, B. J., & Lee, C. (1992). Criteria for diagnosing Parkinson's disease. *Annals of Neurology, 32*(Suppl), 125–127.

Chen, H., Zhang, S. M., Hernán, M. A., Schwarzschild, M. A., Willett, W. C., Colditz, G. A., Speizer, F. E., & Ascherio, A. (2003). Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. *Archives of Neurology, 60*, 1059–1064.

Crippa, P. R., Eisner, M., Morante, S., Stellato, F., Vicentin, F. C., & Zecca, L. (2010). An XAS study of the sulfur environment in human neuromelanin and its synthetic analogs. *European Biophysics Journal, 39*, 959–970.

D'Amato, R. J., Lipman, Z. P., & Snyder, S. H. (1986). Selectivity of the parkinsonian neurotoxin MPTP: Toxic metabolite MPP^+ binds to neuromelanin. *Science, 231*, 987–989.

Dedov, V. N., Griffiths, F. M., Garner, B., Halliday, G. M., & Double, K. L. (2007). Lipid content determines aggregation of neuromelanin granules in vitro. *Journal of Neural Transmission, 72* (Suppl), 35–38.

Dexter, D. T., Carter, C. J., Wells, F. R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P., & Marsden, C. D. (1989). Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *Journal of Neurochemistry, 52*, 381–389.

Double, K. L. (2012). Neuronal vulnerability in Parkinson's disease. *Parkinsonism & Related Disorders, 18*(Suppl 1), 52–54.

Double, K. L., Zecca, L., Costi, P., Mauer, M., Griesinger, C., Ito, S., Ben-Shachar, D., Bringmann, G., Fariello, R. G., Riederer, P., & Gerlach, M. (2000). Structural characteristics of human substantia nigra neuromelanin and synthetic dopamine melanins. *Journal of Neurochemistry, 75*, 2583–2589.

Double, K. L., Gerlach, M., Schunemann, V., Trautwein, A. X., Zecca, L., Gallorini, M., Youdim, M. B., Riederer, P., & Ben-Shachar, D. (2003). Iron-binding characteristics of neuromelanin of the human substantia nigra. *Biochemical Pharmacology, 66*, 489–494.

Double, K. L., Dedov, V. N., Fedorow, H., Kettle, E., Halliday, G. M., Garner, B., & Brunk, U. T. (2008). The comparative biology of neuromelanin and lipofuscin in the human brain. *Cellular and Molecular Life Sciences, 65*, 1669–1682.

Duffy, P. E., & Tennyson, V. M. (1965). Phase and electron microscopic observations of lewy bodies and melanin granules in the substantia nigra and locus coeruleus in Parkinson's disease. *Journal of Neuropathology and Experimental Neurology, 24*, 398–414.

Fasano, M., Bergamasco, B., & Lopiano, L. (2006). Is neuromelanin changed in Parkinson's disease? Investigations by magnetic spectroscopies. *Journal of Neural Transmission, 113*, 769–774.

Faucheux, B. A., Martin, M. E., Beaumont, C., Hauw, J. J., Agid, Y., & Hirsch, E. C. (2003). Neuromelanin associated redox-active iron is increased in the substantia nigra of patients with Parkinson's disease. *Journal of Neurochemistry, 86*, 1142–1148.

Fedorow, H., Tribl, F., Halliday, G., Gerlach, M., Riederer, P., & Double, K. L. (2005a). Neuromelanin in human dopamine neurons: Comparison with peripheral melanins and relevance to Parkinson's disease. *Progress in Neurobiology, 75*, 109–124.

Fedorow, H., Pickford, R., Hook, J., Double, K. L., Halliday, G. M., Gerlach, M., Riederer, P., & Garner, B. (2005b). Dolichol is the major lipid in human substantia nigra neuromelanin. *Journal of Neurochemistry, 92*, 990–995.

Fedorow, H., Pickford, R., Kettle, E., Cartwright, M., Halliday, G. M., Gerlach, M., Riederer, P., Garner, B., & Double, K. L. (2006). Investigation of the lipid component of neuromelanin. *Journal of Neural Transmission, 113*, 735–739.

Foley, J. M., & Baxter, D. (1958). On the nature of pigment granules in the cell of the locus coeruleus and substantia nigra. *Journal of Neuropathology and Experimental Neurology, 17*, 586–598.

Gao, X., Simon, K. C., Han, J., Schwarzschild, M. A., & Ascherio, A. (2009). Genetic determinants of hair color and Parkinson's disease. *Annals of Neurology, 65*, 76–82.

Gao, H. M., Zhou, H., Zhang, F., Wilson, B. C., Kam, W., & Hong, J. S. (2011). HMGB1 acts on microglia Mac1 to mediate chronic neuroinflammation that drives progressive neurodegeneration. *The Journal of Neuroscience, 31*, 1081–1092.

Gaspar, P., Berger, B., Gay, M., Hamon, M., Cesselin, F., Vighy, A., Javoy-Agid, F., & Agid, Y. (1983). Tyrosine hydroxylase and methionine-enkephalin in the human mesencephalon:

Immunocytochemical localization and relationships. *Journal of the Neurological Sciences, 58*, 247–267.

Gelb, J. D., Oliver, E., & Gilman, S. (1999). Diagnostic criteria for Parkinson disease. *Archives of Neurology, 56*, 33–39.

Gerlach, M., Trautwein, A. X., Zecca, L., Youdim, M. B. H., & Riederer, P. (1995). Mossbauer spectroscopic studies of purified human neuromelanin isolated from the substantia nigra. *Journal of Neurochemistry, 65*, 923–926.

Gillilan, L. A. (1943). The nuclear pattern of the non-tectal portions of the midbrain and isthmus in Ungulates. *The Journal of Comparative Neurology, 78*, 289–364.

Graham, D. G. (1979). On the origin and significance of neuromelanin. *Archives of Pathology & Laboratory Medicine, 103*, 359–362.

Greco, G., Panzella, L., Gentile, G., Errico, M. E., Carfagna, C., Napolitano, A., & D'Ischia, M. (2011). A melanin-inspired pro-oxidant system for dopa(mine) polymerization: Mimicking the natural casing process. *Chemical Communications, 47*, 10308–10310.

Halliday, G. M., Ophof, A., Broe, M., Jensen, P. H., Kettle, E., Fedorow, H., Cartwright, M. I., Griffiths, F. M., Shepherd, C. E., & Double, K. L. (2005). α-Synuclein redistributes to neuromelanin lipid in the substantia nigra early in Parkinson's disease. *Brain, 128*, 2654–2664.

Halliday, G., Fedorow, H., Rickert, C. H., Gerlach, M., Riederer, P., & Double, K. L. (2006). Evidence for specific phases in the development of human neuromelanin. *Journal of Neural Transmission, 113*, 721–728.

Hirosawa, K. (1968). Electron microscopic studies on pigment granules in the substantia nigra and locus coeruleus of the Japanese monkey (*Macaca fuscata yakui*). *Zeitschrift für Zellforschung und Mikroskopische Anatomie, 88*, 187–203.

Ikemoto, K., Nagatsu, I., Ito, S., King, R. A., Nishimura, A., & Nagatsu, T. (1998). Does tyrosinase exist in neuromelanin-pigmented neurons in the human substantia nigra? *Neuroscience Letters, 253*, 198–200.

Ito, S. (2003). A chemist's view of melanogenesis. *Pigment Cell Research, 16*, 230–236.

Ito, S. (2006). Encapsulation of a reactive core in neuromelanin. *Proceedings of the National Academy of Sciences USA, 103*, 14647–14648.

Ito, S., Kato, T., & Fujita, K. (1988). Covalent binding of catechols to proteins through the sulphydryl group. *Biochemical Pharmacology, 37*, 1707–1710.

Ito, S., & Wakamatsu, K. (2003). Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: A comparative study. *Pigment Cell Research, 16*, 523–531.

Ito, S., & Wakamatsu, K. (2006). Chemistry of melanins. In J. J. Nordlund, R. E. Boissy, V. J. Hearing, R. A. King, W. S. Oetting, & J. P. Ortonne (Eds.), *The pigmentary system: Physiology and pathophysiology* (pp. 282–310). New York: Blackwell.

Kin, N. M. K., Palo, J., Haltia, M., & Wolfe, L. S. (1983). High levels of brain dolichols in neuronal ceroid-lipofuscinosis and senescence. *Journal of Neurochemistry, 40*, 1465–1473.

Korner, A., & Pawelek, J. (1982). Mammalian tyrosinase catalyzes three reactions in the biosynthesis of melanin. *Science, 217*, 1163–1165.

Land, E. J., Ito, S., Wakamatsu, K., & Riley, P. A. (2003). Rate constants for the first two chemical steps of eumelanogenesis. *Pigment Cell Research, 16*, 487–493.

Lindquist, N. G., Larsson, B. S., & Lyden-Sokolowski, A. (1988). Autoradiography of [^{14}C] paraquat or [^{14}C]diquat in frogs and mice: Accumulation in neuromelanin. *Neuroscience Letters, 93*, 1–6.

Liu, Y., & Simon, J. D. (2003). Isolation and biophysical studies of natural eumelanins: Applications of imaging technologies and ultrafast spectroscopy. *Pigment Cell Research, 16*, 606–618.

Liu, Y., Hong, L., Kempf, V. R., Wakamatsu, K., Ito, S., & Simon, J. D. (2004). Ion-exchange and adsorption of Fe(III) by Sepia melanin. *Pigment Cell Research, 17*, 262–269.

Mann, D. M., & Yates, P. O. (1983). Possible role of neuromelanin in the pathogenesis of Parkinson's disease. *Mechanisms of Ageing and Development, 21*, 193–203.

Mann, D. M., Yates, P. O., & Barton, C. M. (1977). Neuromelanin and RNA in cells of substantia nigra. *Journal of Neuropathology and Experimental Neurology, 36*, 379–383.

Mårs, U., & Larsson, B. S. (1999). Pheomelanin is a binding site for drugs and chemicals. *Pigment Cell Research, 12*, 266–272.

Marsden, C. D. (1961). Pigmentation in the nucleus substantiae nigrae of mammals. *Journal of Anatomy, 95*, 256–261.

Matsunaga, J., Sinha, D., Pannell, L., Santis, C., Solano, F., Wistow, G. J., & Hearing, V. J. (1999). Enzyme activity of macrophage migration inhibitory factor toward oxidized catecholamines. *The Journal of Biological Chemistry, 274*, 3268–3271.

Moos, T. (2000). Absence of ferritin protein in substantia nigra pars compacta neurons. A reappraisal to the role of iron in Parkinson's disease pathogenesis. *Movement Disorders, 15*(Suppl 3), 319A.

Moses, H. L., Ganote, C. E., Beaver, D. L., & Schuffman, S. S. (1966). Light and electron microscopic studies of pigment in human and rhesus monkey substantia nigra and locus coeruleus. *The Anatomical Record, 155*, 167–383.

Okun, M. R. (1997). The role of peroxidase in neuromelanin synthesis: A review. *Physiological Chemistry and Physics and Medical NMR, 29*, 15–22.

Ostergren, A., Annas, A., Skog, K., Lindquist, N. G., & Brittebo, E. B. (2004). Long-term retention of neurotoxic beta-carbolines in brain neuromelanin. *Journal of Neural Transmission, 111*, 141–157.

Paris, I., Perez-Pastene, C., Cardenas, S., Iturra, P., Munõz, P., Couve, E., Caviedes, P., & Segura-Aguilar, J. (2010). Aminochrome induces disruption of actin, alpha-, and beta-tubulin cytoskeleton networks in substantia-nigra-derived cell line. *Neurotoxicity Research, 18*, 82–92.

Prota, G. (1992). *Melanin and melanogenesis*. San Diego: Academic.

Pullarkat, R. K., & Reha, H. (1982). Accumulation of dolichols in brains of elderly. *The Journal of Biological Chemistry, 257*, 5991–5993.

Rosengren, E., Linder-Eliasson, E., & Carlsson, A. (1985). Detection of 5-*S*-cysteinyldopamine in human brain. *Journal of Neural Transmission, 63*, 247–253.

Salazar, M., Sokoloski, T. D., & Patil, P. N. (1978). Binding of dopaminergic drugs by the neuromelanin of the substantia nigra, synthetic melanins and melanin granules. *Federation Proceedings, 37*, 2403–2407.

Samokhvalov, A., Hong, L., Liu, Y., Garguilo, J., Nemanich, R. J., Edwards, G. S., & Simon, J. D. (2005). Oxidation potentials of human eumelanosomes and pheomelanosomes. *Photochemistry and Photobiology, 81*, 145–148.

Scherer, H. J. (1939). Melanin pigmentation of the substantia nigra in primates. *The Journal of Comparative Neurology, 71*, 91–98.

Shima, T., Sarna, T., Swartz, H. M., Stroppolo, A., Gerbasi, R., & Zecca, L. (1997). Binding of iron to neuromelanin of human substantia nigra and synthetic melanin: An electron paramagnetic resonance spectroscopy study. *Free Radical Biology & Medicine, 23*, 110–119.

Simon, J. D., & Peles, D. N. (2010). The red and the black. *Accounts of Chemical Research, 43*, 1452–1460.

Simon, J. D., Peles, D., Wakamatsu, K., & Ito, S. (2009). Current challenges in understanding melanogenesis: Bridging chemistry, biological control, morphology, and function. *Pigment Cell & Melanoma Research, 22*, 563–579.

Spencer, J. P. E., Jenner, P., Daniel, S. E., Lees, A. J., Marsden, D. C., & Halliwell, B. (1998). Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: Possible mechanism of formation involving reactive oxygen species. *Journal of Neurochemistry, 71*, 2112–2122.

Sulzer, D., Bogulavsky, J., Larsen, K. E., Behr, G., Karatekin, E., Kleinman, M. H., Turro, N., Krantzi, D., Edwardsi, R. H., Greene, L. A., & Zecca, L. (2000). Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. *Proceedings of the National Academy of Sciences USA, 97*, 11869–11874.

Thody, A. J., Higgins, E. M., Wakamatsu, K., Ito, S., Burchill, S. A., & Marks, J. M. (1991). Pheomelanin as well as eumelanin is present in human epidermis. *The Journal of Investigative Dermatology, 97*, 340–344.

Tribl, F., Gerlach, M., Marcus, K., Asan, E., Tatschner, T., Arzberger, T., Meyer, H. E., Bringmann, G., & Riederer, P. (2005). "Subcellular proteomics" of neuromelanin granules isolated from the human brain. *Molecular & Cellular Proteomics, 4*, 945–957.

Tse, D. C. S., McCreery, R. L., & Adams, R. N. (1976). Potential oxidative pathways of brain catecholamines. *Journal of Medicinal Chemistry, 19*, 37–40.

Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., & Marth, J. (1999). *Essentials of glycobiology*. Plainview, New York: Cold Spring Harbor Laboratory Press.

Vincensi, M. R., D'Ischia, M., Napolitano, A., Procaccini, E. M., Riccio, G., Monfrecola, G., Santoianni, P., & Prota, G. (1998). Pheomelanin versus eumelanin as a chemical indicator of ultraviolet sensitivity in fair-skinned subjects at high risk for melanoma: A pilot study. *Melanoma Research, 8*, 53–58.

Wakamatsu, K., Fujikawa, K., Zucca, F. A., Zecca, L., & Ito, S. (2003). The structure of neuromelanin as studied by chemical degradative methods. *Journal of Neurochemistry, 86*, 1015–1023.

Ward, W. C., Guan, Z., Zucca, F. A., Fariello, R. G., Kordestani, R., Zecca, L., Raetz, C. R. H., & Simon, J. D. (2007). Identification and quantification of dolichol and dolichoic acid in neuromelanin from substantia nigra of the human brain. *Journal of Lipid Research, 48*, 1457–1462.

Wu, D. C., Jackson-Lewis, V., Vila, M., Tieu, K., Teismann, P., Vadseth, C., Choi, D. K., Ischiropoulos, H., & Przedborski, S. (2002). Blockade of microglial activation is neuroprotective in the 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *The Journal of Neuroscience, 22*, 1763–1771.

Ye, T., Hong, L., Garguilo, J., Pawlak, A., Edwards, G. S., Nemanich, R. J., Sarna, T., & Simon, J. D. (2006). Photoionization thresholds of melanins obtained from free electron laser-photoelectron emission microscopy, femtosecond transient absorption spectroscopy and electron paramagnetic resonance measurements of oxygen consumption. *Photochemistry and Photobiology, 82*, 733–737.

Young, T. E., & Babbitt, B. W. (1983). Electrochemical study of the oxidation of α-methyldopamine, α-methylnoradrenaline, and dopamine. *The Journal of Organic Chemistry, 48*, 562–566.

Zareba, M., Bober, A., Korytowski, W., Zecca, L., & Sarna, T. (1995). The effect of a synthetic neuromelanin on yield of free hydroxyl radicals generated in model systems. *Biochimica et Biophysica Acta, 1271*, 343–348.

Zecca, L., & Swartz, H. M. (1993). Total and paramagnetic metals in human substantia nigra and its neuromelanin. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 5*, 203–213.

Zecca, L., Costi, P., Mecacci, C., Ito, S., Terreni, M., & Sonnino, S. (2000). Interaction of human substantia nigra neuromelanin with lipids and peptides. *Journal of Neurochemistry, 74*, 1758–1765.

Zecca, L., Tampellini, D., Gerlach, M., Riederer, P., Fariello, R. G., & Sulzer, D. (2001a). Substantia nigra neuromelanin: Structure, synthesis, and molecular behaviour. *Molecular Pathology, 54*, 414–418.

Zecca, L., Gallorini, M., Schunemann, V., Trautwein, A. X., Gerlach, M., Riederer, P., Vezzoni, P., & Tampellini, D. (2001b). Iron, neuromelanin and ferritin content in the substantia nigra of normal subjects at different ages: Consequences for iron storage and neurodegenerative processes. *Journal of Neurochemistry, 76*, 1766–1773.

Zecca, L., Fariello, R., Riederer, P., Sulzer, D., Gatti, A., & Tampellini, D. (2002a). The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. *FEBS Letters, 510*, 216–220.

Zecca, L., Tampellini, D., Gatti, A., Crippa, R., Eisner, M., Sulzer, D., Ito, S., Fariello, R., & Gallorini, M. (2002b). The neuromelanin of human substantia nigra and its interaction with metals. *Journal of Neural Transmission, 109*, 663–672.

Zecca, L., Stroppolo, A., Gatti, A., Tampellini, D., Toscani, M., Gallorini, M., Giaveri, G., Arosio, P., Santambrogio, P., Fariello, R. G., Karatekin, E., Kleinman, M. H., Turro, N. J., Hornykiewicz, O., & Zucca, F. A. (2004). The role of iron and copper molecules in the neuronal vulnerability of locus coeruleus and substantia nigra during aging. *Proceedings of the National Academy of Sciences USA, 101*, 9843–9848.

Zecca, L., Bellei, C., Costi, P., Albertini, A., Monzani, E., Casella, L., Gallorini, M., Bergamaschi, L., Moscatelli, A., Turro, N. J., Eisner, M., Crippa, P. R., Ito, S., Wakamatsu, K., Bush, W. D., Ward, W. C., Simon, J. D., & Zucca, F. A. (2008). New melanic pigments in the human brain that accumulate in aging and block environmental toxic metals. *Proceedings of the National Academy of Sciences USA, 105*, 17567–17572.

Zhang, F., & Dryhurst, G. (1994). Effects of L-cysteine on the oxidation chemistry of dopamine: New reaction pathways of potential relevance to idiopathic Parkinson's disease. *Journal of Medicinal Chemistry, 37*, 1084–1098.

Zhang, W., Phillips, K., Wielgus, A. R., Liu, J., Albertini, A., Zucca, F. A., Faust, R., Qian, S. Y., Miller, D. S., Chingell, C. F., Wilson, B., Jackson-Lewis, V., Przedborski, S., Joset, D., Loike, J., Hong, J. S., Sulzer, D., & Zecca, L. (2011). Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: Implications for progression of Parkinson's disease. *Neurotoxicity Research, 19*, 63–72.

Zhou, G. P., & Troy, F. A. (2004). NMR Study of the preferred membrane orientation of polyisoprenols (Dolichol) and the impact of their complex with polyisoprenyl recognition sequence peptides on membrane structure. *Glycobiology, 15*, 347–359.

Zucca, F. A., Giaveri, G., Gallorini, M., Albertini, A., Toscani, M., Pezzoli, G., Lucius, R., Willms, H., Sulzer, D., Ito, S., Wakamatsu, K., & Zecca, L. (2004). The neuromelanin of human substantia nigra: Physiological and pathogenic aspects. *Pigment Cell Research, 17*, 610–617.

Neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as a Parkinson's Disease Model

H. Nakayama, T. Ito, Y. Shibui, T. Sai, K. Uchida, and X. J. He

Contents

Abstract

Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to susceptible animals is the most popular means for modeling the destruction of the nigrostriatal dopaminergic neurons seen in Parkinson's disease (PD). MPTP causes a damage to the substantia nigra pars compacta (SNpc) dopaminergic neurons and depletes striatal dopamine secretion in nonhuman primates and rodents. MPTP passes through the blood–brain barrier (BBB) and is converted, mainly in glial cells, into its active form, 1-methyl-4-phenylpyridinium (MPP^+), by an enzyme, monoamine oxidase B (MAO-B). MPP^+ is selectively transported into dopaminergic nerve terminals through dopamine transporter (DAT) and finally induces dopaminergic cell loss. The selective neurotoxicity of MPTP to

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neural tissues other than the dopaminergic system, such as the subventricular zone (SVZ) and rostral migratory stream (RMS) in the adult brain, has been indicated. The cells undergoing apoptosis in the SVZ are A cells and MAO-B inhibitors completely protected against the neurotoxicity, suggesting that the MPTP neurotoxicity to A cells is mediated by the conversion of MPTP into MPP^+ by MAO-B. The neurotoxicity of MPTP to neuroblasts in the SVZ does not require DAT or other monoamine transporters. There is a strain difference in the susceptibility of the MPTP neurotoxicity between susceptible C57BL/6 and resistant BALB/c mice. However, MAO-B, DAT, neural NOS (nNOS), and inducible NOS (iNOS) expression levels do not influence the strain susceptibility to MPTP. MPTP and MPP^+ can pass through the placenta and embryonic or newborn BBB, and finally reach the brain. The number of MAO-B-positive glial cells in the brain increases in MPTP-treated embryonic and newborn mice, indicating the involvement of MAO-B in the acute neurotoxicity. MPTP or MPP^+ administration into embryonic and newborn mice causes a loss of TH-positive cells and fibers in the nigrostriatal system and increases the number of apoptotic cells in the SVZ. Delayed regeneration of dopaminergic neurons due to the loss of SVZ A cells may be one of possible causes of the pathological condition. With increased attention to dopaminergic neurogenesis in MPTP-induced animal models, such animals become useful as a tool for establishing the contact to PD on neurogenesis.

List of Abbreviations

6-OHDA	6-Hydroxydopamine
BBB	Blood–brain barrier
BrdU	Bromodeoxyuridine
CNS	Central nervous system
DA	Dopamine
DAT	Dopamine transporter
DCX	Doublecortin
DG	Dentate gyrus
icv	Intracerebroventricularly
iNOS	Inducible NOS
MAO-B	Monoamine oxidase B
MMPs	Metalloproteinases
$MPDP^+$	1-Methyl-4-phenyl-2,3-dihydropyridinium
MPP^+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
nNOS	Neural NOS
NOS	Nitric oxide synthase
OB	Olfactory bulb
PCNA	Proliferating cell nuclear antigen
PD	Parkinson's disease
RMS	Rostral migratory stream
Ro 16-6491	*N*-(2-aminoethyl)-4-chlorobenzamide
SERT	Serotonin transporter

SGZ	Subgranular zone
SN	Substantia nigra
SNpc	Substantia nigra pars compacta
SVZ	Subventricular zone
TH	Tyrosine hydroxylase
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling
VMAT2	Vesicular monoamine transporter 2

1 Introduction

Parkinson's disease (PD) is an age-related degenerative disorder of the central nervous system (CNS). It is often characterized by muscle rigidity, tremor, a slowing of physical movement (bradykinesia), and, in extreme cases, a loss of physical movement (akinesia). The symptoms of PD are attributed to the loss of pigmented dopamine-secreting (dopaminergic) neurons in the pars compacta region of the substantia nigra (SNpc) and a subsequent striatal deficiency of dopamine (DA). The idiopathic PD is also pathologically characterized by the presence of cytoplasmic neuronal inclusions, called Lewy bodies, in the affected region of the brain. The etiology of the disease has been poorly understood, and novel non-dopaminergic therapeutic strategies still remain challenging since the initial description was first described by James Parkinson in 1817 (Parkinson 1817). Although pharmacological dopamine replacement strategies provide a temporary symptomatic relief, there are at present no therapeutic methods for stopping the progressive neuronal cell loss (Borta and Hoglinger 2007).

Animal models are important tools in elucidating mechanisms involved in the pathological process and in investigating the new therapeutic strategies in PD. Currently, both genetic and toxic models of PD are available, but the use of neurotoxins such as 6-hydroxydopamine (6-OHDA), paraquat, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and rotenone are still the most popular means for modeling the destruction of the nigrostriatal dopaminergic neurons seen in PD (Przedborski and Ischiropoulos 2005). Among them, 6-OHDA and MPTP have been more extensively used by investigators to produce PD models despite their limitations. 6-OHDA is a structural analog of catecholamines, DA and noradrenaline, and exerts its toxic effects on catecholaminergic neurons (Simola et al. 2007). Since 6-OHDA does not penetrate the blood–brain barrier (BBB) in adult rats, it must be administrated stereotactically into the substantia nigra (SN) or striatum to damage the dopaminergic nigrostriatal system (Perese et al. 1989; Przedbroski et al. 1995). Recognition of MPTP as a neurotoxin occurred early in 1982, when several young drug addicts mysteriously developed a profound Parkinsonian syndrome after the intravenous use of street preparations of meperidine analogs that, unknown to anyone, were contaminated with MPTP (Przedborski and Vila 2003; Langston et al. 1983), an incidental by-product during the chemical synthesis of a meperidine analog (Fig. 1)

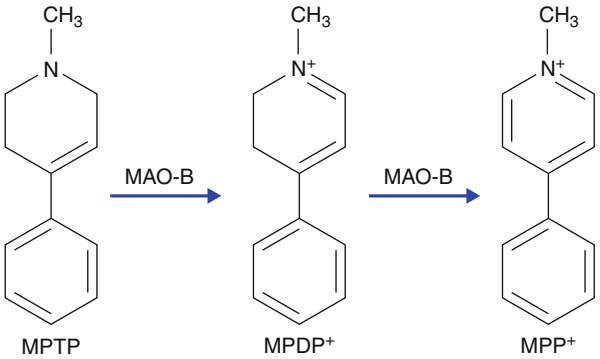

Fig. 1 MPTP is a by-product during the chemical synthesis of a meperidine analog. MPTP neurotoxicity develops only after metabolization to $MPDP^+$ by MAO-B in glial cells, and further to MPP^+, the active toxic compound (He and Nakayama 2009)

with potent heroin-like effects (Przedborski and Vila 2003). MPTP has been found to produce irreversible Parkinsonism in humans almost indistinguishable from PD (Langston et al. 1983; Blume 1983). It is also well known that MPTP depletes striatal dopamine and causes a damage to the SNpc dopaminergic neurons in nonhuman primates and several species of rodents (Burns et al. 1983; Langston et al. 1984; Heikkila et al. 1984; Chiueh et al. 1984).

Recently, a great deal of interest has been focused on the stem cell therapies for PD. In addition to the fetal nigral transplantation, the capability of self-repairing in central nervous system (CNS) in adult mammalian has also been considered as a new insight into the cell-based approach for the treatment of neurodegenerative disorders. In PD or its animal models, it has attracted much attention whether the depletion of dopaminergic neurons triggers an activation of neural stem cells and their subsequent migration into the damaged area, and finally results in repopulation of dopaminergic neurons in SN. We have recently reported several results (He et al. 2006, 2008a, b; He and Nakayama 2009; Popolo et al. 2004), which will provide evidences available for endogenous neurogenesis in response to neurotoxin damages, and provide perspective on the contact of neurotoxin-based animal models on PD. This chapter will mainly describe such MPTP-induced neural damages in the subventricular zone (SVZ) as well as SNpc and striatum, and subsequent neurogenesis in the brain of the mouse. In addition, the strain difference in the susceptibility to MPTP in the mouse, and the relations of MPTP neurotoxicity and aging are mentioned.

2 Neurotoxicity of MPTP to Dopamine Neurons

MPTP is a by-product of the chemical synthesis of a meperidine analog, with potent heroin-like effects (Przedborski and Vila 2003), that causes damage to dopaminergic neurons and depletes dopamine in a manner similar to that seen in PD. When administered to animals, MPTP passes through the BBB and is converted, mainly in glial cells, into its active form, 1-methyl-4-phenylpyridinium (MPP^+), by an enzyme, monoamine oxidase B (MAO-B) (Blum et al. 2001). For the last two decades, MPTP has been widely

used to generate animal models of PD in rodents and nonhuman primates. A few studies have confirmed that MPTP can induce apoptosis of dopaminergic neurons in vivo (Tatton and Kish 1997; Vila et al. 2001; Spooren et al. 1998). When injected intraperitoneally (ip) with a subacute regimen, which consists of one injection per day (30 mg/kg per day) for 5 consecutive days, MPTP induces dopaminergic cell loss by a mechanism mainly involving apoptosis (Przedborski and Vila 2001). The use of this subacute regimen has been the main approach to studying the apoptosis of dopaminergic cells in mice. The induction of apoptosis in mouse nigrostriatal glia has also been demonstrated with this regimen (Serra et al. 2002), indicating that neurotoxicity of MPTP is not specifically restricted to dopaminergic neurons in the mouse brain. Furthermore, results from studies in vitro show that MPTP or MPP^+ induces apoptosis in primary cultured cerebellar granular neurons (Dipasquale et al. 1991; Du et al. 1997), mesencephalic dopaminergic neurons (Mochizuki et al. 1994), and neuroblastoma cells (Itano and Nomura 1995; Sheehan et al. 1997) as well as PC12 cells (Hartley et al. 1994; Desole et al. 1997).

3 Neurotoxicity of MPTP to Neural Stem Cells

3.1 The Origin and Properties of Neural Stem Cells

Neurogenesis continues into adult life in the brain of rodents (Altman 1963; Privat and Leblond 1972), nonhuman primates (Gould et al. 1999a, b; Pencea et al. 2001), and humans (Bernier et al. 2000; Eriksson et al. 1998) (Fig. 2). Neurogenesis has been confined largely to two discrete areas, the subventricular zone (SVZ), and the subgranular zone (SGZ) of the dentate gyrus (DG) (Garcia-Verdugo et al. 1998).

The SVZ, located throughout the inner wall of the lateral ventricle, is the largest germinal region and harbors neural stem cells that retain the capacity to generate multiple cell types (Doetsch et al. 2002). The SVZ is composed of neural progenitor cells (migrating neuroblasts, A cells), neural stem cells (astrocytes, B cells), neural precursor cells (rapidly dividing transit-amplifying cells, C cells), and ependymal cells (Doetsch et al. 1997, 1999). Neural stem B cells divide to give rise to clusters of precursor C cells, which in turn generate neuroblast A cells (Doetsch et al. 1999). Newly generated A cells in the SVZ migrate through a network of tangential pathways in the lateral wall of the lateral ventricle and then converge onto the rostral migratory stream (RMS) to enter the olfactory bulb (OB) with a behavior of "chain migration" (Doetsch and Alvarez-Buylla 1996), where they differentiate into granule cells and interneurons (Doetsch and Alvarez-Buylla 1996; Lois and Alvarez-Buylla 1994). It has been suggested that prokineticin 2 serves as a chemoattractant for SVZ-derived neuroblast A cells, which appears to guide the migration of A cells from the SVZ through the RMS to their final layers in the OB (Ng et al. 2005). The multipotential A cells reside in the SVZ and RMS are eliminated through apoptosis to maintain a balance for proper development of the mammalian nervous system (Brunjes and Armstrong 1996; Levison et al. 2000;

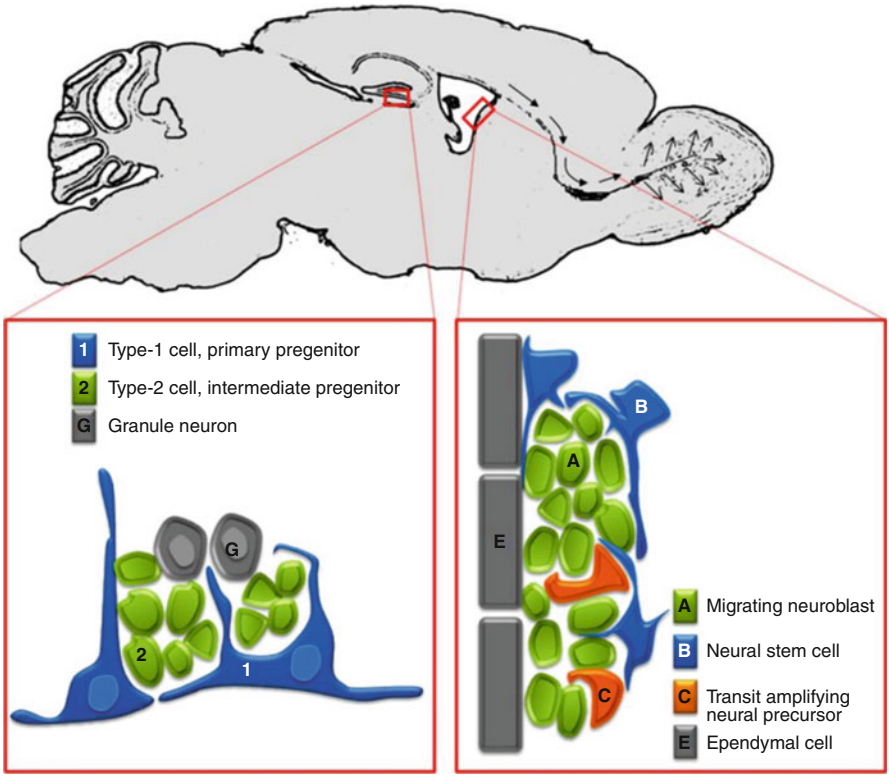

Fig. 2 Schematic representation showing the migratory pattern of newly generated A cells in the SVZ (*up*), and the cell population in the SGZ of DG (*bottom left*) and SVZ (*bottom right*) in adult rodent brain (He and Nakayama 2009)

Biebl et al. 2000). Metalloproteinases (MMPs) may also play a crucial role in the migration of individual A cells since the migration rates are reduced by the presence of inhibitors of MMPs (Bovetti et al. 2007). The architecture and function of the adult human SVZ differs significantly from that described in other mammals. There are four layers with varying thickness and cell densities throughout the lateral ventricular wall: a monolayer of ependymal cells (Layer I), a hypocellular gap (Layer II), a ribbon of cells (Layer III) composed of astrocytes, and a transitional zone (Layer IV) into the brain parenchyma (Quinones-Hinojosa et al. 2006).

The DG is a part of the hippocampal structure. Neuronal progenitors in the adult mammalian hippocampal DG reside in the SGZ, which is located in the hilus immediately beneath the granular layer of the DG (Kaplan and Hinds 1977; Cameron et al. 1993). Neurogenesis in the DG was first demonstrated 40 years ago by autoradiography in rodents (Altman and Das 1965), and thereafter was further demonstrated in all mammalian species including human (Eriksson et al. 1998) and nonhuman primates (Gould et al. 1998, 1999a, b). Two types of neural progenitors can be identified in the SGZ according to their specific morphologies

and expression of unique sets of molecular markers (Zhao et al. 2008). The primary progenitors (type-1 cells) have the appearance of radial glia, which also express glial fibrillary acidic protein (GFAP). They share the similar features with type B cells residing in the adult SVZ and then are suggested as the putative stem cell population (Seri et al. 2001). Type-2 cells (intermediate progenitors) are GFAP negative and highly proliferative (Kempermann et al. 2004), sharing similar features with neuroblast A cells residing in the adult SVZ. Type-2 cells may arise from type 1-cells, but direct evidence delineating this lineage relationship is still lacking (Zhao et al. 2008). The newborn type-2 cells disperse and migrate a short distance into the granule cell layer where they differentiate, extend axons, and express neuronal marker proteins (Cameron et al. 1993; Kuhn et al. 1996). The migration of newborn neurons in the DG may also be controlled by guidance cues, as these cells only migrate to the hilus or the molecular layer under pathological conditions, such as in animal models of temporal lobe epilepsy (Zhao et al. 2008).

3.2 Neurotoxicity of MPTP to SVZ Neural Stem Cells

MPTP neurotoxicity develops only after metabolization to 1-methyl-4-phenyl-2,3-dihydropyridinium ($MPDP^+$) and further to 1-methyl-4-phenylpyridinium (MPP^+), the active toxic compound, by MAO-B (Fig. 1) (Przedborski and Vila 2003). Since MPP^+ is selectively transported into presynaptic dopaminergic nerve terminals through the dopamine transporter (DAT) (Chiba et al. 1985; Javitch et al. 1985; Gainetdinov et al. 1997; Bezard et al. 1999; Pifl et al. 1993), and the absence of the DAT on dopaminergic neurons confers a complete protection against MPTP toxicity (Gainetdinov et al. 1997; Bezard et al. 1999), the neurotoxicity of MPTP is suggested to be selective to dopaminergic neurons in SN. MPTP is mainly used in nonhuman primates and in rodents (Gerlach and Riederer 1996; Przedborski et al. 2001). In rodents, the C57BL/6 mice are widely used to create the PD animal model for understanding the disease pathogenesis in a great deal of reports despite their limitations.

In recent years, the selective neurotoxicity of MPTP to neural tissues other than the dopaminergic system in the adult brain has been elucidated. Several studies (Serra et al. 2002; He et al. 2006, 2008a) have demonstrated that MPTP also destroys forebrain migrating neuroblasts and nigrostriatal glial cells (astrocytes) in the adult mouse brain. In our previous studies (He et al. 2006, 2008a), the number of apoptotic cells in the SVZ and RMS peaked at 24 h after MPTP-injections, and decreased thereafter, paralleling the change in the number of cleaved caspase-3-positive cells (Fig. 3). The cells undergoing apoptosis in the SVZ, RMS, and OB were identified as A cells using immunohistochemistry and ultra-structural analyses, while a few were astrocytes (B cells) and none were transit-amplifying precursors (C cells) (He et al. 2006, 2008a). The decrease in A cell numbers was most marked at day 2 and lasted to day 8 after the administration (He et al. 2006, 2008a). We also demonstrated that MAO-B inhibitors, such as deprenyl or *N*-(2-aminoethyl)-4-chlorobenzamide (Ro 16-6491), completely protected

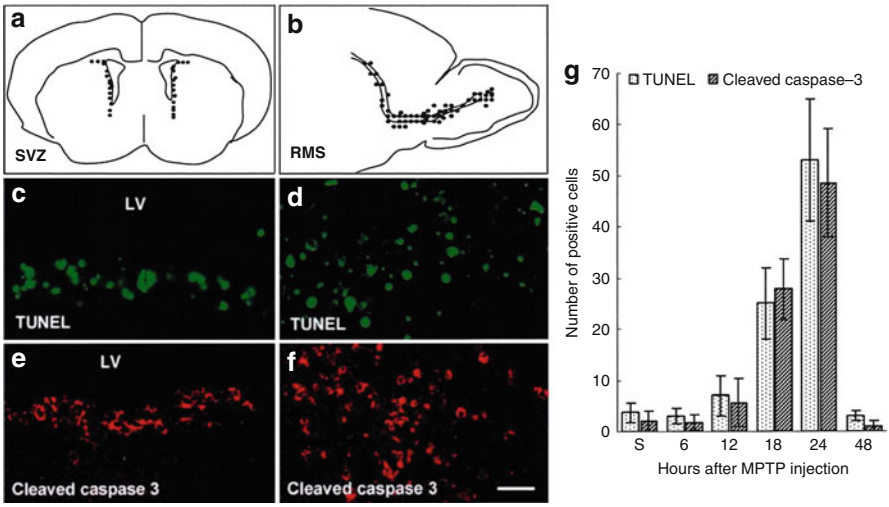

Fig. 3 Detection of apoptotic cells in the SVZ (coronal section) and RMS (sagittal section) with fluorescence TUNEL and immunofluorescence staining for cleaved caspase-3. (**a**, **b**) Schematic distribution of apoptotic cells in the SVZ and RMS with the positions of the cells undergoing apoptosis indicated by *dots*. (**c**, **d**) Apoptotic nucleus (*green*) in the SVZ and RMS revealed by the TUNEL technique, and (**e**, **f**) cleaved caspase-3 staining (*red*) in the same field. Scale bar = 50 μm. (**g**) The results of a quantitative analysis of TUNEL-positive or cleaved caspase-3-immunoreactive cells in the SVZ of MPTP-treated mice. The number of TUNEL-positive or cleave caspase-3-immunoreactive cells peaked at 24 h and had declined to very low levels by 48 h after MPTP injection. Neither TUNEL-positive nor cleaved caspase-3-immunoreactive cells were found in control animals at any time point. Abbreviation: *LV* lateral ventricle (He et al. 2006)

against MPTP neurotoxicity to A cells, suggesting that MPTP-neurotoxicity to A cells is also mediated by the conversion of MPTP into MPP^+ by MAO-B (He et al. 2008b).

3.3 Neurotoxicity of MPTP to Neural Stem Cells Is Not Regulated by Monoamine Transporters

In dopaminergic neurons, MPP^+ enters into the cytoplasm via the dopamine transporter (DAT) (Gainetdinov et al. 1997; Javitch et al. 1985), is concentrated by an active process within the mitochondria, and impairs mitochondrial respiration by inhibiting complex I of the electron transport chain (Vila and Przedborski 2003). DAT is thus thought to play a crucial role in the MPTP neurotoxic process.

On the other hand, other monoamine transporters, including vesicular monoamine transporter 2 (VMAT2), serotonin transporter (SERT), and norepinephrine transporter (NET) may also contribute to MPTP toxicity. It has been reported that the dopaminergic neurotoxicity of MPTP is enhanced by the injection of VMAT2 inhibitors (German et al. 2000; Reinhard et al. 1988; Staal and Sonsalla 2000) as well as in heterozygous VMAT2-knockout mice (Takahashi et al. 1997) due to the

involvement of VMAT2 in the clearance of MPP^+ from dopaminergic neurons and the resultant accumulation of MPP^+ inside the cell (Liu and Edwards 1997). Additionally, the inhibition of SERT or NET by the administration of their inhibitors selectively attenuates MPTP-induced depletion of serotonin and norepinephrine, respectively (Andrews and Murphy 1993). MPTP-induced neurotoxicity is also reduced in NET-knockout mice (Rommelfanger et al. 2004). However, the functions of SERT and NET in MPTP-induced neurotoxicity still remain unknown.

A study is performed to elucidate the role of DAT and other monoamine transporters including VMAT2, SERT, and NET on the SVZ neuroblast apoptosis induced by MPTP administration. The results of the study indicated that there are no DAT-positive neuroblasts in the SVZ (Fig. 4), whereas some neuroblasts were immunopositive for VMAT2 and SERT (Shibui et al. 2009). TUNEL-positive cells were semiquantitatively analyzed after the injection of GBR12909, a DAT inhibitor; tetrabenazine, a VMAT2 inhibitor; fluoxetine, a SERT inhibitor; or desipramine, a NET inhibitor, prior to MPTP injection. However, the injection of the transporter inhibitors had no influence on the MPTP-induced neuroblast apoptosis in the SVZ (Shibui et al. 2009). This finding suggests that the neurotoxicity of MPTP to neuroblasts in the SVZ does not require DAT or other monoamine transporters, and the MPTP-induced apoptosis may be executed through other unknown pathways.

4 Neurogenesis After MPTP-Induced Injury

Neurogenesis is a key event during both physiological and pathological processes, and is regulated by a variety of stimuli, such as hormones, intrinsic growth factors, neurotransmitters, exogenously applied agents, environmental enrichment, exercise, and age.

Dopamine has been shown to regulate cell cycles in the developing brain (Spencer et al. 1998). The activation of dopamine receptors influences cell proliferation in the lateral ganglionic eminence and the neuroepithelium of the frontal cortex in embryonic mice (Ohtani et al. 2003; Popolo et al. 2004). By using immunohistochemical and ultrastructural analyses, it was demonstrated that dopamine 2 like (D2L) receptors are expressed predominately on C cells in the SVZ, whereas A cells express both D1L and D2L receptors in the mouse brain (Hoglinger et al. 2004). The dopaminergic fiber is also confirmed to contact precursor cells in the SVZ of adult human and primates (Hoglinger et al. 2004; Freundlieb et al. 2006). Thus, dopamine seems to play a key role in the regulation of adult mammalian neurogenesis.

Zhao et al. (2003) first provided morphological evidences that the administration with MPTP leads to a twofold increase of bromodeoxyuridine (BrdU) incorporation in nigral dopaminergic neurons 3 weeks after the lesion in mice, which was not detectable in the granular cell layer of DG. The newly generated dopaminergic neurons are demonstrated to be derived from the cells lining the ventricular system, namely, SVZ cells. By using the nestin second intron enhancer-controlled LacZ reporter transgenic mouse model coupled with the MPTP lesion system, Shan et al.

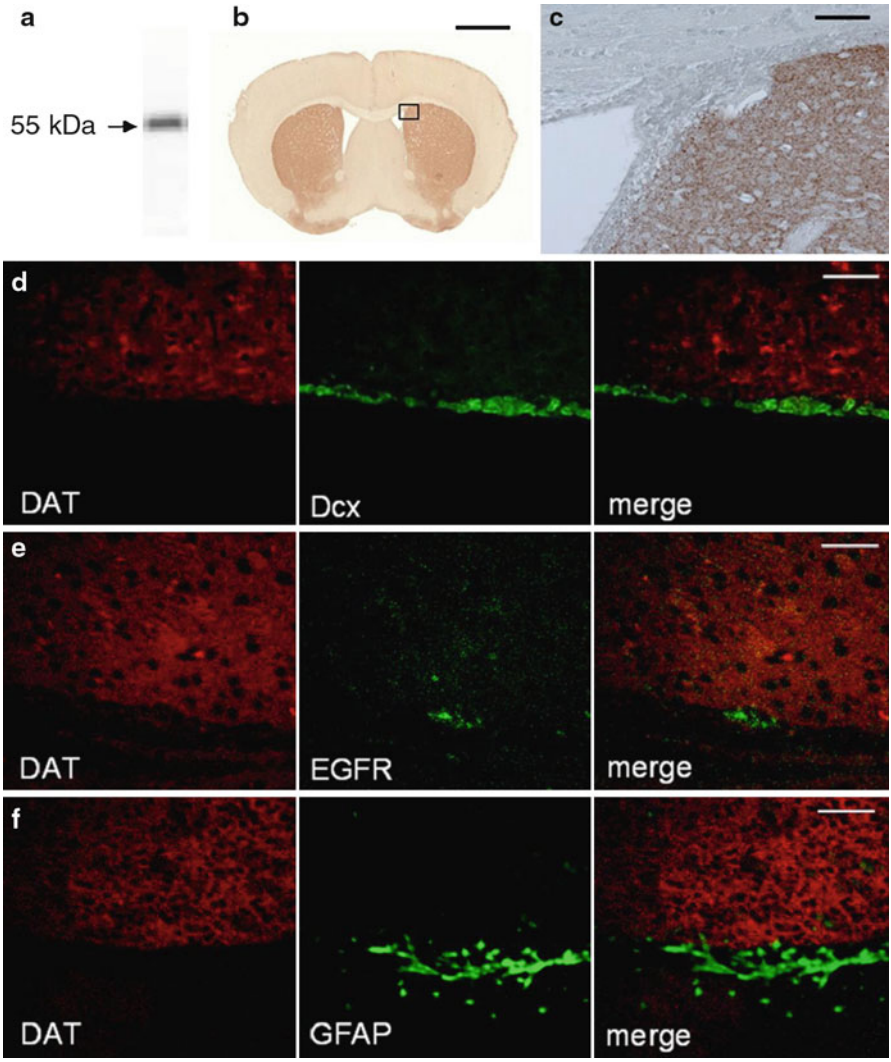

Fig. 4 (**a**) Western blot analysis demonstrates the expression of a 55 kDa DAT protein in the mouse cerebrum. (**b**) A representative coronal section through the striatum and SVZ were immunostained for DAT and showed the expression of DAT in the striatum, but not in the SVZ. (**c**) Higher magnification of the *boxed area* in (**b**). (**d–f**) The sections were also double labeled for DAT and either Dcx, EGFR, or GFAP. GFAP-positive neural stem cells, EGFR-positive neural precursor cells, or Dcx-positive neuroblasts (*green*) do not express DAT (*red*). Scale bars in (**b**) 1.5 mm, (**c**) 30 μm, and (**d–f**) 15 μm (Shibui et al. 2009)

also demonstrated that there are increased dopaminergic neurogenesis in the SN (Shan et al. 2006), supporting the findings by Zhao et al. Increased neurogenesis after the MPTP injury was also confirmed in a very recent article (Peng et al. 2008), in which MPTP lesions in the mouse brain increased the incorporation of BrdU as

well as the number of cells that co-expressed BrdU and the immature neuronal marker, doublecortin (DCX), in the DG, SVZ, and the striatum, but not in the SN, although no differentiation of newly generated cells into dopaminergic neurons was investigated. Thus, neurogenesis in the nigrostriatal system in response to MPTP damage, if it does occur, may be a later event in the self-repairing process of injured SVZ.

5 Strain Differences in the Susceptibility of MPTP

There are species differences in the susceptibility to MPTP injection; for example, human and monkeys are susceptible, whereas rats and hamsters are relatively resistant (Burns et al. 1983; Chiueh et al. 1984; Langston et al. 1984; Mitra et al. 1994). Mice are middle susceptible, but show marked strain differences (Sundstrom et al. 1987; Riachi and Harik 1988; Hamre et al. 1999); C57BL/6 mice are susceptible, while BALB/c mice are resistant, to MPTP neurotoxicity (Sedelis et al. 2000; Yasuda et al. 2008; Filipov et al. 2009). MPTP induces severe degeneration of dopaminergic neurons when administrated to C57BL/6 mice, but such lesions are not observed in BALB/c mice. However, the reason for such strain differences remains unclear.

To clarify which factor(s) cause such strain differences, MPTP or MPP^+ was intracerebroventricularly (icv) injected into adult C57BL/6 (highly susceptible) and BALB/c (resistant) mice (Ito et al. 2013a). Both C57BL/6 and BALB/c mice injected with MPP^+ showed a significant decrease in tyrosine hydroxylase (TH)-immunopositive areas in the striatum and TH-positive cells in the SNpc a few days after injection, compared to saline-injected control mice, and the decrease rates in the BALB/c mice were lower than that in C57BL/6 mice. MPTP-injected C57BL/6 mice, however, showed no lesions in the striatum and SNpc (Fig. 5). These findings indicate that factors other than MAO-B can influence the strain susceptibility between C57BL/6 and BALB/c mice after the conversion from MPTP to MPP^+.

Intact BALB/c mice showed higher DAT protein expression in the striatum than intact C57BL/6 mice (Ito et al. 2013b). MPTP-treated BALB/c mice showed a more significant increase of MAO-B expression than MPTP-treated C57BL/6 mice (Ito et al. 2013b). The increase of neural nitric oxide synthase (nNOS) and inducible NOS (iNOS) protein expressions in MPTP-treated BALB/c mice was more pronounced in the striatum and SNpc than in MPTP-treated C57BL/6 mice (Ito et al. 2013b).

These results indicate that MAO-B, DAT, nNOS, and iNOS expression levels do not influence the different strain susceptibility to MPTP.

6 Aging and MPTP Neurotoxicity

While there have been many researches on the neurotoxicity of MPTP in adult mice including our study (He et al. 2004), there have been only a few in fetal and

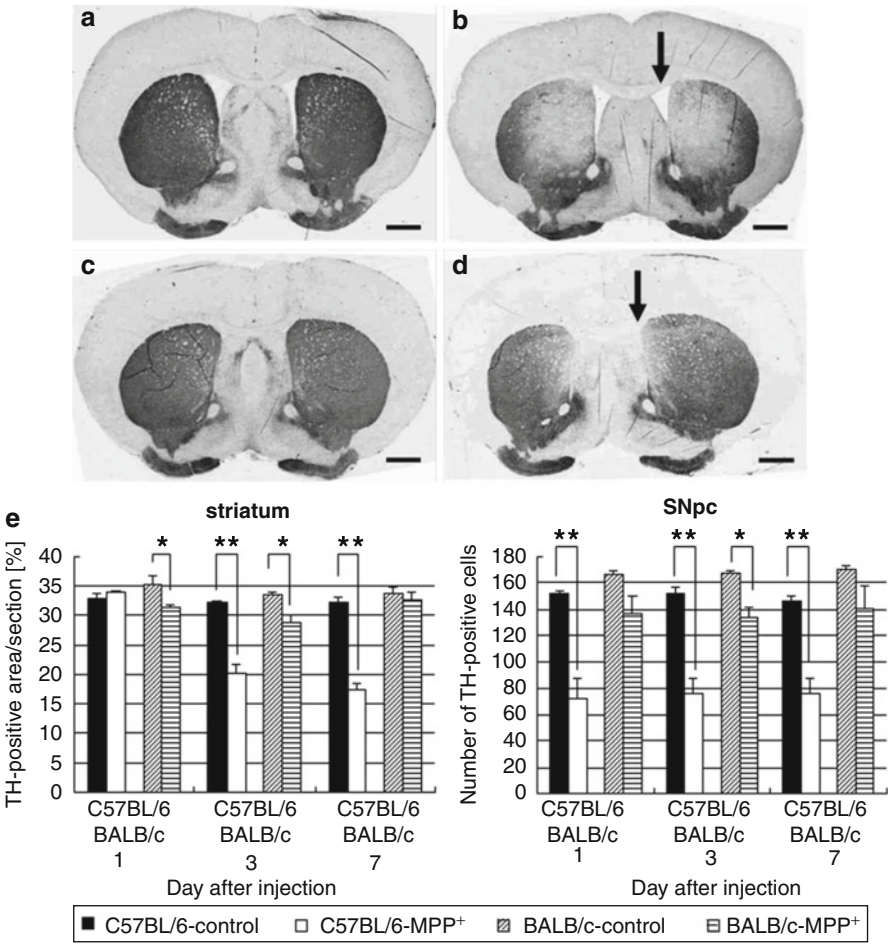

Fig. 5 TH immunoreactivity in the striatum at day 7 after injection. In mice injected icv with MPP^+, a reduction in TH-positive areas was observed at the injection (*arrows*) and contralateral sides of the striatum, especially adjacent to the lateral ventricles in C57BL/6 (**b**) and BALB/c (**d**) mice. However, no lesions were found in control C57BL/6 (**a**) and BALB/c (**c**) mice. Bar = 1 mm. (**e**) Strain difference in susceptibility to MPP^+ of C57BL/6 and BALB/c mice. Significant decreases in TH-positive areas in the striatum and TH-positive cells in the SNpc were documented in C57BL/6 mice at days 3 and 7, and at days 1, 3, and 7, respectively. In BALB/c mice, significant decreases in TH-positive areas and TH-positive cells were observed at days 1 and 3, and day 3 after injection, respectively. The decrease rates of BALB/c mice were smaller compared to those in C57BL/6 mice. Data are expressed as the mean ± standard error ($n = 3$–5). $^*p < 0.05$ and $^{**}p < 0.01$ (Ito et al. 2013a)

newborn mice. Chronic MPTP exposure during the organogenesis period (days 6–15 of gestation) reduced the fetal weight, motor activity, number of striatal TH-positive cells, and amounts of DA and homovanillic acid (Wolfgang and Beat 1991). Such toxicity was enhanced when MPTP was administered postnatally

(Muthian et al. 2010). By contrast, fetal mice treated once with MPTP at gestation day 17 showed unchanged DA concentrations in the brain 24 h after injection and 24 h after birth, and in the striatum 14 and 28 days after birth compared to control mice (Melamed et al. 1990). Recently, we have demonstrated that MPTP and MPP^+ cause a loss of TH-positive cells and fibers and an increase of apoptotic cells in the SVZ of embryonic mice when injected into dams, and of newborn mice when injected ip (Sai et al. 2012). The results indicate that MPTP and MPP^+ pass through the placenta and embryonic or newborn BBB, and finally reach the brain. Further, the results of immunohistochemistry and double-labeling immunofluorescent stainings demonstrated an increase of MAO-B-positive glial cells in the brain in MPTP-treated mice, indicating the involvement of MAO-B in the acute neurotoxicity of MPTP also in both embryonic and newborn mice. The expression of DAT was not observed in the nigrostriatal area of embryonic mice and in the area and SVZ of newborn mice.

7 Conclusion

The administration of MPTP to susceptible animals such as C57BL/6 mice induces SVZ neuroblastic A cell apoptosis as well as SNpc and striatal dopaminergic cell injury. The conversion from MPTP to its active form, MPP^+, is conducted mainly in MAO-B-positive ependymal cells and GFAP-positive cells in the SVZ. MPTP-induced neuroblast apoptosis in the SVZ is not prevented by the inhibition of monoamine transporters such as DAT, VMAT2, SERT, or NET, indicating that these transporters are not necessarily required to exert the neurotoxicity.

Recent results (Ito et al. 2012b) indicate that the nNOS or iNOS expression level is not involved in the different susceptibility to MPTP between susceptible C57BL/6 and resistant BALB/c, though there are a few reports (Chalimoniuk et al. 2006; Joniec et al. 2009) describing the significant increase of nNOS and iNOS expressions in the striatum and SNpc in C57BL/6 mice after MPTP administration. Factors influencing the strain difference in the susceptibility to MPTP toxicity are still unknown at present.

Both MPTP and MPP^+ can pass through the placenta and BBB and cause acute toxicity to the SN, striatum, and SVZ in the brain of embryonic and newborn mice as well as adult mice. The involvement of MAO-B in the acute neurotoxicity is confirmed regardless of the age of animals.

The replacement of lost neurons in a damaged brain area is the best way of therapeutic development for neurodegenerative diseases. Neurogenesis is, however, still a most controversial issue in studies of neurodegenerative diseases and their animal models. As mentioned, MPTP destroys neuroblastic A cells in the SVZ in the adult brain, followed by a transiently impaired neurogenesis. Though it still remains to be investigated whether and how multiple potential neural stem cells migrate into the nigrostriatal system and replace the lost dopaminergic neurons, delayed regeneration of dopaminergic neurons due to the loss of SVZ A cells may be one of possible causes of the pathological condition. With

increased attention to dopaminergic neurogenesis in MPTP-induced animal models for PD, it is expected that such animals become useful as a tool for establishing the contact to PD on neurogenesis.

References

Altman, J. (1963). Autoradiographic investigation of cell proliferation in the brains of rats and cats. *Anatomical Record, 145*, 573–591.

Altman, J., & Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *The Journal of Comparative Neurology, 124*, 319–335.

Andrews, A. M., & Murphy, D. L. (1993). Fluoxetine and desipramine selectively attenuate 2′-NH_2-MPTP-induced depletions in serotonin and norepinephrine. *European Journal of Pharmacology, 250*, 215–221.

Bernier, P. J., Vinet, J., Cossette, M., & Parent, A. (2000). Characterization of the subventricular zone of the adult human brain: Evidence for the involvement of Bcl-2. *Neurosciences Research, 37*, 67–78.

Bezard, E., Gross, C. E., Fournier, M. C., Dovero, S., Bloch, B., & Jaber, M. (1999). Absence of MPTP-induced neuronal death in mice lacking the dopamine transporter. *Experimental Neurology, 155*, 268–273.

Biebl, M., Cooper, C. M., Winkler, J., & Kuhn, H. G. (2000). Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. *Neuroscience Letters, 291*, 17–20.

Blum, D., Torch, S., Lambeng, N., et al. (2001). Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: Contribution to the apoptotic theory in Parkinson's disease. *Progress in Neurobiology, 65*, 135–172.

Blume, E. (1983). Street drugs yield primate Parkinson's model. *The Journal of the American Medical Association, 250*, 13–14.

Borta, A., & Hoglinger, G. U. (2007). Dopamine and adult neurogenesis. *Journal of Neurochemistry, 100*, 587–595.

Bovetti, S., Bovolin, P., Perroteau, I., & Puche, A. C. (2007). Subventricular zone-derived neuroblast migration to the olfactory bulb is modulated by matrix remodelling. *European Journal of Neuroscience, 25*, 2021–2033.

Brunjes, P. C., & Armstrong, A. M. (1996). Apoptosis in the rostral migratory stream of the developing rat. *Brain Research. Developmental Brain Research, 92*, 219–222.

Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M., & Kopin, I. J. (1983). A primate model of Parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proceedings of the National Academy of Sciences of the United States of America, 80*, 4546–4550.

Cameron, H. A., Woolley, C. S., McEwen, B. S., & Gould, E. (1993). Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience, 56*, 337–344.

Chalimoniuk, M., Lukacova, N., Marsala, J., & Langfort, J. (2006). Alterations of the expression and activity of midbrain nitric oxide synthase and soluble guanylyl cyclase in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism in mice. *Neuroscience, 141*, 1033–1046.

Chiba, K., Trevor, A. J., & Castagnoli, N., Jr. (1985). Active uptake of MPP^+, a metabolite of MPTP, by brain synaptosomes. *Biochemical and Biophysical Research Communications, 128*, 1228–1232.

Chiueh, C. C., Markey, S. P., Burns, R. S., Johannessen, J. N., Pert, A., & Kopin, I. J. (1984). Neurochemical and behavioral effects of systemic and intranigral administration of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the rat. *European Journal of Pharmacology, 100*, 189–194.

Desole, M. S., Sciola, L., Delogu, M. R., Sircana, S., Migheli, R., & Miele, E. (1997). Role of oxidative stress in the manganese and 1-methyl-4-(2-ethylphenyl)- 1,2,3,6-tetrahydropyridine-induced apoptosis in PC12 cells. *Neurochemistry International, 31*, 169–176.

Dipasquale, B., Mariani, A. M., & Youle, R. J. (1991). Apoptosis and DNA degradation induced by 1-methyl-4-phenylpyridinium in neurons. *Biochemical and Biophysical Research Communications, 181*, 1442–1448.

Doetsch, F., & Alvarez-Buylla, A. (1996). Network of tangential pathways for neuronal migration in adult mammalian brain. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 14895–14900.

Doetsch, F., Garcia-Verdugo, J. M., & Alvarez-Buylla, A. (1997). Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *Journal of Neuroscience, 17*, 5046–5061.

Doetsch, F., Caille, I., Lim, D. A., Garcia-Verdugo, J. M., & Alvarez-Buylla, A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell, 97*, 703–716.

Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J. M., & Alvarez-Buylla, A. (2002). EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron, 36*, 1021–1034.

Du, Y., Dodel, R. C., Bales, K. R., Jemmerson, R., Hamilton-Byrd, E., & Paul, S. M. (1997). Involvement of a caspase-3-like cysteine protease in 1-methyl-4-phenylpyridinium-mediated apoptosis of cultured cerebellar granule neurons. *Journal of Neurochemistry, 69*, 1382–1388.

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine, 4*, 1313–1317.

Filipov, N. M., Norwood, A. B., & Sistrunk, S. C. (2009). Strain-specific sensitivity to MPTP of C57BL/6 and BALB/c mice is age dependent. *Neuroreport, 20*, 713–717.

Freundlieb, N., Francois, C., Tande, D., Oertel, W. H., Hirsch, E. C., & Hoglinger, G. U. (2006). Dopaminergic substantia nigra neurons project topographically organized to the subventricular zone and stimulate precursor cell proliferation in aged primates. *Journal of Neuroscience, 26*, 2321–2325.

Gainetdinov, R. R., Fumagalli, F., Jones, S. R., & Caron, M. G. (1997). Dopamine transporter is required for in vivo MPTP neurotoxicity: Evidence from mice lacking the transporter. *Journal of Neurochemistry, 69*, 1322–1325.

Garcia-Verdugo, J. M., Doetsch, F., Wichterle, H., Lim, D. A., & Alvarez-Buylla, A. (1998). Architecture and cell types of the adult subventricular zone: In search of the stem cells. *Journal of Neurobiology, 36*, 234–248.

Gerlach, M., & Riederer, P. (1996). Animal models of Parkinson's disease: An empirical comparison with the phenomenology of the disease in man. *Journal of Neural Transmission, 103*, 987–1041.

German, D. C., Liang, C. L., Manaye, K. F., Lane, K., & Sonsalla, P. K. (2000). Pharmacological inactivation of the vesicular monoamine transporter can enhance 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine-induced neurodegeneration of midbrain dopaminergic neurons, but not locus coeruleus noradrenergic neurons. *Neuroscience, 101*, 1063–1069.

Gould, E., Tanapat, P., McEwen, B. S., Flugge, G., & Fuchs, E. (1998). Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 3168–3171.

Gould, E., Reeves, A. J., Fallah, M., Tanapat, P., Gross, C. G., & Fuchs, E. (1999a). Hippocampal neurogenesis in adult old world primates. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 5263–5267.

Gould, E., Reeves, A. J., Graziano, M. S., & Gross, C. G. (1999b). Neurogenesis in the neocortex of adult primates. *Science, 286*, 548–552.

Hamre, K., Tharp, R., Poon, K., Xiong, X., & Smeyne, R. J. (1999). Differential strain susceptibility following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration acts in

an autosomal dominant fashion: Quantitative analysis in seven strains of *Mus musculus*. *Brain Research, 828*, 91–103.

Hartley, A., Stone, J. M., Heron, C., Cooper, J., & Schapira, A. H. V. (1994). Complex I inhibitors induce dose-dependent apoptosis in PC12 cells: Relevance to Parkinson's disease. *Journal of Neurochemistry, 63*, 1987–1990.

He, X. J., & Nakayama, H. (2009). Neurogenesis in neurotoxin-induced animal models for Parkinson's disease – a review of the current status. *Journal of Toxicologic Pathology, 22*, 101–108.

He, X. J., Nakayama, H., Ueno, M., & Doi, K. (2004). Age-dependent susceptibility to MPTP neurotoxicity in C57BL mice: A tyrosine hydroxylase-immunohistochemical evaluation. *Journal of Toxicologic Pathology, 17*, 239–244.

He, X. J., Nakayama, H., Dong, M., Yamauchi, H., Ueno, M., Uetsuka, K., & Doi, K. (2006). Evidence of apoptosis in the subventricular zone and rostral migratory stream in the MPTP mouse model of Parkinson disease. *Journal of Neuropathology and Experimental Neurology, 65*, 873–882.

He, X. J., Yamauchi, H., Uetsuka, K., & Nakayama, H. (2008a). Neurotoxicity of MPTP to migrating neuroblasts: Studies in acute and subacute mouse models of Parkinson's disease. *Neurotoxicology, 29*, 413–420.

He, X. J., Uetsuka, K., & Nakayama, H. (2008b). Neural progenitor cells are protected against MPTP by MAO-B inhibitors. *Neurotoxicology, 29*, 1141–1146.

Heikkila, R. E., Cabbat, F. S., Manzino, L., & Duvoisin, R. C. (1984). Effects of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine on neostriatal dopamine in mice. *Neuropharmacology, 23*, 711–713.

Hoglinger, G. U., Rizk, P., Muriel, M. P., Duyckaerts, C., Oertel, W. H., Caille, I., & Hirsch, E. C. (2004). Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nature Neuroscience, 7*, 726–735.

Itano, Y., & Nomura, Y. (1995). 1-methyl-4-phenylpyridinium ion (MPP^+) causes DNA fragmentation and increases the Bcl-2 expression in human neuroblastoma, SH-SY5Y cells through different mechanism. *Brain Research, 704*, 240–245.

Ito, T., Suzuki, K., Uchida, K., & Nakayama, H. (2013a). Different susceptibility to 1-methyl-4-phenylpyridium (MPP^+)-induced nigro-striatal dopaminergic cell loss between C57BL/6 and BALB/c mice is not related to the difference of monoamine oxidase-B (MAO-B). *Experimental and Toxicologic Pathology, 65*, 153–158.

Ito, T., Uchida, K., & Nakayama, H. (2013b). Neuronal or inducible nitric oxide synthase (NOS) expression level is not involved in the different susceptibility to nigro-striatal dopaminergic neurotoxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) between C57BL/6 and BALB/c mice. *Experimental and Toxicologic Pathology, 65*, 121–125.

Javitch, J. A., D′Amato, R. J., Strittmatter, S. M., & Snyder, S. H. (1985). Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proceedings of the National Academy of Sciences of the United States of America, 82*, 2173–2177.

Joniec, I., Ciesielska, A., Kurkowska-Jastrzebska, I., Przybylkowski, A., Czlonkowska, A., & Czlonkowski, A. (2009). Age- and sex-differences in the nitric oxide synthase expression and dopamine concentration in the murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Brain Research, 1261*, 7–19.

Kaplan, M. S., & Hinds, J. W. (1977). Neurogenesis in the adult rat: Electron microscopic analysis of light radioautographs. *Science, 197*, 1092–1094.

Kempermann, G., Jessberger, S., Steiner, B., & Kronenberg, G. (2004). Milestones of neuronal development in the adult hippocampus. *Trends in Neurosciences, 27*, 447–452.

Kuhn, H. G., Dickinson-Anson, H., & Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation. *Journal of Neuroscience, 16*, 2027–2033.

Langston, J. W., Ballard, P., Tetrud, J. W., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science, 219*, 979–980.

Langston, J. W., Forno, L. S., Rebert, C. S., & Irwin, I. (1984). Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. *Brain Research, 292*, 390–394.

Levison, S. W., Rothstein, R. P., Brazel, C. Y., Young, G. M., & Albrecht, P. J. (2000). Selective apoptosis within the rat subependymal zone: A plausible mechanism for determining which lineages develop from neural stem cells. *Developmental Neuroscience, 22*, 106–115.

Liu, Y., & Edwards, R. H. (1997). The role of vesicular transport proteins in synaptic transmission and neural degeneration. *Annual Review of Neuroscience, 20*, 125–156.

Lois, C., & Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science, 264*, 1145–1148.

Melamed, E., Rosenthal, J., & Youdim, M. B. (1990). Immunity of fetal mice to prenatal administration of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine. *Journal of Neurochemistry, 55*, 1427–1431.

Mitra, N., Mohanakumar, K. P., & Ganguly, D. K. (1994). Resistance of golden hamster to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Relationship with low levels of regional monoamine oxidase B. *Journal of Neurochemistry, 62*, 1906–1912.

Mochizuki, H., Nakamura, N., Nishi, K., & Mizuno, Y. (1994). Apoptosis is induced by 1-methyl-4-phenylpyridinium ion (MPP^+) in ventral mesencephalic-striatal co-culture in rat. *Neuroscience Letters, 170*, 191–194.

Muthian, G., Mackey, V., King, J., & Charlton, C. G. (2010). Modeling a sensitization stage and a precipitation stage for Parkinson's disease using prenatal and postnatal 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine administration. *Neuroscience, 169*, 1085–1093.

Ng, K. L., Li, J. D., Cheng, M. Y., Leslie, F. M., Lee, A. G., & Zhou, Q. Y. (2005). Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. *Science, 308*, 1923–1927.

Ohtani, N., Goto, T., Waeber, C., & Bhide, P. G. (2003). Dopamine modulates cell cycle in the lateral ganglionic eminence. *Journal of Neuroscience, 23*, 2840–2850.

Parkinson, J. (1817). An essay on the shaking palsy. *The Journal of Neuropsychiatry and Clinical Neurosciences, 14*, 223–236.

Pencea, V., Bingaman, K. D., Freedman, L. J., & Luskin, M. B. (2001). Neurogenesis in the subventricular zone and rostral migratory stream of the neonatal and adult primate forebrain. *Experimental Neurology, 172*, 1–16.

Peng, J., Xie, L., Jin, K., Greenberg, D. A., & Andersen, J. K. (2008). Fibroblast growth factor 2 enhances striatal and nigral neurogenesis in the acute 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine model of Parkinson's disease. *Neuroscience, 153*, 664–670.

Perese, D. A., Ulman, J., Viola, J., Ewing, S. E., & Bankiewicz, K. S. (1989). A 6-hydroxydopamine-induced selective Parkinsonian rat model. *Brain Research, 494*, 285–293.

Pifl, C., Giros, B., & Caron, M. G. (1993). Dopamine transporter expression confers cytotoxicity to low doses of the Parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium. *Journal of Neuroscience, 13*, 4246–4253.

Popolo, M., McCarthy, D. M., & Bhide, P. G. (2004). Influence of dopamine on precursor cell proliferation and differentiation in the embryonic mouse telencephalon. *Developmental Neuroscience, 26*, 229–244.

Privat, A., & Leblond, C. P. (1972). The subependymal layer and neighboring region in the brain of the young rat. *The Journal of Comparative Neurology, 146*, 277–302.

Przedborski, S., & Ischiropoulos, H. (2005). Reactive oxygen and nitrogen species: Weapons of neuronal destruction in models of Parkinson's disease. *Antioxidants & Redox Signaling, 7*, 685–693.

Przedborski, S., & Vila, M. (2001). MPTP: A review of its mechanism of neurotoxicity. *Clinical Neuroscience Research, 1*, 407–418.

Przedborski, S., & Vila, M. (2003). The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model: A tool to explore the pathogenesis of Parkinson's disease. *Annals of the New York Academy of Sciences, 991*, 189–198.

Przedborski, S., Jackson-Lewis, V., Naini, A. B., Jakowec, M., Petzinger, G., Miller, R., & Akram, M. (2001). The Parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): A technical review of its utility and safety. *Journal of Neurochemistry, 76*, 1265–1274.

Przedbroski, S., Leviver, M., Jiang, H., Ferreira, M., Jackson-Lewis, V., Donaldson, D., & Togasaki, D. M. (1995). Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by instrastriatal injection of 6-hydroxydopamine. *Neuroscience, 67*, 631–647.

Quinones-Hinojosa, A., Sanai, N., Soriano-Navarro, M., Gonzalez-Perez, O., Mirzadeh, Z., Gil-Perotin, S., Romero-Rodriguez, R., Berger, M. S., Garcia-Verdugo, J. M., & Alvarez-Buylla, A. (2006). Cellular composition and cytoarchitecture of the adult human subventricular zone: A niche of neural stem cells. *The Journal of Comparative Neurology, 494*, 415–434.

Reinhard, J. F., Jr., Daniels, A. J., & Viveros, O. H. (1988). Potentiation by reserpine and tetrabenazine of brain catecholamine depletion by MPTP (1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine) in the mouse; evidence for subcellular sequestration as basis for cellular resistance to the toxicant. *Neuroscience Letters, 90*, 349–353.

Riachi, N. J., & Harik, S. I. (1988). Strain differences in systemic 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine neurotoxicity in mice correlate best with monoamine oxidase activity at the blood–brain barrier. *Life Sciences, 42*, 2359–2363.

Rommelfanger, K. S., Weinshenker, D., & Miller, G. W. (2004). Reduced MPTP toxicity in noradrenaline transporter knockout mice. *Journal of Neurochemistry, 91*, 1116–1124.

Sai, T., Uchida, K., & Nakayama, H. (2013). Acute toxicity of MPTP and MPP^+ in the brain of embryo and newborn mice. *Experimental and Toxicologic Pathology, 65*, 113–119.

Sedelis, M., Hofele, K., Auburger, G. W., Morgan, S., Huston, J. P., & Schwarting, R. K. (2000). MPTP susceptibility in the mouse: Behavioral, neurochemical, and histological analysis of gender and strain differences. *Behavior Genetics, 30*, 171–182.

Seri, B., Garcia-Verdugo, J. M., McEwen, B. S., & Alvarez-Buylla, A. (2001). Astrocytes give rise to new neurons in the adult mammalian hippocampus. *Journal of Neuroscience, 21*, 7153–7160.

Serra, P. A., Sciola, L., Delogu, M. R., Spano, A., Monaco, G., Miele, E., Rocchitta, G., Miele, M., Migheli, R., & Desole, M. S. (2002). The neurotoxin 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine induces apoptosis in mouse nigrostriatal glia. Relevance to nigral neuronal death and striatal neurochemical changes. *Journal of Biological Chemistry, 277*, 34451–34461.

Shan, X., Chi, L., Bishop, M., Luo, C., Lien, L., Zhang, Z., & Liu, R. (2006). Enhanced de novo neurogenesis and dopaminergic neurogenesis in the substantia nigra of 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine-induced Parkinson's disease-like mice. *Stem Cells, 24*, 1280–1287.

Sheehan, J. P., Palmer, P. E., Helm, G. A., & Tuttle, J. B. (1997). MPP^+ induced apoptotic cell death in SH-SY5Y neuroblastoma cells: An electron microscope study. *Journal of Neuroscience Research, 48*, 226–237.

Shibui, Y., He, X. J., Uchida, K., & Nakayama, H. (2009). MPTP-induced neuroblast apoptosis in the subventricular zone is not regulated by dopamine or other monoamine transporters. *Neurotoxicology, 30*, 1036–1044.

Simola, N., Morelli, M., & Carta, A. R. (2007). The 6-hydroxydopamine model of Parkinson's disease. *Neurotoxicity Research, 11*, 151–167.

Spencer, G. E., Klumperman, J., & Syed, N. I. (1998). Neurotransmitters and neurodevelopment. Role of dopamine in neurite outgrowth, target selection and specific synapse formation. *Perspectives on Developmental Neurobiology, 5*, 451–467.

Spooren, W. P., Gentsch, C., & Wiessner, C. (1998). TUNEL-positive cells in the substantia nigra of C57BL/6 mice after a single bolus of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neuroscience, 85*, 649–651.

Staal, R. G., & Sonsalla, P. K. (2000). Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata. *Journal of Pharmacology and Experimental Therapeutics, 293*, 336–342.

Sundstrom, E., Stromberg, I., Tsutsumi, T., Olson, L., & Jonsson, G. (1987). Studies on the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on central catecholamine neurons in C57BL/6 mice. Comparison with three other strains of mice. *Brain Research, 405*, 26–38.

Takahashi, N., Miner, L. L., Sora, I., Ujike, H., Revay, R. S., Kostic, V., Jackson-Lewis, V., Przedborski, S., & Uhl, G. R. (1997). VMAT2 knockout mice: Heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 9938–9943.

Tatton, N. A., & Kish, S. J. (1997). In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labelling and acridine orange staining. *Neuroscience, 77*, 1037–1048.

Vila, M., & Przedborski, S. (2003). Targeting programmed cell death neurodegenerative diseases. *Nature Reviews Neuroscience, 4*, 365–375.

Vila, M., Jackson-Lewis, V., Vukosavic, S., et al. (2001). Bax ablation prevents dopaminergic neurodegeneration in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 2837–2842.

Wolfgang, S., & Beat, U. (1991). Placental toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice. *Toxicology, 67*, 63–74.

Yasuda, Y., Shimoda, T., Uno, K., Tateishi, N., Furuya, S., Yagi, K., Suzuki, K., & Fujita, S. (2008). The effects of MPTP on the activation of microglia/astrocytes and cytokine/chemokine levels in different mice strains. *Journal of Neuroimmunology, 204*, 43–51.

Zhao, M., Momma, S., Delfani, K., Carlen, M., Cassidy, R. M., Johansson, C. B., Brismar, H., Shupliakov, O., Frisen, J., & Janson, A. M. (2003). Evidence for neurogenesis in the adult mammalian substantia nigra. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 7925–7930.

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell, 132*, 645–660.

N-Methyl-(R) Salsolinol and the Enzymes Catalyzing its Synthesis and Metabolism in Parkinson's Disease

Makoto Naoi and Wakako Maruyama

Contents

Abstract

Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is an endogenous catechol isoquinoline detected in the human brain. It is produced by the

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nonenzymatic Pictet-Spengler reaction as the racemic mixture. However, a novel enzyme, (*R*)salsolinol synthase, was isolated from the human brain, which catalyzes the enantio-specific synthesis of (*R*)salsolinol from dopamine and acetaldehyde or keto acids. *R*-Salsolinol is *N*-methylated by (*R*)salsolinol *N*-methyltransferase (*N*-MT) into 2(*N*)-methyl-(*R*)salsolinol [*N*M-(*R*)Sal], which is oxidized by an oxidase sensitive to semicarbazide. *N*M-(*R*)Sal induces cell death in the dopaminergic cells of the rat substantia nigra after the systemic administration. The behavioral and pathological changes in rats were quite similar to those in other animal models of Parkinson's disease, using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or 6-hydroxydopamine. In cellular experiments, *N*M-(*R*)Sal binds to type A monoamine oxidase and induces apoptosis through activation of mitochondrial death signaling. Furthermore, *N*M-(*R*)Sal levels increase in the cerebrospinal fluid from parkinsonian patients, and the activity of its synthesizing neural (*R*)Sal *N*-MT is significantly higher in the lymphocytes prepared from parkinsonian patients than in control. The enantioselective neurotoxicity of *N*M-(*R*)Sal suggests that the enzymes related to the synthesis of this toxin might be associated with the etiology for PD. This chapter reviews the role of *N*-methylated isoquinolines and enzymes catalyzing *N*-methylation of small molecules in the pathogenesis of Parkinson's disease.

Keywords

Dopamine • Neurodegeneration • Parkinson's disease • Salsolinol

List of Abbreviations

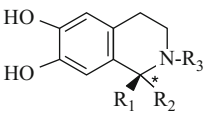
CSF	The cerebrospinal fluid
DA	Dopamine
$DMDHIQ^+$	1,2-dimethyl-6,7-dihydroxyisoquinolinium ion
IQ	Isoquinoline
IVF	Intraventricular fluid
MAO-A and MAO-B	Type A and B monoamine oxidase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
*N*M-(*R*)Sal	*N*-methyl-(*R*)salsolinol
N-MT	*N*-methyltransferase
Norsal	Norsalsolinol
PD	Parkinson's disease
ROS and RNS	Reactive oxygen and nitrogen species
Sal	Salsolinol
Sal-1-CA	Salsolinol-1-carboxylic acid
SAM	*S*-adenosyl-L-methionine
SN	Substantia nigra
TH	Tyrosine hydroxylase
TIQ	1,2,3,4-tetrahydroisoquinoline
β-CB	β-carboline

1 Introduction

The pathological hallmark of Parkinson's disease (PD) is the selective loss of dopamine (DA) neurons in the substantia nigra (SN) and the accumulation of inclusion body called Lewy body in the brain. Several genes have been identified to be responsive for the familial form of PD. However, the molecular mechanism to develop the disease remains enigmatic, and various epidemiologic conditions have been proposed as risk factors in PD. The selective cell loss of DA neurons suggests that DA itself may be associated with the pathogenesis of PD. DA is oxidized enzymatically by monoamine oxidase [MAO, EC 1.4.3.4] and produces hydrogen peroxide and also nonenzymatically oxidized into toxic semiquinone with superoxide anion. DA oxidation induces oxidative stress, dysfunction of mitochondria and ubiquitin-proteasome system, and inflammation and activates death signal pathway in DA neurons. In addition, the discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) suggested that endogenous DA-derived toxins might be involved in the neuronal loss in PD (Collins 2002).

DA-derived isoquinolines (IQs) have been proposed as endogenous neurotoxin candidates and intensively investigated in cellular and animal experiments. Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, Sal) is one of DA-derived 1,2,3,4-tetrahydroisoquinolines (TIQs) and was identified in urine from PD patients treated with L-DOPA (Sandler et al. 1973) and the human brain. Sal derivatives, β-phenylethylamine-derived TIQs, and indoleamine-derived β-carbolines (β-CBs) were found in the urine, cerebrospinal fluid (CSF), and brain (Collins 1980; Sjöquist et al. 1981). Figure 1 summarizes the chemical structure of major IQs detected in the human brain.

Racemic forms of IQs are produced by nonenzymatic condensation of monoamines with aldehydes or keto acids in the Pictet-Spengler reaction. However, the



	R_1	R_2	R_3
Salsolinol	CH_3	H	H
N-Methyl-salsolinol	CH_3	H	CH_3
Norsalsolinol	H	H	H
N-Methyl-norsalsolinol	H	H	CH_3
Salsolinol-l-carboxylic acid	CH_3	COOH	H
1,2-Dehydrosalsolinol	CH_3	H	H
6- or 7-Methyl-salsolinol	H	H	H
1,2-Dimethyl-6,7-dihydroxy-isoquinolinium ion	H	CH_3	CH_3
2-Methyl-6,7-didroxy-isoquinolinium ion	H	H	CH_3
Tetrahydropapaveroline	H	(3',4'-dihydroxy-benzyl)	H

Fig. 1 Chemical structure of Sal derivatives identified in the human brain. * Indicates asymmetric C_1 position in isoquinoline structure

predominant occurrence of the (*R*)enantiomers was confirmed in mammalian tissues (Dostert et al. 1889, 1990), suggesting the enzymatic synthesis of Sal. From the human brain, we isolated three major enzymes in Sal metabolism: (*R*)salsolinol synthase catalyzing the enantioselective synthesis *R*-Sal, a (*R*)salsolinol *N*-methyltransferase (*N*-MT) methylating *R*-Sal at 2(*N*) position into N-methyl-(R)salsolinol [*N*M-(*R*)Sal], and *N*M-(*R*)Sal oxidase producing 1,2-dimethyl-6, 7-dihydroxy-1,2,3,4-tetrahydroisoquinoline ion ($DMDHIQ^{\pm}$).

This chapter summarizes the results on the enzymatic biosynthesis and metabolism of (*R*)Sal and *N*M-(*R*)Sal, the occurrence of Sal derivatives in the human brain, the preparation of in vivo model of PD in rat, and the molecular mechanism behind the programmed cell death induced by *N*M-(*R*)Sal. Finally the analyses of clinical samples from PD patients are presented to show the possible association of *N*M-(*R*)Sal with the pathogenesis of PD. The role of *N*M-(*R*)Sal and a neutral (*R*)Sal *N*-MT and other *N*-MTs in the selective degeneration of DA neurons in PD is discussed.

2 Biosynthesis of (*R*)salsolinol Derivatives in Human Brain

2.1 (*R*)Salsolinol Synthase Catalyzing the Enantio-Specific Synthesis of (*R*)salsolinol

In foods and beverages, Sal occurs as racemic mixture produced by the nonenzymatic condensation in the Pictet-Spengler reaction. However, Sal has an asymmetric center at 1 position, and (*R*)-enantiomer was dominant in urine from healthy volunteers, suggesting the stereoselective biosynthesis (Dostert et al. 1989). Dostert et al. (1990) proposed the biosynthesis pathway of (*R*)Sal in human, as shown in Fig. 2. The condensation of DA with pyruvic acid yields salsolinol-1-carboxylic acid (Sal-1-CA), which was detected in the human brain caudate nucleus, the CSF, and urine, but the enantiomeric structure was not determined (Ung-Chhun et al. 1985). The nonenzymatic decarboxylation of Sal-1-CA produces 1,2-dehydrosalsolinol, which was also identified in human urine (Dostert et al. 1990). The presence of an enzyme catalyzing the oxidative decarboxylation of Sal-1-CA into 1,2-dehydrosalinol was reported in rat kidney (Collins and Cheng 1988), but the enzyme has neither been characterized nor detected in the human brain. The enantioselective reduction of 1,2-dehydrosalsolinol or the direct decarboxylation of Sal-1-CA into (*R*)Sal has never been confirmed in animals or plants.

The enzyme participating in the enantio-specific biosynthesis of (*R*)Sal was isolated from the human brain. (*R*)Sal synthase was purified from the cytosolic fraction prepared from human gray matter as a monomer with molecular mass of 34.3 kDa. Table 1 summarizes the basic characteristics of this enzyme. (*R*)Sal synthase catalyzes the enantioselective synthesis of (*R*)Sal and (*R*)Sal-1-CA from DA with acetaldehyde or pyruvic acid (Naoi et al. 1996b). This enzyme synthesizes also norsalsolinol (6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, Norsal) from DA and formaldehyde, but adrenalin and noradrenalin are not the substrate.

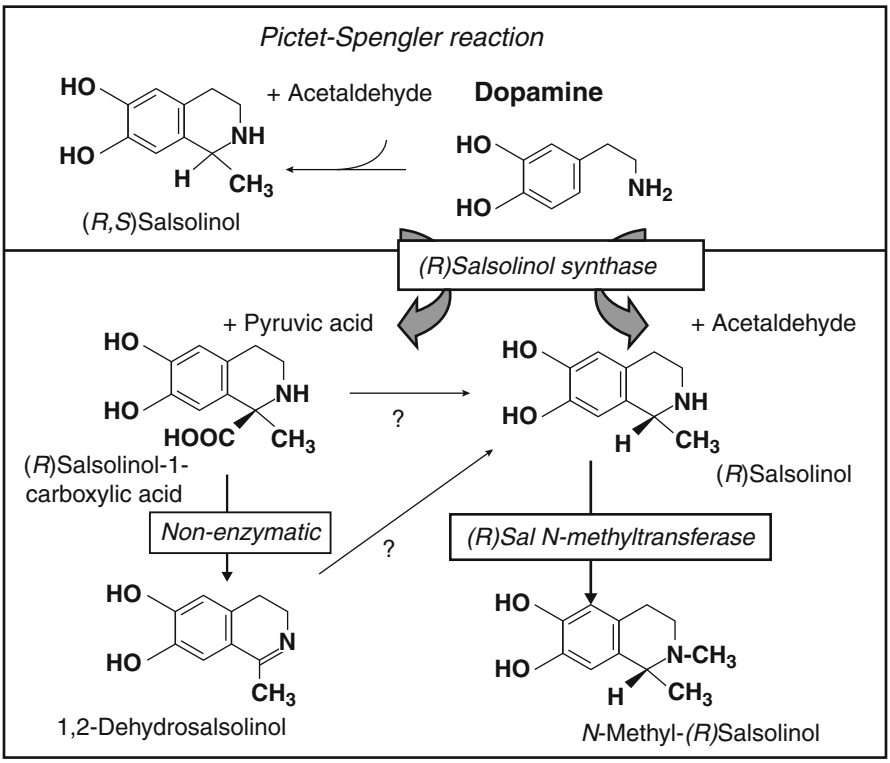


Fig. 2 Nonenzymatic and enzymatic synthesis of (*R*)-enantiomer of Sal derivatives in the human brain. Dopamine is condensed with acetaldehyde into (*R*/*S*)Sal by the Pictet-Spengler reaction. (*R*) Salsolinol synthase condenses DA with acetaldehyde or pyruvic acid to yield (*R*)Sal and (*R*)Sal-1-carboxylic acid, which is nonenzymatically converted into1,2-dehydrosalsolinol. The enantioselective synthesis of (*R*)Sal from (*R*)Sal-1-carboxylic acid or 1,2-dehydrosalsolinol has not been confirmed. SAM-dependent *N*-methyltransferase catalyzes the *N*-methylation of (*R*)Sal into *N*M-(*R*)Sal. *N*M-(*R*) Sal is further oxidized into $DMDHIQ^+$ enzymatically by *N*M-(*R*)Sal oxidase or nonenzymatically

The enzymatic activity to produce Norsal is the highest, followed by that to (*R*)Sal-1-CA and (*R*)Sal. However, this synthase does not produce *N*M-(*R*)Sal directly from *N*-methyldopamine (epinine) with acetaldehyde, suggesting that *N*M-(*R*)Sal is synthesized by two-step reactions with (*R*)Sal synthase and an *N*-MT.

2.2 (*R*)Salsolinol *N*-Methyltransferase

The conversion of N-methylation of (R)Sal into 132 NM-(R)Sal was con rmed in our studies using in vivo microdialysis in rat brain, and the highest activity was in the SN (Maruyama et al. 1992). In the sample from the human brain, (*R*)Sal *N*-MT activity was detected with two peaks at pH 6–7 and 8–9, and the activity of sample prepared from the striatum was higher at pH 7.0 than at the more alkaline pH

Table 1 Characteristics of enzymes related to the metabolism of *N*-methyl-(*R*)salsolinol

	(*R*)salsolinol synthase	Neutral (*R*)salsolinol *N*-Methyltransferase
Substrate specificity	Dopamine, acetaldehyde, pyruvic acid	(*R*)salsolinol, norsalsolinol
	Not *N*-methyl-DA, adrenaline, noradrenaline	
Methyl donor		SAM
Optimal pH	7.4	7.0
Molecular weight	34.3 kDa[a]	35.6 kDA[b]
Intracellular localization	Cytoplasm	Cytoplasm
Michaelis constant (Km)	For dopamine; 5.11 ± 0.62 mM	For (*R*)Sal; 126 ± 73 μM
	For acetaldehyde; 0.59 ± 0.12 mM	For SAM; 57.6 ± 2.3 μM
Enzymatic activity	(*R*)Sal; 342.4 ± 40.5	*N*M-(*R*)Sal; 28.4 ± 13.6
(pmol/min/mg protein)	(*R*)Sal-1-carboxylic acid; 1 149 ± 95	*N*M-Norsal; 9.1 ± 3.0
	Norsal; 3 715 ± 207	

(*R*)Sal synthase and (*R*)Sal *N*-methyltransferase were isolated from the cytoplasm fraction of human brain gray matter and the striatum, respectively

[a](*R*)Sal synthase was purified by stepwise column chromatograph with twice large DEAE, once small DEAE, and finally gel-filtration column, to a single protein with 1 700-fold purification from the cytoplasm fraction (Naoi et al. 1996a, b)

[b](*R*)Sal *N*-methyltransferase was purified by stepwise column chromatograph with a DEAE, and finally gel-filtration column, to a single protein with 130-fold purification from the cytoplasm fraction

(Fig. 3a, inserted). A neutral (*R*)Sal *N*-MT with the optimum pH at 7.0 was isolated from the cytoplasm fraction of the human brain as a single protein with the molecular mass of 35.6 kDa (Fig. 3a) (Naoi et al. 1997). *S*-Adenosyl-L-methionine (SAM) was a methyl donor. This neutral *N*-MT had the strict affinity to (*R*)Sal, and (*S*)Sal was not the substrate. Previously, a *N*-MT for 1,2,3,4-TIQ was isolated from the human brain, and the reaction was optimal at pH 8.25 (Naoi et al. 1989), which might correspond to alkaline (*R*)Sal *N*-MT. There was no correlation between the activities of the neutral and alkaline *N*-MT, and the age and sex did not affect the activities in any brain regions (Maruyama et al. 1997c).

Amine *N*-MT A from bovine liver was reported to *N*-methylate stereospecifically (*R*)-norsalsoline (7-hydroxy-6-methoxy-1-methyl-1,2,3,4-TIQ) and (*R*)salsolidine (6,7-dimethoxy-1-methyl-1,2,3,4-TIQ) (Bahnmaier et al. 1999). Recombinant human amine *N*-MT [tryptamine *N*-MT, EC 2.1.1.49] concerts benzylisoquinoline, tetrahydropapaveroline (THP), 6-O-methyl-THP and norreticuline into the *N*-methylated derivatives with a strict preference for (*R*)-configuration (Grobe et al. 2011). At present, it is not confirmed whether they can *N*-methylate Sals.

2.3 *N*-Methyl-(*R*)salsolinol Oxidase and Other Enzymes Related to the Metabolism

An enzyme catalyzing the oxidation of *N*M-(*R*)Sal into $DMDHIQ^{\pm}$ was identified in the human brain (Naoi et al. 1995). This oxidase is sensitive to semicarbazide,

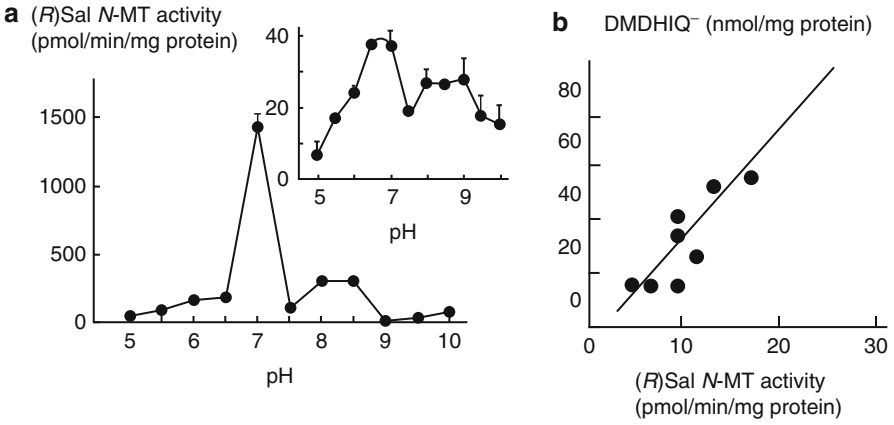


Fig. 3 The pH-dependent activity of (*R*)Sal *N*-MT and relation to Sal derivatives in the human brain. (**a**) The effect of pH on neutral (*R*)Sal *N*-MT activity purified from the human striatum. Inserted figure represents the effect of pH on the activity of (*R*)Sal *N*-MT in the cytoplasm fraction for the human striatum. (**b**) The relationship of $DHDMIQ^+$ levels in the SN with neutral (*R*)Sal *N*-MT activity in the caudate from the control human brains. Significant correlation was confirmed between the $DMDHIQ^+$ level in the substantia nigra and the activities of neutral *N*-methyltransferase in the caudate ($r = 0.905$, $p < 0.001$) and putamen ($r = 0.616$, $p < 0.05$)

but not inhibited by clorgyline and deprenyl, inhibitors of type A and B monoamine oxidase (MAO-A, MAO-B). It is localized in cytoplasm, but not in mitochondria as in the case with MAO. It catalyzes also the oxidation of *N*M-(*S*)Sal and 2(*N*)-methyl Norsal (*N*M-Norsal) with much less activity than to *N*M-(*R*)Sal. (*R*)- and (*S*)-Sal and Norsal were not the substrate of this enzyme. *N*M-(*R*)Sal is also nonenzymatically oxidized (Ung-Chhun et al. 1985; Maruyama et al. 1995).

3 The Occurrence of Salsolinol Derivatives in the Human Brain

3.1 Occurrence of (*R*)- and (*S*)-Enantiomers

The reported contents of Sal derivatives in the human brain basal nuclei were varied from 0.2 to 10 ng/g wet tissue weight, indicating the difficulty in the quantitative analyses of human materials. The occurrence of (*R*)- and (*S*)-Sal derivatives in the human brain was reported, but the results are contracting, as summarized in Table 2. We quantitatively determined (*R*)- and (*S*)-Sal derivatives in the human brain from control without the history of any neurological disorder (Maruyama et al. 1997a). (*R*)Sal was detected ubiquitously in the frontal lobe, caudate, putamen, and SN, but the (*S*)Sal was under detection limit. *N*M-(*R*)Sal content was significantly high in the SN and $DMDHIQ^+$ was detected only in the SN. Others reported similar results. Sal derivative contents were high

Table 2 Occurrence of (*R*)- and (*S*)-enantiomers of Sal derivatives in the human brain, CSF, and IVF (Reported later than 1990)

Reference	Tissue, brain regions	Isoquinolines	Enantiomer
Moser and Kämpf (1992)	CSF	Sal, *N*M-Norsal	
Moser et al. (1995)	CSF	*N*M-Norsal[a]	
Maruyama et al. (1996a)	CSF	Sal, *N*M-Sal[b]	only (*R*)-
Maruyama et al. (1999)	CSF	Sal, *N*M-Sal[a]	only (*R*)-
Maruyama et al. (1996b)	IVF	*N*M-Sal[a], (Sal), $DMDHIQ^+$	only (*R*)-
Maruyama et al. (1997c)	Frontal lobe, caudatus, putamen, substantia nigra	Sal, *N*M-Sal[a]	only (*R*)-
Müller et al. (1999)	CSF	(*R*), (*S*)-Sal[c]	(*R*) = (*S*)
Musshoff et al. (1999)	Hypothalamus, accumbens, caudatus, putamen	Sal, Norsal	Not determined
Musshoff et al. (2000)	N. accumbens, caudatus, putamen, SN, hypothalamus	Sal, Norsal	$(R) > S$ $(R/S = 2)$
DeCuypere et al. (2008a)	Putamen, substantia nigra,	Sal[a], *N*M-Sal[a], Norsal	(*R*) = (*S*)
	Cerebellar cortex, frontal lobe		$R > S$
DeCuypere et al. (2008b)	SN, striatum	*N*M-Norsal[a]	

[a]Decreased, [b]increased, or [c]not changed in PD and aging

in the striatum followed by the SN and hypothalamus (Musshoff et al. 1999; DeCuypere et al. 2008a). However, Musshoff et al. (1999) reported the presence of minor amounts of (*S*)Sal in addition to (*R*)Sal with ration of about 1–2, and DeCuypere et al. (2008a) found the same amounts of (*R*)- and (*S*)-enantiomers of Sal and *N*M-Sal in the caudate and putamen, SN, and frontal lobe. At present, the reason of the discrepancy has not been well clarified. However, it is clear that (*R*)-enantiomers of Sal and *N*M-Sal are synthesized in the brain, and the enzymatic synthesis of (*R*)Sal derivatives is predominant in the brain regions with low DA contents, suggesting that nonenzymatic synthesis of racemic Sal might occur in the regions containing high levels of free DA. In addition, Sal derivatives may be transported into the brain through the blood–brain barrier (BBB) (Thümen et al. 2002), and racemic Sal derivatives derived from food and beverages might contribute to the presence of (*S*)Sal derivatives in the brain.

In the human brain, Norsal was also detected in the striatum, followed by the SN and hypothalamus (Musshoff et al. 1999). *N*M-Norsal was identified also in the same regions and immunochemically co-localized with a neuronal marker, neuron-specific enolase, and a catecholamine marker, tyrosine hydroxylase (TH) (DeCuypere et al. 2008b).

In neurons, Sal derivatives are localized in distinct intracellular compartments depending on their chemical structure. Sal derivatives are accumulated in the mitochondrial and membrane-nuclear fraction, 90–60 % of the total amounts, after 3-day incubation of (*R*)Sal and Norsal with PC12 cells (Maruyama et al. 1993). These results might be relevant with the recent result that *N*M-(*R*)Sal binds to MAO-A in the mitochondria (Yi et al. 2006). $DMDHIQ^+$ binds to melanin, which may cause its high contents in the SN (Naoi et al. 1994a). ^{3}H-Sal was reported to bind to a selective binding site in the striatum, cortex, median eminence, and hypothalamus of the rat brain, and the binding was displaced with DA, L-DOPA, carbidopa, and apomorphine, but not by DA receptor ligands, inhibitors of DA transport (Homicsko et al. 2002). The presence of this Sal-binding protein has not been confirmed in the human brain.

3.2 Factors Affecting the Salsolinol Levels

In human brain samples, (*R*)Sal levels decreased in the caudate, putamen, and SN according to the age, and a negative correlation was confirmed between *N*M-(*R*)Sal levels in the striatum and the age (Maruyama et al. 1997c). Alcohol intake markedly increased racemic Sal in blood and maybe also in the brain, but this issue has not been settled. The presence of alcohol did not affect the levels of (*R*)-enantiomer of Sal and *N*M-Sal in any brain regions (Naoi et al. 2004).

These results suggest that the concentration of the (*R*)-enantiomers may depend on the activity of the synthesizing enzymes. The relationship between the activities of Sal-related enzymes and Sal derivative levels was examined in human brain regions (Naoi et al. 1997). A good correlation was confirmed between the activity of neutral (*R*)Sal *N*-MT, but not that of alkaline *N*-MT, in the striatum (putamen and caudate) and the concentration of $DMDHIQ^+$ in the SN (Fig. 3b). *N*M-(*R*)Sal is synthesized in the striatum, oxidized into $DMDHIQ^+$, and transported and accumulated in the SN, as in the case with the rat PD model, where $DMDHIQ^+$ was detected in the SN after injection of *N*M-(*R*)Sal in the striatum (Naoi et al. 1996b). There was no other relationship that was confirmed between other enzyme activities and Sal derivative levels in frontal cortex and thalamus. In rat brain, the levels of racemic *N*M-Sal reached to the maximum within 2–6 h after the intraperitoneal injection, but *N*M-Sal was quickly eliminated from the brain (Lorenc-Koci et al. 2008). This rapid clearance of Sal may account for the discrepancy of the Sal contents with the activity of synthesizing enzymes in the human brain regions.

Sal derivatives are O-methylated by catechol-O-methyltransferase (COMT) (Hötzl and Thomas 1997) and oxidized by FAD-containing monooxygenase (Wu et al. 1992) and debrisoquine 4-monooxygenase (Iwahashi et al. 1993). However, it has not been studied whether the same enzymes are associated with the catabolism of Sal derivatives in the human brain.

4 Neurotoxicity of *N*-Methyl-(*R*)salsolinol: In Vivo and In Vitro Studies

4.1 An Animal Model of Parkinson's Disease

A PD model was established with *N*M-(*R*)Sal. After 1-week continuous injection in the rat striatum, *N*M-(*R*)Sal caused behavioral changes, such as akinesia, twitching, postural disturbance, and spontaneous rotation (Naoi et al. 1996b). The number of DA neurons stained with TH antibody and the contents of DA, noradrenaline, and their metabolites reduced markedly in the SN of the injected site. On the other hand, *N*M-(*S*)Sal, (*R*)Sal and (*S*)Sal, Norsal, and *N*M-Norsal did not induce significant biochemical-histopathological changes in rats. $DMDHIQ^+$ caused severe necrotic reaction at the injected site, but the behavioral and neurochemical changes were only transit.

The preparation of animal models of PD with Sal and other endogenous IQs has been also reported. Chronic administration of racemic Sal and TIQ caused a decrease in DA metabolism in the SN and striatum of rats, suggesting the loss of DA neurons (Antkiewicz-Michaluk et al. 2000). Systematic administration of racemic *N*M-Sal into rat perturbed CA metabolism but failed to cause the cell death in the SN (Lorenc-Koci et al. 2008). TIQ and *N*-methyl-6,7-dimethoxyisoquinolinium ion were infused in rat supra-nigral region and produced 42 % and 20 % SN cell loss, respectively, and reduced DA contents in the striatum, but behavioral change was not observed (McNaught et al. 1996). Intraperitoneal injection of TIQ reduced TH-immunoreactive neurons and DA levels in the striatum (Lorenc-Koci et al. 2000). Administration of indoleamine-derived 2(*N*)-methyl-β-CB into the rat SN induced the reduction of DA metabolites and the massive necrotic features (Neafsey et al. 1989). The presence of a methyl group at 9(*N*) position of β-CB increased the in vivo neurotoxicity markedly (Matsubara et al. 1998).

4.2 Induction of Apoptosis by *N*-Methyl-(*R*)salsolinol and Its Molecular Mechanism

In PD, apoptosis of dopamine neurons was confirmed in the SN by detection of activated caspase 3, increased Bax, and nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Hartmann et al. 2000; Tatton 2000). In human dopaminergic neuroblastoma SH-SY5Y cells, *N*M-(*R*)Sal induces apoptosis (Akao et al. 1999, 2002; Maruyama et al. 1997b, 2001a, b). Among Sal derivatives, *N*M-(*R*)Sal is the most potent to induce DNA damage, whereas *N*M-(*S*)Sal, (*R*)- and (*S*)-Sal, and 1-carboxy-Sal are much less cytotoxic. Apoptosis is initiated with increased membrane permeability in mitochondria, as shown by the collapse in membrane potential, $\Delta\Psi m$, and swelling of mitochondria. Then, cytochrome c is released in the cytoplasm, caspase 3 is activated, GAPDH is translocated in nuclei, and finally nucleosomal DNA is fragmented and condensed. Only (*R*)-enantiomer of *N*M-Sal induced $\Delta\Psi m$ collapse in SH-SY5Y cells (Maruyama et al. 2001a, b) and also in isolated mitochondria (Akao et al. 2002), suggesting the presence of a binding protein of *N*M-(*R*)Sal. *N*M-(*R*)Sal binding to MAO-A located in mitochondria

outer membrane initiates apoptosis signaling (Yi et al. 2006). *N*M-(*R*)Sal binds to MAO-A at the substrate-binding site, which is suppressed by knockdown of MAO-A expression by short-interfering RNA (siRNA) and also by serotonin (5-hydroxytryptamine, 5-HT) and clorgyline, a MAO-A-specific substrate and inhibitor.

Sal derivatives cause also oxidative stress and mitochondrial dysfunction, proposed causal factors for dopaminergic neural loss in PD. *N*M-(*R*)Sal increases reactive oxygen and nitrogen species (ROS, RNS) in the dialysate of in vivo microdialysis at the rat striatum, but (*R*)Sal reduces the ROS levels at the distinct concentrations (Maruyama et al. 1995). (*R*)Sal, *N*M-(*R*)Sal, and $DHDMIQ^+$ inhibit the activities of complexes I (NADH-Q reductase) and complex II (succinate-Q reductase) in mitochondrial electron transfer pathway, and $DHDMIQ^+$ is the most potent inhibitor (Morikawa et al. 1998). *N*-Methylation of Sal and TIQ increases the inhibition of complex I and the cytotoxicity, as shown with *N*M-isoquinolinium ion ($NM\text{-}IQ^+$) and *N*M-TIQ (Suzuki et al. 1992), 1,2,3,4-tetrahydro-2-methyl-4,6,7-isoquinolinetriol (Willets et al. 1996), and *N*M-Norsal (Storch et al. 2002). Increased ROS production plays a major role in the cytotoxicity of racemic Sal, which particularly inhibits complex II (Storch et al. 2000). TIQ and IQ induced apoptosis in PC12 and SK-N-MC cells through the inhibition of complex I and increased ROS production (Seaton et al. 1997).

5 *N*-Methyl-(*R*)salsolinol and *N*-Methyltransferases in Parkinson's Disease

5.1 Salsolinol Derivatives in the Parkinsonian Brain

The changes in the amounts of Sal derivatives have been scarcely reported in the brain and CSF from PD patients. In the caudate nuclei of the brains from PD patients, the lower levels of (*R*)Sal, (*S*)Sal, *N*M-(*R*)Sal, and *N*M-(*S*)Sal were reported in comparison with the normal brain (DeCuypere et al. 2008a). On the other hand, in the CSF, *N*M-(*R*)Sal was increased in PD compared to control and patients with multiple system atrophy (MSA) (Maruyama et al. 1996a). The *N*M-(*R*)Sal contents in PD ($n = 16$) were 8.32 ± 2.89 nM (4.54–15.7), whereas in control ($n = 29$) and MSA ($n = 5$) 4.53 ± 2.08 (0.62–11.9) and 3.59 ± 1.52 (1.33–5.25). The relative concentration of *N*M-(*R*)Sal versus homovanillic acid (HVA), a DA metabolite, is significantly higher in PD than in control and MSA, suggesting the increased synthesis of *N*M-(*R*)Sal in PD. In the PD CSF, Sal was detected (Moser and Kämpf 1992) and Sal was increased in PD patients with dementia (Antkiewics-Michaluk et al. 1997). In the intraventricular fluid (IVF) from parkinsonian patients treated with L-DOPA, *N*M-(*R*)Sal and a trace amount of (*R*)Sal were detected in addition to $DMDHIQ^+$, but the (*S*)-enantiomers were not (Maruyama et al. 1996b). The amounts of *N*M-(*R*)Sal in the CSF and IVF had a tendency to increase at the early Hoehn and Yahr stage and decrease according to the advancing Hoehn and Yahr stage by disease duration (Maruyama et al. 1999) (Fig. 4a). In the CSF from the PD patients ($n = 9$), *N*M-*R*-Sal levels

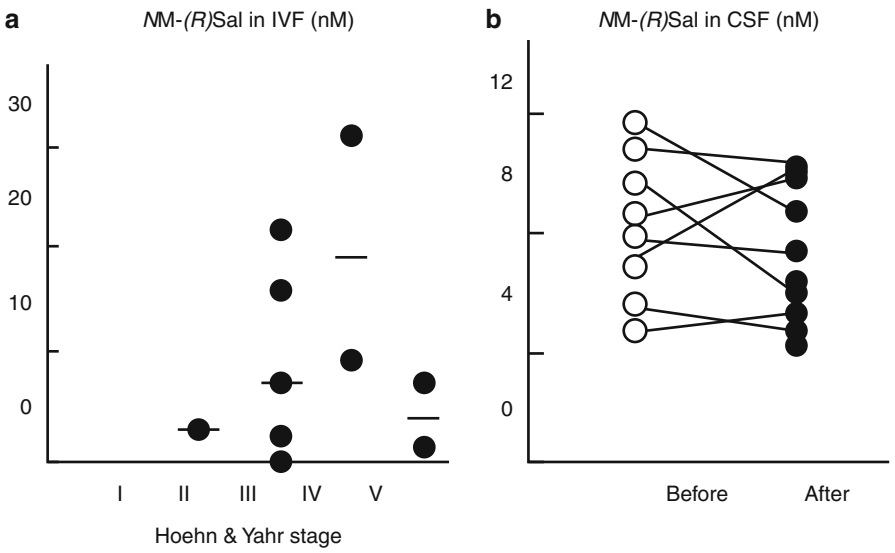


Fig. 4 The effects of Hoehn and Yahr stage and disease duration on *N*M-(*R*)Sal levels in the IVF and CSF. (**a**) The effects of the Hoehn and Yahr stage on the *N*M-(*R*)Sal levels in the IVF from parkinsonian patients. (**b**) Decline of *N*M-(*R*)Sal levels in the CSF of the same PD patients after 2 years of disease duration

decreased after 2 years of L-DOPA medication (Fig. 4b). The levels of Sal and *N*M-Norsal in the CSF were negatively correlated with the duration of PD (Moser et al. 1995; Müller et al. 1999). In plasma from de novo parkinsonian patients, (*R*)- and (*S*)-Sal levels were not increased, but (*R*)Sal levels were inversely related to the duration and intensity of PD, in contrast to (*S*)Sal and DA (Müller et al. 1998), whereas L-DOPA therapy increases Sal, Norsal, and *N*M-Norsal in urine (Scholz et al. 2004).

These results indicate that Sal derivative increase at the early phase of PD and reduce according to the disease progression. The loss of DA neurons caused by the disease progression and also aging reflects the Sal levels in the postmortem brain samples and in the CSF and IVF. Significant reduction of *N*M-(*R*)Sal in the SN for PD brains compared to control (DeCuypere et al. 2008a) may indicate the accelerated cell loss of DN neurons by this neurotoxin.

5.2 Neutral (*R*)salsolinol and Other *N*-Methyltransferases

The enzyme activities for Sal synthesis and metabolism were investigated in lymphocytes isolated from PD patients (n = 24) and control subjects without neurological disease (n = 56) (Naoi et al. 1998). The enzymatic activities of neural (*R*)Sal *N*-MT were found to increase significantly in PD lymphocytes, whereas the activities of alkaline (*R*)Sal *N*-MT and *N*M-*R*-Sal oxidase were not changed (Fig. 5a). The relationship between (*R*)Sal *N*-MT activity in the lymphocytes and

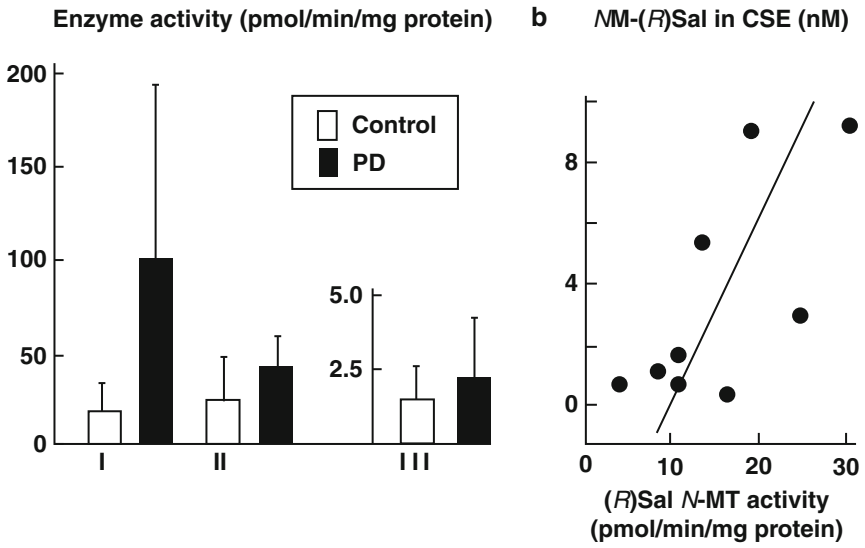


Fig. 5 The activities of (*R*)Sal *N*-MT and related enzymes in lymphocytes from PD patients and its relation to *N*M-(*R*)Sal levels in the CSF. (**a**) The enzymatic activity of neutral (I) and alkaline (*R*)Sal *N*-MT and *N*M-(*R*)Sal oxidase (III) in lymphocytes prepared from control (*white column*) and PD patients (*black column*). The column and bar represent the mean and SD. (**b**) The relationship of neutral (*R*)Sal *N*-MT activity in lymphocytes with *N*M-(*R*)Sal levels in the CSF from the same PD patients

*N*M-(*R*)Sal levels in the CSF was investigated (Fig. 5b). A weak correlation between them was observed ($y = -3.65 + 0.62$, $R = 0.71$, $p < 0.05$), but the limited number of the samples ($n = 9$) could not yield a conclusive result.

The increase in (*R*)Sal *N*-MT in PD lymphocytes was reexamined in other groups of control and PD patients (Fig. 6a, b). The activities of (*R*)Sal *N*-MT in PD lymphocytes ($n = 41$) were mainly distributed as being higher than 35 pmol/min/mg protein, whereas in control lymphocytes ($n = 205$) the activities were mostly lower than 30 pmol/min/mg protein. The activities did not show significant relationship to Hoehn-Yahr stage and the duration of illness, in contrast to the *N*M-(*R*)Sal contents in the CSF and IVF (Fig. 6c, d). The activities in lymphocytes were not affected by the gender, the age of PD onset, and the age of the patients when the samples were taken, and the dose of L-DOPA administered. These results suggest that (*R*)Sal *N*-MT activity may be genetically determined and the increased activity observed in PD may be neither secondary to the disease process nor to the treatment. (*R*)Sal *N*-MT was isolated and purified from the control brain, but we have no chance to investigate whether this enzyme changes its enzymatic properties in the PD brain.

N-Methylation of Sal, TIQs, and β-CB increases the cytotoxicity, as discussed above (Naoi et al. 1994b; Maruyama et al. 1997b; Matsubara et al. 2002; Williams and Ramasden 2005). Mammalian SAM-dependent *N*-MT [EC. 2.1.1] is a large family of enzymes catalyzing *N*-methylation of small molecules, and these *N*-MTs have similar molecular weights in the range of 25–35 kDa and share common enzymatic

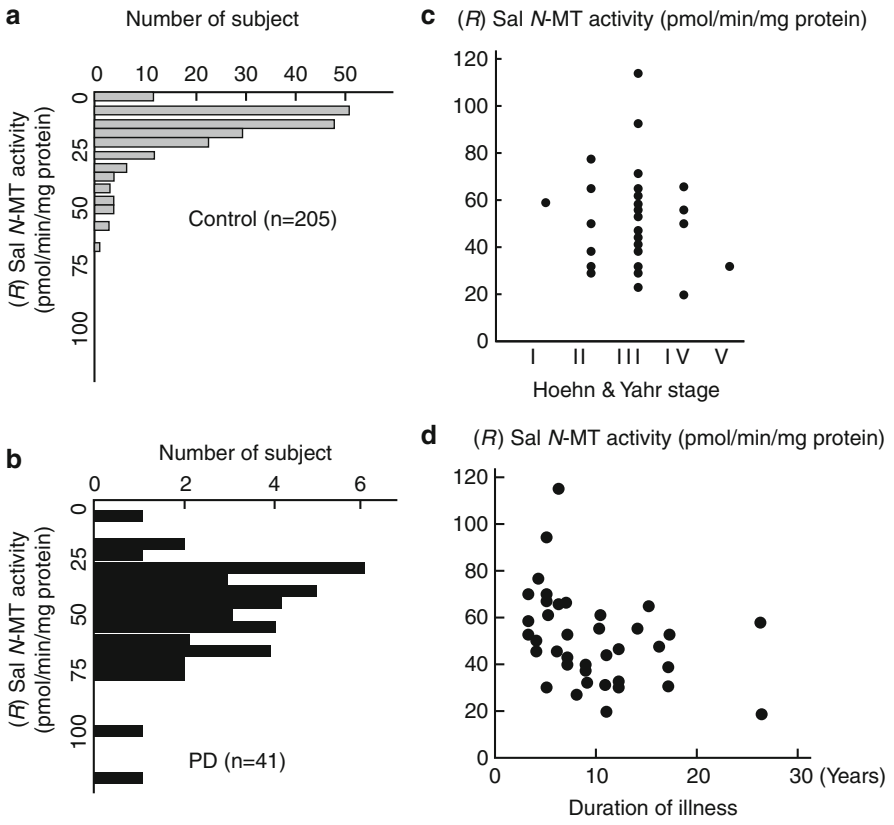


Fig. 6 Distribution of activity of neutral (*R*)Sal *N*-MT in lymphocytes and the effects of disease progress. (**a**) The activity in control subjects (n = 205). (**b**) In lymphocytes from PD patients (n = 41), the activity was distributed to higher than 35 pmol/min/mg protein. (**c**) The effects of the Hoehn and Yahr stage on the activity of neutral (*R*)Sal *N*-MT in lymphocyte from PD patients. (**d**) The effects of disease duration on the activities of neutral (*R*)Sal *N*-MT in lymphocytes from PD patients

characteristics. Nicotinamide *N*-MT [EC 2.1.1.1] is isolated from human liver, has a molecular weight of 29kDa as a monomer, and *N*-methylates nicotinamide and other pyridine (Aksoy et al. 1994). This *N*-MT converts 4-phenyl-1,2,3,6-tetrahydropyridine and 4-phenylpyridine into MPTP and MPP^+, suggesting the biosynthesis of MPTP derivatives from endogenous or environmental precursors (Ansher et al. 1986). The *N*-methylation of nicotinamide produces *N*-methylnicotinamide, which has MPP^+-like cytotoxicity (Fukushima et al. 2002). The protein levels of nicotinamide *N*-MT were higher in the CSF from PD patients (65 years old or younger) than control and decreased with aging (Aoyama et al. 2001). In the cerebella and caudate from PD patients, high expression of this *N*-MT protein and activity was confirmed, and the disease duration was negatively correlated with the expression (Parsons et al. 2002). The increased nicotinamide *N*-MT in PD may be relevant with the increased levels

of *N*-methylnicotinamide in parkinsonian brain (Williams et al. 1993) and high urinary excretions of nicotinamide metabolites in younger PFD patients, which declined with aging (Aoyama et al. 2000).

In PD brain, the activity of β-CB 9(*N*)-MT, which methylates nitrogen at 9 position in β-CB, but not at 2, increased in the frontal cortex in PD (Gearhart et al. 2000). Phenylethanolamine (PEA) *N*-MT (noradrenalin *N*-MT), which catalyzes the methylation of noradrenalin into adrenalin [EC 2.1.1.28], was reported to have β-CB 2(*N*)-MT, which methylates nitrogen at 2 position of β-CB (Gearhart et al. 2002). The thr15Ile polymorphism of a histamine *N*-MT [EC 2.1.1.8] was reported to be associated with PD (Palada et al. 2011) and the increase in histamine, which is related to DA neuron loss in PD (Anichtchik et al. 2000). However, recent papers did not confirm the association of this functional polymorphism with PD and essential tremor (Keeling et al. 2010) and AD (Marasovic-Susnjara et al. 2011).

6 Conclusion

These presented results from the basic research using cellular and animal models to the analyses of clinical samples from parkinsonian patients prove that *N*M-(*R*)Sal may be involved in the pathogenesis of PD as an endogenous dopaminergic toxin. However, these results cannot be free from criticism. The quite high concentrations of these IQs are used for very short time of experiment, which might not reasonably reflect the slowly processing neuronal loss in PD. The postmortem studies of IQ contents in the parkinsonian brain cannot draw the common consensus because of unavoidable variations in the quantitative data. These results seem to suggest that the neurotoxin hypothesis might pass away as the pathogenesis of PD, if we cannot establish new approach.

However, the results on (*R*)Sal *N*-MT, nicotinamide *N*-MT, and other *N*-MT suggest that we should investigate the enzymes in the metabolism of Sal derivatives in relation to the pathogenesis of PD. *N*-MT might be a good candidate for this study, since this enzyme can convert small molecules of endogenous and environmental protoxins into cytotoxic *N*-methylated toxins. The activity is regulated genetically and also by environmental factors, protoxin levels in diets, and cellular signals, such as interferon-γ (Yamada et al. 2010). These results suggest that the study on *N*-MT brings us further insights on the genetic and environmental factors as the pathogenesis of PD. If we can find the methods to suppress the synthesis of toxins with inhibitors, we can develop novel neuroprotective therapy to prevent the onset and progress of degeneration of DA neurons.

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Declaration of Approval from the Ethical Committee

The Ethical Committee of Iwate Medical University, Aichi Medical University, National Institute for Longevity Science, and Neurological Clinic (Dr. H. Narabayashi) approved the protocol for the examination of the CSF samples and lymphocytes and of the IVF. The patients were fully informed about the risk and the benefits of the examination.

References

Akao, Y., Nakagawa, Y., Maruyama, W., Takahashi, T., & Naoi, M. (1999). Apoptosis induced by an endogenous neurotoxin, *N*-methyl(*R*)salsolinol, is mediated by activation of caspase 3. *Neuroscience Letters, 267*, 153–156.

Akao, Y., Maruyama, W., Shimizu, S., Yi, H., Nakagawa, Y., Shamoto-Nagai, M., Youdim, M. B. H., Tsujimoto, Y., & Naoi, M. (2002). Mitochondrial permeability transition mediates apoptosis induced by *N*-methyl(*R*)salsolinol, an endogenous neurotoxin, and is inhibited by Bcl-2 and rasagiline, *N*-propargyl-1(*R*)-aminoindan. *Journal of Neurochemistry, 82*, 913–923.

Aksoy, S., Szumlanski, C. L., & Weinshiboum, R. M. (1994). Human liver nicotinamide *N*-methyltransferase. cDNA cloning, expression, and biochemical characterization. *The Journal of Biological Chemistry, 269*, 14835–14840.

Anichtchik, O. V., Rinne, J. O., Kalimo, H., & Panula, P. (2000). An altered histaminergic innervation of the substantia nigra in Parkinson's disease. *Experimental Neurology, 163*, 20–30.

Ansher, S. S., Cadet, J. L., Jakpby, W. B., & Baker, J. K. (1986). Role of N-methyltransferases in the neurotoxicity associated with the metabolites of 1-methyl-4-phenyl-1,2,3,6-tetrahydopyridine (MPTP) and other 4-substituted pyridines present in the environment. *Biochemical Pharmacology, 35*, 3359–3363.

Antkiewics-Michaluk, L., Krygowska-Wajs, A., Szczudlik, A., Romanska, I., & Vetulani, J. (1997). Increase in salsolinol level in the cerebrospinal fluid of parkinsonian patients is related to dementia: Advantage of a new high-performance liquid chromatography methodology. *Biological Psychiatry, 42*, 514–518.

Antkiewicz-Michaluk, L., Romanska, I., Papla, I., Michaluk, J., Bakalrz, M., Vetulani, J., Krygowska-Wajs, A., & Szczudlik, A. (2000). Neurochemical changes induced by acute and chronic administration of 1,2,3,4-tetrahydroisoquinoline and salsolinol in dopaminergic structures of rat brain. *Neuroscience, 96*, 59–64.

Aoyama, K., Matsubara, K., Okada, K., Fukushima, S., Shimizu, K., Yamaguchi, S., Uezono, T., Satomi, M., Hayase, N., Ohta, S., Shiono, H., & Kobayashi, S. (2000). N-methylation ability for azaheterocyclic amines is higher in Parkinson's disease. *Journal of Neural Transmission, 107*, 985–995.

Aoyama, K., Matsubara, K., Kondo, M., Murakawa, Y., Suno, M., Yamashita, K., Yamaguchi, S., & Kobayashi, S. (2001). Nicotinamide-*N*-methyltransferase is higher in the lumbar cerebrospinal fluid of patients with Parkinson's disease. *Neuroscience Letters, 298*, 78–80.

Bahnmaier, A. H., Woesle, B., & Thomas, H. (1999). Stereospecific *N*-methylation of the tetrahydroisoquinoline alkaloids isosalsoline and salsolidine by amine N-methyltransferase A from bovine liver. *Chirality, 11*, 160–165.

Collins, M. A. (1980). Neuroamine condensations in human subjects. *Advances in Experimental Medicine and Biology, 126*, 87–102.

Collins, M. A. (2002). Alkaloids, alcohol and Parkinson's disease. *Parkinson Related Disorder, 8*, 417–422.

Collins, M. A., & Cheng, B. Y. (1988). Oxidative decarboxylation of salsolinol-1-carboxylic acid to 1,2-dehydrosalsolinol: Evidence for exclusive catalysis by particulate factors in rat kidney. *Archives of Biochemistry and Biophysics, 263*, 86–95.

DeCuypere, M., Lu, Y., Miller, D. D., & Leboux, M. S. (2008a). Regional distribution of tetrahydroisoquinoline derivatives in rodent, human, and Parkinson's disease brain. *Journal of Neurochemistry, 107*, 1398–1413.

DeCuypere, M., Kalabokis, V. N., Hao, R., Schroeder, D., Miller, D. D., & LeDoux, M. S. (2008b). Localization of N-methyl-norsalsolinol within rodent and human brain. *Journal of Neuroscience Research, 86*, 2543–2552.

Dostert, P., Strolin Benedetti, M., Dordain, G., & Vernay, D. (1889). Enantiomeric composition of urinary salsolinol in Parkinsonian patients after Madopar. *Journal of Neural Transmission [P-D Sect], 1*, 269–278.

Dostert P, Strolin Benedetti M, Dordain G, Vernay D (1989). Enantiomeric composition of urinary salsolinol in parkinsonian patients after Madopar. *J Neural Transm Park Dis Dement Sect. 1*(4), 269–278.

Dostert, P., Strolin Benedetti, M., Bellotti, V., Allievi, C., & Dordain, G. (1990). Biosynthesis of salsolinol, a tetrahydroisoquinoline alkaloid, in healthy subjects. *Journal of Neural Transmission, 81*, 215–223.

Fukushima, T., Kaetsu, A., Lim, H., & Moriyama, M. (2002). Possible role of 1-methylnicotinamide in the pathogenesis of Parkinson's disease. *Experimental and Toxicologic Pathology, 53*, 469–473.

Gearhart, D. A., Collins, M. A., Lee, J. M., & Neafsey, E. J. (2000). Increased β-carboline 9*N*-methyltransferase activity in the frontal cortex in Parkinson's disease. *Neurobiology of Disease, 7*, 201–211.

Gearhart, D. A., Neafsey, E. J., & Collins, M. A. (2002). Phenylethanolamine *N*-methyltransferase has β-carboline *N*-methyltransferase activity: Hypothetical relevance to Parkinson's disease. *Neurochemistry International, 40*, 611–620.

Grobe, N., Ren, X., Kutchan, T. M., & Zenk, M. H. (2011). An (*R*)-specific *N*-methyltransferase involved in human morphine biosynthesis. *Archives of Biochemistry and Biophysics, 506*, 42–47.

Hartmann, A., Hunot, S., Michel, P. P., Muriel, M. P., Vyas, S., Faucheux, B. A., Mouatt-Prigent, A., Turmel, H., Srinivasan, A., Ruberg, M., Evan, G. I., Agid, Y., & Hirsch, E. C. (2000). Caspase-3: A vulnerability factor and final effector in apoptotic death of dopaminergic neurons in Parkinson's disease. *Proceedings of the National Academy of Sciences USA, 97*, 2875–2880.

Homicsko, K. G., Kertesz, I., Radnai, B., Toth, B. E., Tosh, G., Fülop, F., Fekete, M. I. K., & Nagy, G. M. (2002). Binding site of salsolinol: Its properties in different regions of the brain and the pituitary gland of the rat. *Neurochemistry International, 1232*, 1–8.

Hötzl, B. K., & Thomas, H. (1997). O-methylation of (+)-(R)- and (-)-(S)-6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydrosioquinoline (salsolinol) in the presence of pig brain catechol-O-methyltransferase. *Chirality, 9*, 367–372.

Iwahashi, K., Matsuo, Y., Tsuneoka, Y., Ichikawa, Y., Hosokawa, K., & Suwaki, H. (1993). Histamine and 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroioquinoline are competitive inhibitors of debrisoquine 4-monooxygenase in rat liver. *Archives of Toxicology, 67*, 514–516.

Keeling, B. H., Vilaino-Güell, C., Soto-Ortolaza, A. I., Ross, O. A., Uitti, R. J., Rajput, A., Wszolek, Z. K., & Farrer, M. J. (2010). *Histamine N-methyltransferase* Thr105Ile is not associated with Parkinson's disease or essential tremor. *Parkinsonism and Related Disorders, 16*, 112–114.

Lorenc-Koci, E., Smiakowska, M., Antikiewicz-Michaluk, L., Golembiowska, K., Bajkowska, M., & Wolfarth, S. (2000). Effect of acute and chronic administration of 1,3,4-tetrahydroisoquinoline on muscle tone, metabolism of dopamine in the striatum and tyrosine hydroxylase immunocytochemistry in the substantia nigra in rats. *Neuroscience, 95*, 1049–1059.

Lorenc-Koci, E., Antikiewicz-Michaluk, L., Kaminska, A., Lenda, T., Zieba, B., Wieronska, J., Smiakowska, M., Schulze, G., & Rommelspacher, H. (2008). The influence of acute and chronic administration of 1,2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydro-isoquinoline on the function of the nigrostriatal dopaminergic system in rats. *Neuroscience, 156*, 973–986.

Marasovic-Susnjara, I., Palada, V., Marinovic-Terzic, I., et al. (2011). No association between histamine N-methyltransferase functional polymorphism Thr105Ile and Alzheimer's disease. *Neuroscience Letters, 489*, 119–121.

Maruyama, W., Nakahara, D., Ota, M., Takahashi, T., Takahashi, A., Nagatsu, T., & Naoi, M. (1992). *N*-Methylation of dopamine-derived 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, (*R*)-salsolinol in rat brains; In vivo microdialysis study. *Journal of Neurochemistry, 59*, 395–400.

Maruyama, W., Takahashi, T., Minami, M., Takahashi, A., Dostert, P., Nagatsu, T., & Naoi, M. (1993). Cytotoxicity of dopamine-derived 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines. *Advances in Neurology, 60*, 224–230.

Maruyama, M., Dostert, P., & Naoi, M. (1995). Dopamine-derived 1-methyl-6,7-dihydroxyisoquinolines as hydroxyl radical promoters and scavengers in the rat brain: In vivo and in vitro studies. *Journal of Neurochemistry, 64*, 2635–2643.

Maruyama, W., Abe, T., Tohgi, H., Dostert, P., & Naoi, M. (1996a). A dopaminergic neurotoxin, (*R*)-N-methylsalsolinol, increases in parkinsonian cerebrospinal fluid. *Annals of Neurology, 40*, 119–122.

Maruyama, W., Narabayashi, H., Dostert, P., & Naoi, M. (1996b). Stereospecific occurrence of a parkinsonian-inducing catechol isoquinoline, *N*-methyl(*R*)-salsolinol, in the human intraventricular fluid. *Journal of Neural Transmission, 103*, 1069–1076.

Maruyama, W., Deng, Y., Dostert, P., & Naoi, M. (1997a). Analysis of endogenous enantiomers of neurotoxins in clinical samples by a multiple-electrode detection system with a chiral column. In I. N. Acworth, M. Naoi, H. Parvez, & S. Parvez (Eds.), *Coulometric electrode array detectors for HPLC* (Progress in HPLC-HPCE, Vol. 6, pp. 339–350). VSP: Utrecht.

Maruyama, W., Naoi, M., Kasamatsu, T., Hashizume, Y., Takahashi, T., Kohda, K., & Dostert, P. (1997b). An endogenous dopaminergic neurotoxin, *N*-methyl(*R*)salsolinol, induces DNA damage in human dopaminergic neuroblastoma SH-SY5Y cells. *Journal of Neurochemistry, 69*, 322–329.

Maruyama, W., Sobue, G., Matsubara, K., Hashizume, Y., Dostert, P., & Naoi, M. (1997c). A dopaminergic neurotoxin, 1(*R*),2(*N*)-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, *N*-methyl(*R*)-salsolinol, and its oxidation product, 1,2(*N*)-dimethyl-6,7-dihydroxy isoquinolinium ion, accumulate in the nigrostriatal system of the human brain. *Neuroscience Letters, 223*, 61–64.

Maruyama, W., Abe, T., Tohgi, H., & Naoi, M. (1999). An endogenous MPTP-like dopaminergic neurotoxin, *N*-methyl(*R*)salsolinol, in the cerebrospinal fluid decreases with progression of Parkinson's disease. *Neuroscience Letters, 262*, 13–16.

Maruyama, W., Akao, Y., Youdim, M. B. H., Davis, G. A., & Naoi, M. (2001a). Transfection-enforced Bcl-2 overexpression and an antiparkinson drug, rasagiline, prevent nuclear accumulation of glyceraldehydes-3-phosphate dehydrogenase induced by an endogenous dopaminergic neurotoxin, *N*-methyl(*R*)salsolinol. *Journal of Neurochemistry, 78*, 727–735.

Maruyama, W., Boulton, A. A., Davis, B. A., Dostert, P., & Naoi, M. (2001b). Enantio-specific induction of apoptosis by an endogenous neurotoxin, *N*-methyl(*R*)salsolinol, in dopaminergic SH-SY5Y cells: Suppression of apoptosis by *N*-(2-heptyl)-*N*-methyl-propargylamine. *Journal of Neural Transmission, 108*, 11–24.

Matsubara, K., Gonda, T., Sawada, H., Uezono, T., Kobayashi, Y., Kawamura, T., Ohtani, K., Kimura, K., & Alaike, A. (1998). Endogenously occurring β-carboline induces parkinsonism in nonprimate animals: A possible causative protoxin in idiopathic Parkinson's disease. *Journal of Neurochemistry, 70*, 727–735.

Matsubara, K., Aoyama, K., Suno, M., & Awaya, T. (2002). N-methylation underlying Parkinson's disease. *Neurotoxicology and Teratology, 24*, 593–598.

McNaught, K. S., Thull, U., Carrupt, P. A., Allomare, C., Cellamare, S., Carotti, A., Testa, B., Jenner, P., & Marsden, C. D. (1996). Nigral cell loss produced by infusion of isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurodegeneration, 5*, 265–274.

Morikawa, N., Naoi, M., Maruyama, W., Ohta, S., Kotake, Y., Kawai, H., Niwa, T., Dostert, P., & Mizuno, Y. (1998). Effects of various tetrahydroisoquinoline derivatives on mitochondrial respiration and the electron transfer complexes. *Journal of Neural Transmission, 105*, 677–688.

Moser, A., & Kämpf, D. (1992). Presence of methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, derivatives of the neurotoxin isoquinoline, in parkinsonian lumbar CSF. *Life Sciences, 50*, 1885–1891.

Moser, A., Scholz, J., Nobbe, F., Vieregge, P., Böhme, V., & Bamberg, H. (1995). Presence of N-methyl-norsalsolinol in the CSF: Correlation with dopamine metabolites of patients with Parkinson's disease. *Journal of the Neurological Sciences, 131*, 183–189.

Müller, T., Sällström Baum, S., Häussermann, P., Woitalla, D., Rommelspacher, H., Przuntek, H., & Kuhn, W. (1998). Plasma levels of R- and S-salsolinol are not increased in "de-novo" Parkinsonian patients. *Journal of Neural Transmission, 105*, 239–246.

Müller, T., Sällström Baum, S., Haussermann, P., Przuntek, H., Rommelspacher, H., & Kuhn, W. (1999). R- and S-salsolinol are not increased in cerebrospinal fluid of Parkinsonian patients. *Journal of the Neurological Sciences, 164*, 158–162.

Musshoff, R., Schmidt, P., Dettmeyer, R., Priemer, F., Witting, H., & Madae, B. (1999). A systematic regional study of dopamine and dopamine-derived salsolinol and norsalsolinol levels in human brain areas. *Forensic Science International, 105*, 1–11.

Musshoff, F., Schmidt, P., Dettmeyer, R., Priemer, F., Jachau, K., & Madae, B. (2000). Determination of dopamine and dopamine-derived (*R*)-/(*S*)-salsolinol in various human brain area using solid-phase extraction and gas chromatography/mass spectrometry. *Forensic Science In, 113*, 359–366.

Naoi, M., Matsuura, S., Takahashi, T., & Nagatsu, T. (1989). A N-methyltransferase in human brain catalyses N-methylation of 1,2,3,4-tetrahydroisoquinoline into N-methyl-1,2,3,4-tetrahydroisoquinoline, a precursor of a dopaminergic neurotoxin, N-methylisoquinolinium ion. *Biochemical and Biophysical Research Communications, 1161*, 1213–1219.

Naoi, M., Maruyama, W., & Dostert, P. (1994a). Binding of 1,2(*N*)-dimethyl-6,7-dihydroxy-isoquinolinium ion to melanin: Effects of ferrous and ferric ion on the binding. *Neuroscience Letters, 171*, 9–12.

Naoi, M., Maruyama, W., Niwa, T., & Nagatsu, T. (1994b). Novel toxins and Parkinson's disease: *N*-methylation and oxidation as metabolic bioactivation of neurotoxins. *Journal of Neural Transmission, 41*(Suppl), 197–205.

Naoi, M., Maruyama, W., Zhang, J. H., Takahashi, T., Deng, Y., & Dostert, P. (1995). Enzymatic oxidation of the dopaminergic neurotoxin, 1(*R*),2(*N*)-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, into 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion. *Life Sciences, 57*, 1061–1066.

Naoi, M., Maruyama, W., Dostert, P., Kohda, K., & Kaiya, T. (1996a). A novel enzyme enantioselectively synthesizes (*R*)salsolinol, a precursor of a dopaminergic neurotoxin, *N*-methyl(*R*) salsolinol. *Neuroscience Letters, 212*, 183–186.

Naoi, M., Maruyama, W., Dostert, P., Hashizume, Y., Nakahara, D., Takahashi, T., & Ota, M. (1996b). Dopamine-derived endogenous 1(*R*),2(*N*)-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoqunoline, *N*-methyl-(*R*)-salsolinol, induced parkinsonism in rat: Biochemical, pathological and behavioral studies. *Brain Research, 709*, 285–295.

Naoi, M., Maruyama, W., Matsubara, K., & Hashizume, Y. (1997). A neutral *N*-methyltransferase activity in the striatum determines the level of an endogenous MPP^+-like neurotoxin, 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion, in the substantia nigra of human brains. *Neuroscience Letters, 235*, 81–84.

Naoi, M., Maruyama, W., Nakao, N., Ibi, T., Sahashi, K., & Strolin, B. M. (1998). (*R*)Salsolinol *N*-methyltransferase activity increases in parkinsonian lymphocytes. *Annals of Neurology, 43*, 212–216.

Naoi, M., Maruyama, W., & Nagy, G. M. (2004). Dopamine-derived salsolinol derivatives as endogenous monoamine oxidase inhibitors: Occurrence, metabolism and function in human brains. *NeuroToxicology, 25*, 193–204.

Neafsey, E. J., Drucker, G., Raikoff, K., & Collins, M. A. (1989). Striatal dopaminergic toxicity following intranigral injection in rats of 2-methyl-norharman, a β-carbolinium analogue of *N*-methyl-4-phenylpyridinium ion (MPP^+). *Neuroscience Letters, 105*, 344–349.

Palada, V., Terzic, J., Mazzulli, J., et al. (2011). Histamine *N*-methyltransferase Thr105Ile polymorphism is associated with Parkinson's disease. *Neurobiology of Aging, 33*, 836.

Parsons, R. B., Smith, M. L., Williams, A. C., Waring, R. H., & Ramsden, D. B. (2002). Expression of nicotinamide N-methyltransferase (E.C. 2.1.1.1) in the Parkinsonian brain. *Journal of Neuropathology and Experimental Neurology, 61*, 111–124.

Sandler, M., Bonham Carter, S., Hunter, K. R., & Stern, G. M. (1973). Tetrahydroisoquinoline alkaloids: In vive metabolites of L-DOPA in man. *Nature, 1241*, 439–443.

Scholz, J., Klinemann, I., & Moser, A. (2004). Increased systemic levels of norsalsolinol derivatives are induced by levodopa treatment and do not represent biological markers of Parkinson's disease. *Journal of Neurology Neurosurgery and Psychiatry, 75*, 634–636.

Seaton, T. A., Cooper, J. M., & Schapira, A. H. V. (1997). Free radical scavengers protect dopaminergic cell lines from apoptosis induced by complex I inhibitors. *Brain Research, 777*, 110–118.

Sjöquist, B., Borg, S., & Kvande, H. (1981). Salsolinol and methylated salsolinol in urine and cerebrospinal fluid from healthy volunteers. *Substance and Alcohol Actions/Misuse, 2*, 73–77.

Storch, A., Kaftan, A., Burkhardt, K., & Schwarz, J. (2000). 1-Methyl-6,7-dihydroxy- 1,2,3,4-tetrahydroisoqunoline (salsolinol) is toxic to dopaminergic neuroblastoma SH-SY5Y cells via impairment of cellular energy metabolism. *Brain Research, 855*, 67–75.

Storch, A., Ott, S., Hwang, Y., Ortmann, R., Hein, A., Frenzel, S., Matsubara, K., Ohta, S., Wolf, H.-U., & Schwarz, J. (2002). Selective dopaminergic neurotoxicity of isoquinoline derivatives related to Parkinson's disease: Studies using heterologous expression systems of the dopamine transporter. *Biochemical Pharmacology, 63*, 909–920.

Suzuki, K., Mizuno, Y., Yamauchi, Y., Nagatsu, T., & Yoshida, M. (1992). Selective inhibition of complex I by N-methylisoquinolinium ion and N-methyl-1,2,3,4-tetrahydroisoquinoline in isolated mitochondrial prepared from mouse brain. *Journal of the Neurological Sciences, 109*, 219–222.

Tatton, N. A. (2000). Increased caspase 3 and Bax immunoreactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson's disease. *Experimental Neurology, 166*, 29–43.

Thümen, A., Behnecke, A., Quadri, F., Bäuml, E., & Moser, A. (2002). N-methyl-norsalsolinol, a putative dopaminergic neurotoxin, passes through the blood-brain-barrier in vivo. *Neuroreport, 13*, 25–28.

Ung-Chhun, N., Cheng, B. Y., Pronger, D. A., Serrano, P., Chavez, C., Perez, R. F., Morales, J., & Collins, M. A. (1985). Alkaloid adducts in human brain: coexistence of 1-carboxylated and noncarboxylated isoquinolines and ß-carbolines in alcoholics and nonalcoholics. In M. A. Collins (Ed.), *Aldehyde adducts in alcoholism* (pp. 125–136). New York: Alan T. Liss.

Willets, J. M., Lambert, D. G., Lunec, J., Griffiths, H. R., & Phillipson, O. (1996). Neurotoxicity of 1,2,3,4-tetrahydro-2-methyl-4,6,7-isoquinolinetriol (TMIQ) and effects on catecholamine homeostasis in SH-SY5Y cells. *Environmental Toxicology and Pharmacology, 2*, 59–68.

Williams, A. C., & Ramasden, D. B. (2005). Autotoxicity, methylation and a road to the prevention of Parkinson's disease. *Journal of Clinical Neuroscience, 12*, 6–11.

Williams, A. C., Pall, H. S., Steventon, G. B., Green, S., Buttrum, S., Molloy, H., & Waring, R. H. (1993). *N*-Methylation of pyridines and Parkinson's disease. *Advances in Neurology, 60*, 194–196.

Wu, R. F., Miura, S., & Ichikawa, Y. (1992). Neurotoxins: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 1,2,3,4-tetrahydroisoquinoline and 1-methyl-6,7-dihydroxy-tetrahydroisoquinoline as substrates for FAD-containing monooxygenase of porcine liver microsomes. *Biochemical Pharmacology, 44*, 2079–2081.

Yamada, K., Miyazaki, T., Hara, N., & Tsuchiya, M. (2010). Interferon-γ elevates nicotinamide N-methyltransferase activity in human glioma cells. *Journal of Nutritional Science and Vitaminology, 56*, 83–86.

Yi, H., Akao, Y., Maruyama, W., Chen, K., Shih, J., & Naoi, M. (2006). Type A monoamine oxidase is the target of an endogenous dopaminergic neurotoxin, *N*-methyl(*R*)salsolinol, leading to apoptosis in SH-SY5Y cells. *Journal of Neurochemistry, 96*, 541–549.

Regulation of DA Homeostasis and Role of VMAT2 in DA-Induced Neurodegeneration

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Contents

Abstract

Metabolic turnover of dopamine (DA) and other monoamines is tightly regulated by their synthesis, degradation, and compartmentalization. DA transmission plays important roles in learning and behavior, while abnormal DA tone is implicated in multiple neurological disorders, including Parkinson's disease (PD), schizophrenia, and psychoses. Free cytosolic DA can produce oxidative stress and protein damage and is suspected to contribute to the development of PD. Vesicular monoamine transporter 2 (VMAT2) sequesters DA into synaptic vesicles, thereby occupying a unique position where it facilitates DA synaptic transmission while preventing the deleterious effects of DA presence in the cytosol. This review summarizes recent findings on the regulation of DA cellular homeostasis and on the relationship between VMAT2 activity and DA-mediated neurotoxicity.

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List of Abbreviations

AADC	Aromatic L-amino acid decarboxylase
DA	Dopamine
DA_{cyt}	Cytosolic DA concentration
DAT	DA uptake transporter
DOPAC	3,4-dihydroxyphenylacetate
DOPAL	3,4-dihydroxyphenylacetaldehyde
LC	Locus coeruleus
L-DOPA	L-3,4-dihydroxyphenylalanine, levodopa
MAO	Monoamine oxidase
METH	Methamphetamine
MPP^+	1-methyl-4-phenylpyridinium
MSN	Medium spiny neurons
PD	Parkinson's disease
SNpc	Substantia nigra pars compacta
ROS	Reactive oxygen species
TH	Tyrosine hydroxylase
VGLUT2	Vesicular glutamate transporter 2
VTA	Ventral tegmental area
VMAT	Vesicular monoamine transporter

1 Introduction

Dopaminergic transmission in the brain plays important role in movement control, decision making, motivation, reward, learning, and other essential behavioral and cognitive functions. The three most thoroughly characterized dopaminergic projections are the nigrostriatal, mesolimbic, and mesocortical systems that each originate in the ventral midbrain and project to the striatum, limbic system, and cortex, respectively. These systems are targeted by drugs of abuse and psychostimulants, and abnormal DA neurotransmission in these pathways is linked to a number of diseases. Altered sensitivity to DA in mesolimbic and mesocortical pathways has been implicated in schizophrenia, narcolepsy, and effective disorders (van Rossum 1966; Gamo and Arnsten 2011), whereas degeneration of nigrostriatal neurons in PD represents the best-studied link between DA neurotransmission and neuropathology (Fahn 2008; Obeso et al. 2008).

PD is characterized by the preferential loss of DA neurons in the substantia nigra pars compacta (SNpc), although other neuronal populations, including locus coeruleus (LC), raphe nuclei, the dorsal motor nucleus of the vagus nerve, and the peripheral autonomic neurons, are also affected. Neurodegeneration in SNpc

with the concomitant depletion of striatal DA is responsible for the cardinal motor symptoms of PD: tremor, rigidity, bradykinesia, and postural instability (Fahn 2008; Obeso et al. 2008). Other features of PD include inflammation and the appearance of alpha-synuclein-positive "inclusions" known as Lewy bodies and Lewy neurites. Age is the greatest risk factor of PD; the likelihood that an individual will develop the disease is ~0.5 % at the age of 65 and increases by ~0.2 % each subsequent year (de Rijk et al. 1997; Baldereschi et al. 2000; Fahn and Sulzer 2004). The majority of PD cases are idiopathic, although at least 10 % of patients are now thought to have "familial" forms of the disease (reviewed in Martin et al. 2010); research to unveil new genetic causes of PD still continues.

SNpc and LC contain large quantities of neuromelanin, a dark pigment that gives names to these brain areas. Neuromelanin appears in SNpc neurons within 3 years of birth and increases linearly with age (Zecca et al. 2002). The pigment is derived from the oxidized metabolites of DA that form stable complex with iron, copper, and other metals (Sulzer et al. 2000; Zucca et al. 2006) and can be produced by DA neurons in vitro within several days of treatment with DA precursor L-3,4-dihydroxyphenylalanine (L-DOPA) (Sulzer and Zecca 2000). Because of the selective vulnerability of neuromelanin-containing neurons, dysregulation of DA homeostasis has been suggested to underlie neurodegeneration in PD (Edwards 1993; Gainetdinov et al. 1998a; Uhl 1998; Sulzer 2001; Lotharius and Brundin 2002).

2 DA Metabolism and Toxicity

Accumulation of cytosolic DA (DA_{cyt}) is toxic to cells in vitro (Mytilineou et al. 1993; Pardo et al. 1995; Sulzer et al. 2000; Fuentes et al. 2007; Mosharov et al. 2009) and several mechanisms for DA-mediated toxicity have been proposed. Spontaneous oxidation of DA in the presence of molecular oxygen produces hydrogen peroxide, hydroxyl radical, and superoxide (Graham 1978; Sulzer and Zecca 2000). Oxidation of the catechol moiety of DA yields DA-o-quinone and dopaminochrome (Sulzer and Zecca 2000); the latter can undergo a one-electron reduction to leukoaminochrome o-semiquinone radical that has been shown to produce other reactive species (Paris et al. 2009). Furthermore, DA-o-quinone reacts with free cysteine and exposed cysteine residues of proteins and glutathione, producing 5-S-cysteinyl-DA following cleavage by proteases. 5-S-cysteinyl-DA can undergo further oxidation and is toxic to cultured cells (Spencer et al. 2002) or when injected into the mouse brain (Zhang and Dryhurst 1994). 5-S-cysteinyl adducts of DA and its metabolites are readily detected in human SNpc confirming the physiological relevance of DA-induced protein damage (Fornstedt et al. 1989; Montine et al. 1995; Hastings and Berman 1999).

Other mechanisms of DA-mediated neurotoxicity include stabilization of toxic α-synuclein protofibrils (Rochet et al. 2004; Mosharov et al. 2006), inhibition of chaperone-mediated autophagy by DA-synuclein adducts (Martinez-Vicente et al. 2008), and reactions of DA with nitric oxide (Daveu et al. 1997), peroxynitrite

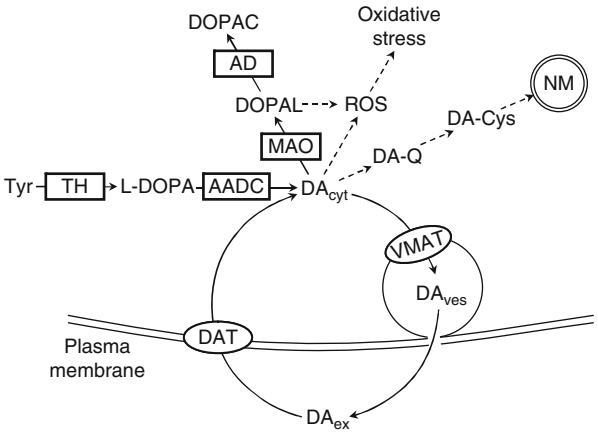

Fig. 1 DA homeostasis. Abbreviations: *AADC* aromatic L-amino acid decarboxylase, *AD* aldehyde dehydrogenase, *DA* dopamine (cytosolic, vesicular, and extracellular pools), *DAT* DA uptake transporter, *DA-Cys* 5-S-cysteinyl-DA, *DA-Q* DA-o-quinone, *DOPAC* 3,4-dihydroxyphenylacetate, *DOPAL* 3,4-dihydroxyphenylacetaldehyde, *L-DOPA* 3,4-dihydroxy-L-phenylalanine, *MAO* monoamine oxidase, *NM* neuromelanin inside autophagic vacuoles, *ROS* reactive oxygen species, *TH* tyrosine hydroxylase, *Tyr* tyrosine, *VMAT* vesicular monoamine transporter

(Daveu et al. 1997; Vauzour et al. 2008), and aldehydes (Collins and Bigdeli 1975; Deitrich and Erwin 1980; Naoi et al. 1993; Marchitti et al. 2007). Several recent reports confirm that a buildup of DA_{cyt} is indeed sufficient to induce progressive nigrostriatal neurodegeneration in rodents ((Caudle et al. 2007; Chen et al. 2008); also see below), although clinical studies of L-DOPA toxicity produced controversial results (Fahn et al. 2004; Holford et al. 2006; Chan et al. 2007).

Considering the toxic potential of DA, it is not surprising that multiple cellular mechanisms exist to ensure that DA concentration in the cytosol does not increase uncontrollably. It has been suggested that neuromelanin biosynthesis acts as one of the mechanisms for regulating toxic DA by-products by sequestering them into autophagic vacuoles (Sulzer and Zecca 2000). Other mechanisms include feedback inhibition of DA synthesis, catabolic DA cleavage, and synaptic vesicle sequestration.

2.1 Synthesis

Catecholamine neurotransmitters DA, norepinephrine, and epinephrine are defined by the presence of a catechol ring and an amine side-chain; together with indoleamine serotonin, these compounds are referred to as monoamines. DA and other catecholamines are synthesized from the nonessential amino acid tyrosine by a series of enzymatic reactions (Fig. 1).

In the first and typically rate-limiting step, tyrosine hydroxylase (TH) attaches a hydroxyl group to the aromatic ring of tyrosine using molecular oxygen,

tetrahydropterin, and ferrous iron (Fe^{2+}) as substrates and cofactors (Ramsey and Fitzpatrick 2000). TH activity is regulated on transcriptional, translational, and posttranslational levels (reviewed in (Goldstein and Lieberman 1992; Kumer and Vrana 1996; Fitzpatrick 2000; Daubner et al. 2011). The latter includes phosphorylation-dependent activation of TH by various kinases and its inhibition by DA, which competes for the binding site with tetrahydrobiopterin. This feedback mechanism limits DA production when its cytosolic concentration increases.

The second enzyme in DA biosynthesis, aromatic L-amino acid decarboxylase (AADC), uses pyridoxal phosphate as a cofactor and converts L-DOPA to DA. AADC activity can be regulated by second messenger systems; antagonists acting on extracellular DA receptors enhance AADC activity and agonists diminish it (reviewed in Hadjiconstantinou and Neff (2008)). Again, these mechanisms act to decrease DA production when its extracellular concentration increases. We recently found that AADC activity is increased by cytosolic Ca^{2+} (Mosharov et al. 2009). Although under normal conditions this mechanism may help to boost DA availability during high exocytotic activity, it may also increase the vulnerability of catecholaminergic cell populations that rely on Ca^{2+}- driven pacemaking, i.e., SNpc and LC neurons (Hetzenauer et al. 2006; Surmeier et al. 2010).

Importantly, AADC is not saturated under physiological conditions and increased L-DOPA concentration leads to its immediate conversion to DA. As L-DOPA is able to cross the blood–brain barrier, in the early 1960s it was introduced as an effective medication for PD (Hornykiewicz 2010). L-DOPA (levodopa), frequently in combination with the peripheral inhibitors of AADC carbidopa or benserazide, alleviates cardinal symptoms of PD and has remained the most commonly used drug for the treatment of the disease. L-DOPA treatment, however, does not halt the progression of PD, due to the ongoing loss of striatal terminals that store and release DA.

2.2 Cleavage

DA can be catabolized in the brain in a three step reaction that yields homovanillic acid (HVA) as a final product. First, monoamine oxidase (MAO) deaminates DA to produce 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is further oxidized to 3,4-dihydroxyphenylacetate (DOPAC) by aldehyde dehydrogenase. In the next reaction catalyzed by catechol-O-methyltransferase (COMT), a methyl residue is attached to one of the hydroxyl groups of the catechol ring preventing its possible auto-oxidation to quinone. COMT however is located in postsynaptic neurons and glial cells thus playing limited role in the clearance of DA in catecholaminergic neurons (Kaakkola et al. 1987; Myohanen et al. 2010).

Of the first two reactions, MAO-mediated deamination of DA that takes place on the outer membrane of the mitochondria is the rate-limiting step. There are two isoforms of the enzyme: MAO-A is expressed in catecholaminergic cells and MAO-B is more abundant in glial cells and serotonergic and histaminergic neurons (Westlund et al. 1985). Even though MAO constitutes the first step of detoxification

of DA, it produces two highly reactive compounds, DOPAL and hydrogen peroxide (H_2O_2), which can react with each other producing hydroxyl radical. Although aldehyde dehydrogenase activity is very high and maintains the steady-state concentration of DOPAL at low levels, possible neurotoxicity of the aldehyde metabolites of amines was predicted 60 years ago due to their extremely reactive nature (Blaschko 1952). Indeed, the presence of DOPAL and its conjugation products in the brain and their toxicity has been experimentally demonstrated both in vitro and in vivo (reviewed in (Burke et al. 2004)). The proposed mechanism of toxicity involves formation of free radicals followed by the opening of the mitochondrial permeability transition pore and apoptosis.

DOPAC, the final product of intracellular DA cleavage, possesses a catechol ring and, similar to DA and L-DOPA, can auto-oxidize to a quinone that further reacts with cysteine residues of proteins and glutathione. Cysteine conjugated catechols, 5-S-cysteinyl-DOPAC, 5-S-cysteinyl-DA, and 5-S-cysteinyl-DOPA, are considered to be markers of excess cytosolic DA and oxidative stress in vivo (Hastings and Berman 1999; Caudle et al. 2007).

Overall, intracellular DA catabolism may not provide neuroprotection because of the reactive nature of the DA cleavage products. Interestingly, two MAO inhibitors that cross the blood–brain barrier, selegiline and rasagiline, have been successfully tested as neuroprotective therapy in PD either as monotherapy or in combination with L-DOPA. It has been suggested, however, that the neuroprotective effect of these drugs does not depend on inhibition of MAO but rather is ascribed to the stabilization of mitochondria and the induction of antiapoptotic Bcl-2 protein family and neurotrophic factors (reviewed in Naoi and Maruyama (2010); Weinreb et al. (2010)).

2.3 Synaptic Vesicle Sequestration

Monoamines are stored at high concentrations in secretory vesicles. These organelles provide protection against degradation by metabolic enzymes and enable regulated release via exocytosis. Moreover, acidic pH of the vesicles prevents auto-oxidation of DA allowing to accumulate high intravesicular neurotransmitter concentrations. Vesicular monoamine transporters (VMAT) responsible for DA uptake into synaptic vesicles are members of the mammalian major facilitator superfamily (MFS) which show structural and functional homology to bacterial toxin-extruding exporters (TEXAN) (Yelin and Schuldiner 1995; Fluman and Bibi 2009). In prokaryotes, multidrug resistance transporters are located on the plasma membrane and remove toxins from cytosol into the extracellular milieu, whereas in eukaryotes they are found on membranes of various cellular organelles and transport their substrates from cytosol into endocytic compartments.

In accordance with the toxin-extruding nature of VMAT, mammalian cDNA of the transporter was cloned by Robert Edward's laboratory on the basis of the protection that it provided against 1-methyl-4-phenylpyridinium (MPP^+).

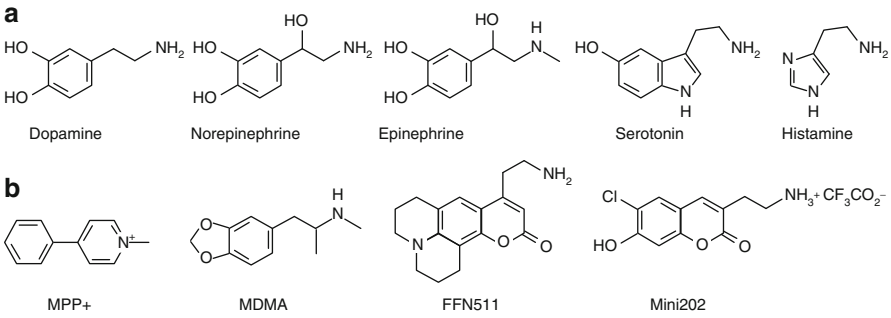

Fig. 2 Examples of (**a**) native and (**b**) synthetic substrates of VMAT. Abbreviations: *MPP*$^+$ 1-methyl-4-phenylpyridinium, *MDMA* 3,4-Methylenedioxymethamphetamine, ecstasy, *FFN511* false fluorescent transmitter 511, the first published optical probe designed to be a VMAT substrate, *Mini202* a fluorescent ratiometric VMAT substrate sensitive to changes in vesicular pH

This neurotoxin is an active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound that induces parkinsonism in humans and is used in animal models of PD. VMAT-mediated resistance results from the removal of the neurotoxin from cytosol into vesicular organelles and is blocked by VMAT inhibitor reserpine (Liu et al. 1992a, b).

There are two isoforms of mammalian VMAT that are encoded by two separate genes that share 60 % homology (reviewed in (Guillot and Miller 2009; Wimalasena 2011)). The more abundantly expressed VMAT2 is found in central and sympathetic neurons, mast cells, and histamine-releasing cells in the gut, whereas VMAT1 expression is limited to the large dense core vesicles of peripheral endocrine cells, including adrenal chromaffin cells where it is co-expressed with VMAT2 (Weihe et al. 1994; Peter et al. 1995; Erickson et al. 1996).

Substrate specificity of VMATs is surprisingly poor, and in addition to native transmitters, it can transport a diverse range of synthetic substrates (Fig. 2; see (Wimalasena 2011) for review). It appears that two principle structural constrains that define whether a substance is a VMAT substrate are the presence of the aminoethyl group and of an aromatic ring. Such substrate promiscuity was recently exploited to develop a new set of fluorescent VMAT substrates, FFN511 (Gubernator et al. 2009) and Mini202 (Lee et al. 2010), that provide optical tools for studies of stimulation-dependent exocytosis in catecholaminergic cells (Fig. 2).

VMAT-mediated translocation of DA across secretory vesicles membrane depends on the electrochemical gradient (Johnson 1988):

$$\mathrm{Log}\left(\frac{\mathrm{DA_{ves}}}{\mathrm{DA_{cyt}}}\right) = \frac{\Delta\Psi \cdot F}{R \cdot T} + 2 \cdot \Delta\mathrm{pH},$$

where $\mathrm{DA_{ves}}$ and $\mathrm{DA_{cyt}}$ are catecholamine concentrations inside and outside the vesicle, $\Delta\Psi$ is electrical gradient, F is Faraday's constant, R is the universal gas

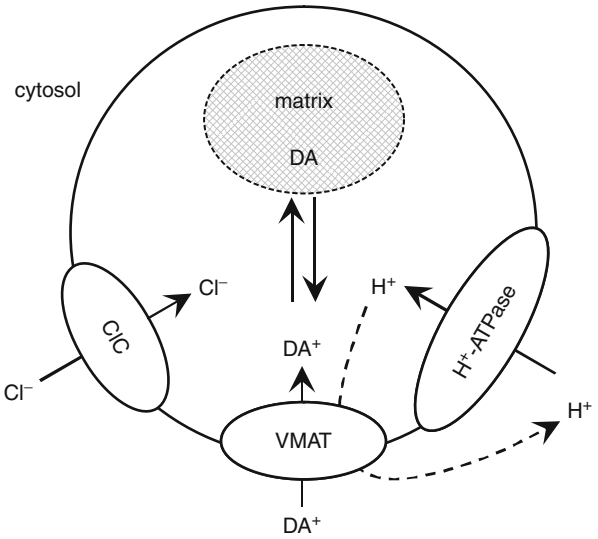

Fig. 3 VMAT-mediated uptake of DA into synaptic vesicles. VMAT exchanges two protons that move from vesicle to the cytosol for one positively charged DA molecule that moves from cytosol into the vesicle. Steep intravesicular proton gradient is generated by V-type H^+-ATPase that uses the energy of ATP hydrolysis to achieve vesicular pH of ~5.5. Chloride channels (*ClC*) help to dissipate positive charge resulting from the accumulation of protons and catecholamines inside the vesicles. Similarly, vesicular uptake of negatively charged glutamate in some midbrain DA neurons that co-express VMAT2 and VGLUT2 increases the amount of stored DA. As small synaptic vesicles have diameters of ~50 nm and release 1,000–3,000 molecules of DA during exocytosis (Pothos et al. 1998), vesicular concentration of neurotransmitter should reach 25–75 mM. Interestingly, in such a small organelle, pH 5.5 corresponds to 0.1 protons per vesicle

constant, T is absolute temperature, and ΔpH is the difference in pH between the cytosol and the vesicle.

Both the electrical and the chemical parts of the gradient are important for the transport (reviewed in (Edwards 2007; Guillot and Miller 2009)). The chemical gradient of protons is created by the vesicular proton pump, V-type H^+-ATPase (Fig. 3), and VMAT uses this energy by exchanging two protons for one DA molecule. As DA is positively charged, the electrical gradient also becomes important. The influx of Cl^- via the chloride channels neutralizes the buildup of positive charges due to the accumulation of DA and protons inside the vesicles therefore promoting neurotransmitter uptake. Work on isolated vesicles demonstrated that vesicle acidification is indeed much more efficient in the presence of chloride ions (Johnson 1988). Similarly, it has been shown that the knockout of the ClC-3 channel expressed on the vesicular membrane impairs vesicle acidification and induces degeneration of neuronal terminals in vivo (Stobrawa et al. 2001; Jentsch et al. 2005).

Recent data (Hnasko et al. 2010; Stuber et al. 2010) indicate that a subpopulation of midbrain neurons that project to the nucleus accumbens shell co-express VMAT2 and vesicular glutamate transporter 2 (VGLUT2). Vesicular uptake of negatively charged glutamate was found to promote accumulation of DA in the same vesicles, probably by dissipating $\Delta\Psi$ and increasing the pH gradient that drives VMAT2-mediated monoamine transport. This suggests yet another presynaptic mechanism that regulates dopaminergic transmission.

At a steady state, when $\Delta\Psi$ and pH in the cytosol are constant, a linear correlation is expected (i) between DA_{ves} and DA_{cyt} and (ii) between DA_{ves} and the square of the proton concentration in the vesicle:

$$DA_{ves} = A \cdot DA_{cyt} \cdot [H^+]^2_{ves}, \text{ where } A = 10^{\frac{\Delta\Psi \cdot F}{R \cdot T}} / [H^+]^2_{cyt}$$

Indeed, both relationships have been confirmed using optical measurements of vesicular pH and electrochemical detection of DA_{cyt} and the amount of the transmitter released during stimulation-dependent vesicle exocytosis (Pothos et al. 1998; Markov et al. 2008; Mosharov et al. 2009). It is important to note, however, that the total amount of stored DA can be much higher than the amount calculated from DA_{ves} because of the presence of the vesicular "matrix" (Fig. 3). In large dense core vesicles that store norepinephrine and epinephrine, negatively charged chromogranins and secretogranins bind catecholamines allowing up to 0.5 M concentrations of vesicular transmitter (Albillos et al. 1997), which would be impossible if the osmotic and the electrical gradient were not dissipated. It remains an open question whether similar polyelectrolyte proteins play a role in DA storage in small synaptic vesicles, although the high concentrations of vesicular transmitter seem to imply this (Fig. 3).

2.4 Reuptake

DA released by stimulation-dependent exocytosis acts on pre- and postsynaptic DA receptors, and its action is terminated by (1) passive diffusion, (2) COMT-mediated catabolism, and (3) reuptake by the dopamine uptake transporter (DAT).

DAT-mediated reuptake of DA has received considerable attention because this transporter is targeted by psychostimulants and drugs of abuse (reviewed in (Schmitt and Reith 1187; Zhu and Reith 2008; Sulzer 2011)). DAT activity and surface expression is regulated by phosphorylation/dephosphorylation in response to changes in extracellular DA concentration. Reuptake of DA and termination of its action on extracellular receptors also plays an important role in regulating intracellular DA pools, as illustrated in DAT-deficient mice (Gainetdinov et al. 1998b; Jones et al. 1998). In addition to expected significant elevation of extracellular DA levels, the tissue content of dopamine in DAT knockout striatum was less than 5 % of wild-type levels. It was suggested that recycled DA contributes

significantly to the maintenance of vesicular stores of DA in striatal projections of midbrain neurons. Alternatively, the observed reduction of DA intracellular stores may be due to hyperactivation of DA autoreceptors and the consequent inhibition of DA synthesis discussed above.

The importance of balanced maintenance of DA concentration in neuronal cytosol was demonstrated in a transgenic mouse that was engineered to express DAT on the plasma membrane of glutamatergic medium spiny neurons (MSNs) (Chen et al. 2008). MSNs constitute the majority of the neuronal population in the striatum and normally are exposed to DA only from the outside. In these transgenic animals however, MSNs can take up DA released by striatal projections of the midbrain dopaminergic neurons. Within several weeks following the induction of DAT expression, the mice develop motor dysfunctions, significant striatal and cortical atrophy, and progressive neurodegeneration of MSNs accompanied by oxidative stress, protein damage, and decreased glutathione levels. L-DOPA treatment worsened these symptoms, while reducing DA input to the striatum by lesioning the medial forebrain bundle had the opposite effect, demonstrating the dependence of the pathology on DA supply.

2.5 Spatial Considerations

The contribution of vesicular DA uptake differs significantly between the cell bodies and axonal terminals of neurons as the later have smaller cytosolic volume and higher concentration of vesicles. Because of this difference in stored-to-total DA ratio, DA_{cyt} in synaptic terminals will be more dependent on VMAT-mediated DA uptake, which can be illustrated by a simple calculus. If one synaptic vesicle releases all its content inside the neuronal soma, the change in DA_{cyt} will equal $DA_{ves}/N_A/V_{cell}$, where DA_{ves}, vesicular quantal size, is ~2,000 molecules (Pothos et al. 1998); N_A is Avogadro's Constant ($6.023*10^{23}$ molecules/mole); and V_{cell} is the cell soma volume (~1.77 pL for a cell with a diameter of 15 μm). It follows that emptying one vesicle would increase DA_{cyt} by ~2 nM. If, however, vesicular content is emptied inside a synaptic terminal with the diameter of ~1 μm, DA_{cyt} will be increased by >6 μM: a 3,000-fold difference.

We have recently investigated the contribution of DA metabolic pathways in the maintenance of cytosolic neurotransmitter concentration in cultured chromaffin cells (Mosharov et al. 2003) and neuronal cell bodies (Mosharov et al. 2009). Adrenal chromaffin cells, which lack neurites and are filled with secretory vesicles that each contain ~1,000,000 epinephrine or norepinephrine molecules (Wightman et al. 1991), are closer to synaptic terminals in their stored-to-total DA ratio. Cytosolic catecholamine concentration in these cells is 10–20 μM and can be increased severalfold by L-DOPA treatment. Consistent with strict control of the level of cytosolic catecholamine concentration, inhibition of either MAO or VMAT does not alter cytosolic transmitter levels, presumably because one pathway compensates for decreased activity of the other. When, however, both MAO and VMAT are blocked simultaneously, cytosolic catecholamine levels increase by more than sixfold.

In contrast to chromaffin cells, DA_{cyt} in neuronal cell bodies is below the limits of detection (<0.1 μM) unless augmented by L-DOPA pretreatment (Mosharov et al. 2009). Importantly, DA_{cyt} increases readily upon treatment with MAO inhibitors, but no contribution of somatic VMAT-mediated uptake can be detected either in the absence or in the presence of MAO blockade. Obviously, some DA secretory organelles reside in or close to neuronal cell bodies, as evident from somatodendritic DA release from midbrain neurons (Rice et al. 1997; Jaffe et al. 1998); however, the number of DA storage vesicles in the soma is probably insufficient to have significant effect on DA_{cyt} following VMAT blockade.

Amphetamine and its derivatives are often used to disrupt vesicular and DA_{cyt} pools. These psychostimulants have multiple intracellular sites of action, including inhibition of MAO, stimulation of catecholamine synthesis, redistribution of catecholamines from secretory vesicles to the cytosol, and induction of catecholamine reverse transport from the cytosol to the extracellular milieu via dopamine and norepinephrine uptake transporters (reviewed in (Sulzer 2011)). Chromaffin cells acutely treated with methamphetamine (METH) display decreased quantal size (Sulzer et al. 1995; Anderson et al. 1998; Mundorf et al. 1999) and increased cytosolic catecholamine levels, indicating that effects of METH on DA metabolism and the leakage of DA from vesicles are the dominant processes in these cells (Mosharov et al. 2003). In contrast, neurons exposed to METH showed decreased DA_{cyt} in the somas (Mosharov et al. 2009), demonstrating that DAT-mediated reverse transport governs the steady-state DA concentration, while redistribution of neurotransmitter from vesicles has negligible effect in neuronal cell bodies. While it is currently impossible to measure cytosolic DA in axonal terminals due to their small size, their similarity to chromaffin cells suggests a possibility that an equally large increase in DA_{cyt} occurs in METH-treated neuronal terminals. If so, this may explain a significant increase in striatal 5-S-cysteinyl-DA levels (LaVoie and Hastings 1999) and the pattern of METH-induced neurodegeneration in which neurites but not cell bodies are damaged in vitro (Cubells et al. 1994; Larsen et al. 2002), in animal models in vivo (Ricaurte et al. 1982), and in imaging studies of METH abusers after prolonged abstinence (Sang et al. 2007).

2.6 Duration of Exposure

The time during which a cell is exposed to the toxic effect of DA and its metabolites is another parameter that significantly affects neurotoxicity. In vitro, DA-mediated neurotoxicity depends linearly on the dose (concentration*time) of cell exposure to elevated DA_{cyt} levels, i.e., neurons can be quite resistant to short bursts of high DA_{cyt} but are compromised if small elevations in cytosolic transmitter persist for sufficiently long time (Mosharov et al. 2009). This consideration becomes very important when comparing data obtained from experiments in cellular and animal models with neuropathology in human as even the early onset type of PD develops over decades of life, which is orders of magnitude longer than the life span of rodents and cells in culture.

3 VMAT Expression and DA-Mediated Neurotoxicity

3.1 Less VMAT, More Toxicity

Considerable experimental evidence supports the idea that decreased vesicular uptake correlates with enhanced toxicity. Reserpine, an inhibitor of VMAT, induces parkinsonism by depleting releasable pool of DA from striatal terminals (Carlsson et al. 1957). The striatum of animals treated with reserpine shows marked rise in 5-S-cysteinyl-DA and oxidized glutathione levels, indicative of elevated cytosolic DA production and an ongoing oxidation stress (Fornstedt and Carlsson 1989; Spina and Cohen 1989; Bilska et al. 2007). Although no dopaminergic cell loss has been reported in reserpinized animals, a 7-day treatment with tetrabenazine, another VMAT inhibitor, induces irreversible histological changes in the neurons of SNpc (Satou et al. 2001). In the amphetamine-induced model of axonal degeneration in the striatum, reserpine exacerbated drug-induced toxicity, whereas pretreatment with TH inhibitor α-methyl-p-tyrosine provided protection (Wagner et al. 1983). Additionally, reserpine causes concentration-dependent death of RCSN-3 cells, a catecholaminergic cell line derived from adult rat SNpc (Fuentes et al. 2007).

Several genetic mouse models with various levels of VMAT2 expression have been created to study the role of the transporter in neurotoxicity (see (Guillot and Miller 2009; Taylor et al. 2011) for comprehensive reviews). Mice with a homozygous deletion of VMAT2 transporter ($VMAT2^{-/-}$) do not show any gross anatomical abnormalities, including normal number of neurons in SNpc, but die within a few days after birth, probably because they do not feed (Fon et al. 1997; Takahashi et al. 1997; Wang et al. 1997). The brains of $VMAT2^{-/-}$ animals manifest severely compromised DA storage (~1.5 % of wild-type levels). Midbrain neuronal cultures from these mice show normal morphology and TH fibers density but contain only 3 % of the intracellular DA levels of the $VMAT2^{+/+}$ cultures and are unable to accumulate DA within synaptic vesicles (Fon et al. 1997; Yamamoto et al. 2007). Importantly, $VMAT2^{-/-}$ neurons are significantly more sensitive to METH-induced neurodegeneration of primarily neurites and display intense labeling for ROS formation upon drug treatment (Larsen et al. 2002). These data indicate that although METH-induced oxidative stress does not require the presence of a vesicular pool of DA, vesicular sequestration of the neurotransmitter provides significant neuroprotection.

In contrast to animals with complete VMAT2 deletion, VMAT2 heterozygous mice ($VMAT2^{+/-}$) are viable to adulthood. $VMAT2^{+/-}$ mice display ~50 % reduced total DA, diminished levels of extracellular striatal DA, as well as decreased potassium- and amphetamine-evoked DA release in vivo and in postnatally cultured cells (Fon et al. 1997; Takahashi et al. 1997; Wang et al. 1997). Although the animals perform normally on motor tests, they show depressive-like phenotype and impairments in learned helplessness and conditioned place preference paradigms (Takahashi et al. 1997; Wang et al. 1997; Fukui et al. 2007). As expected, mice are more sensitive to neurodegeneration induced by

methamphetamine and MPTP (Gainetdinov et al. 1998a; Fumagalli et al. 1999), confirming the ability of VMAT2 to protect cells by removing toxins from the cytosol. Though no PD-like neuropathology was observed in VMAT2$^{+/-}$ mice up to 7-month of age, further studies are required to determine whether neurodegeneration of the nigrostriatal system may occur later in life. Interestingly, while no loss of the nigrostriatal biochemical markers was found in VMAT2$^{+/-}$ animals injected with L-DOPA for 28 days (Reveron et al. 2002), cultured DA neurons from these mice show increased vulnerability to the toxic effect of L-DOPA (Kariya et al. 2005).

Another line of mice that proved extremely helpful in PD research expresses only 5 % of the normal level of VMAT2. The first generation of mice that have a hypomorphic allele of the VMAT2 (VMAT2-deficient KA1 line (Mooslehner et al. 2001)) was later found to have a spontaneous deletion of the alpha-synuclein gene (Specht and Schoepfer 2001; Colebrooke et al. 2006). As this protein plays an important role in the pathology of PD (reviewed in (Vila and Przedborski 2004; Eriksen et al. 2005; Sulzer 2007; Dawson et al. 2010)), further breeding was done to remove all traces of alpha-synuclein deficiency.

The resulting VMAT2-LO mice show 95 % reduction of VMAT2 expression and 85 % reduction in DA levels in the striatum (Caudle et al. 2007). As the animals age, they show signs of PD-like progressive neurodegeneration, including L-DOPA-responsive motor deficits; oxidative stress and protein damage; decreased DA, DAT, and TH levels in the striatum; and pathological accumulations of alpha-synuclein and a reduced number of DA neurons in the SNpc (Caudle et al. 2007; Taylor et al. 2011). Importantly, animals do not manifest motor symptoms and some of the biochemical markers until they are 12–28-month-old (Taylor et al. 2011), highlighting the slow nature of progressive neurodegeneration in this animal model.

The onset of the motor symptoms in PD is accompanied and generally preceded by a variety of nonmotor disturbances, including but not limited to olfactory deficits, constipation, sleep disorders, and depression. It has been suggested that degeneration of other monoamine systems, such as norepinephrine neurons in LC and serotonin neurons in the dorsal raphe nucleus, may contribute to the development of the nonmotor symptoms (reviewed in (Dauer and Przedborski 2003; Fornai et al. 2007; Rommelfanger and Weinshenker 2007; Fahn 2008; Obeso et al. 2008; Delaville et al. 2011; Lang 2011)). Biochemical characterization of VMAT2-LO mice revealed progressive depletion of all monoamines (Caudle et al. 2007; Taylor et al. 2009) as well as age-dependent neurodegeneration in the LC that preceded the loss of neurons in the SNpc (Taylor et al. 2011), similar to the clinical data from PD patients. Moreover, VMAT2-LO mice demonstrate early deficits in olfactory discrimination, delayed gastric emptying, altered sleep latency, anxiety-like behavior, and age-dependent depressive behavior (Taylor et al. 2009), confirming the involvement of monoamine dysfunction in the manifestation of nonmotor symptoms of PD. Overall, the VMAT2-LO mouse model recapitulated the majority of motor and nonmotor symptoms of PD and demonstrates that

a reduced capacity of cells to sequester DA and other monoamines inside storage vesicles is sufficient to cause PD-like neurodegeneration of neurons and their axonal projections.

3.2 More VMAT, Less Toxicity

As VMAT2 should prevent DA-induced toxicity by sequestering neurotransmitter inside synaptic vesicles, enhancing VMAT-mediated transport is expected to be neuroprotective. Clinical data indirectly support this claim as neurons in the ventral tegmental area, a dopaminergic cell population that is spared in PD, express higher levels of VMAT2 and produce lower amounts of neuromelanin compared to neurons in SNpc (Hirsch et al. 1988; Zecca et al. 2001; Liang et al. 2004). Furthermore, higher expression level of VMAT2 compared to that of DAT has been suggested to predict lower vulnerability of neuronal populations in PD (Uhl 1998; Miller et al. 1999), while gain-of-function haplotypes in the human VMAT2 gene reduce the risk of developing PD in women (Glatt et al. 2006).

The link between neurotoxicity and the activity of VMAT2 has been directly studied in vitro using cultured cells. As described above, mouse midbrain neurons treated for several days with DA precursor L-DOPA produce neuromelanin due to accumulation of cytosolic DA and downstream oxidation products (Sulzer et al. 2000). Virus-mediated overexpression of VMAT2 in these cultures prevents neuromelanin synthesis, increases the number of DA molecules packaged per synaptic vesicle, decreases DA_{cyt}, and efficiently protects neurons from L-DOPA-induced cell death (Pothos et al. 2000; Sulzer and Zecca 2000; Mosharov et al. 2009). Similarly, overexpression of VMAT2 in primary rat mesencephalic cultures and PC12 cells, a pheochromocytoma-derived cell line, increases intracellular DA content, augments stimulation-dependent DA release, and attenuates cell death induced by METH; downregulation of VMAT2 using virally delivered shRNAs produces an opposite effect (Vergo et al. 2007). Additionally, increased expression of VMAT2 in vivo is able to mitigate the oxidative stress and gliosis caused by METH (Guillot et al. 2008). Together these data strongly support the inverse relationship between DA-induced neurotoxicity and the activity of VMAT2.

4 Conclusion

Overview of enzymes and transporters involved in DA homeostasis illustrates that all metabolic steps work to maximize the amount of DA available for exocytosis while at the same time limiting the accumulation of potentially toxic cytosolic transmitter. Importantly, all except one metabolic step in DA turnover affect neurotoxicity and neurotransmission in the same direction, i.e., by altering the levels of cytosolic and vesicular DA in synchrony. For example, increased activities of TH and AADC will enhance DA-mediated toxicity and the amount of DA stored

in vesicles, whereas increased catabolism will diminish them both. The sole exception is the activity of VMAT, which has opposing effects on cytosolic and vesicular DA levels. This unique position of VMAT in DA handling provides therapeutic advantages that can be explored in future drug development.

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References

Albillos, A., Dernick, G., Hostmann, H., Almers, W., Alvarez de Toledo, G., & Lindau, M. (1997). The exocytotic event in chromaffin cells revealed by patch amperometry. *Nature, 389*, 509–512.

Anderson, B. B., Chen, G., Gutman, D. A., Ewing, A. G. (1998). Dopamine levels of two classes of vesicles are differentially depleted by amphetamine. Brain Res 788:294–301.

Baldereschi, M., Di Carlo, A., Rocca, W. A., Vanni, P., Maggi, S., Perissinotto, E., et al. (2000). Parkinson's disease and parkinsonism in a longitudinal study: Two-fold higher incidence in men. ILSA Working Group. Italian Longitudinal Study on Aging. *Neurology, 55*(9), 1358–1363.

Bilska, A., Dubiel, M., Sokolowska-Jezewicz, M., Lorenc-Koci, E., & Wlodek, L. (2007). Alpha-lipoic acid differently affects the reserpine-induced oxidative stress in the striatum and prefrontal cortex of rat brain. *Neuroscience, 146*(4), 1758–1771.

Blaschko, H. (1952). Amine oxidase and amine metabolism. *Pharmacological Reviews, 4*(4), 415–458.

Burke, W. J., Li, S. W., Chung, H. D., Ruggiero, D. A., Kristal, B. S., Johnson, E. M., et al. (2004). Neurotoxicity of MAO metabolites of catecholamine neurotransmitters: Role in neurodegenerative diseases. *Neurotoxicology, 25*(1–2), 101–115.

Carlsson, A., Lindqvist, M., & Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature, 180*(4596), 1200.

Caudle, W. M., Richardson, J. R., Wang, M. Z., Taylor, T. N., Guillot, T. S., McCormack, A. L., et al. (2007). Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *Journal of Neuroscience, 27*(30), 8138–8148.

Chan, P. L., Nutt, J. G., & Holford, N. H. (2007). Levodopa slows progression of Parkinson's disease: External validation by clinical trial simulation. *Pharmaceutical Research, 24*(4), 791–802.

Chen, L., Ding, Y., Cagniard, B., Van Laar, A. D., Mortimer, A., Chi, W., et al. (2008). Unregulated cytosolic dopamine causes neurodegeneration associated with oxidative stress in mice. *Journal of Neuroscience, 28*(2), 425–433.

Colebrooke, R. E., Humby, T., Lynch, P. J., McGowan, D. P., Xia, J., & Emson, P. C. (2006). Age-related decline in striatal dopamine content and motor performance occurs in the absence of nigral cell loss in a genetic mouse model of Parkinson's disease. *European Journal of Neuroscience, 24*(9), 2622–2630.

Collins, M. A., & Bigdeli, M. G. (1975). Tetrahydroisoquinolines in vivo. I. Rat brain formation of salsolinol, a condensation product of dopamine and acetaldehyde, under certain conditions during ethanol intoxication. *Life Sciences, 16*(4), 585–601.

Cubells, J. F., Rayport, S., Rajendran, G., & Sulzer, D. (1994). Methamphetamine neurotoxicity involves vacuolation of endocytic organelles and dopamine-dependent intracellular oxidative stress. *Journal of Neuroscience, 14*, 2260–2271.

Daubner, S. C., Le, T., & Wang, S. (2011). Tyrosine hydroxylase and regulation of dopamine synthesis. *Archives of Biochemistry and Biophysics, 508*(1), 1–12.

Dauer, W., & Przedborski, S. (2003). Parkinson's disease: Mechanisms and models. *Neuron, 39*(6), 889–909.

Daveu, C., Servy, C., Dendane, M., Marin, P., & Ducrocq, C. (1997). Oxidation and nitration of catecholamines by nitrogen oxides derived from nitric oxide. *Nitric Oxide, 1*(3), 234–243.

Dawson, T. M., Ko, H. S., & Dawson, V. L. (2010). Genetic animal models of Parkinson's disease. *Neuron, 66*(5), 646–661.

de Rijk, M. C., Tzourio, C., Breteler, M. M., Dartigues, J. F., Amaducci, L., Lopez-Pousa, S., et al. (1997). Prevalence of parkinsonism and Parkinson's disease in Europe: The EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry, 62*(1), 10–15.

Deitrich, R., & Erwin, V. (1980). Biogenic amine-aldehyde condensation products: Tetrahydroisoquinolines and tryptolines (beta-carbolines). *Annual Review of Pharmacology and Toxicology, 20*, 55–80.

Delaville, C., Deurwaerdere, P. D., & Benazzouz, A. (2011). Noradrenaline and Parkinson's disease. *Frontiers in Systems Neuroscience, 5*(31), 31.

Edwards, R. H. (1993). Neural degeneration and the transport of neurotransmitters. *Annals of Neurology, 34*(5), 638–645.

Edwards, R. H. (2007). The neurotransmitter cycle and quantal size. *Neuron, 55*(6), 835–858.

Erickson, J. D., Schafer, M. K., Bonner, T. I., Eiden, L. E., & Weihe, E. (1996). Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. *Proceedings of the National Academy of Sciences of the United States of America, 93*(10), 5166–5171.

Eriksen, J. L., Przedborski, S., & Petrucelli, L. (2005). Gene dosage and pathogenesis of Parkinson's disease. *Trends in Molecular Medicine, 11*(3), 91–96.

Fahn, S. (2008). The history of dopamine and levodopa in the treatment of Parkinson's disease. *Movement Disorders, 23*(Suppl 3), S497–S508.

Fahn, S., & Sulzer, D. (2004). Neurodegeneration and neuroprotection in Parkinson disease. *NeuroRx, 1*(1), 139–154.

Fahn, S., Oakes, D., Shoulson, I., Kieburtz, K., Rudolph, A., Lang, A., et al. (2004). Levodopa and the progression of Parkinson's disease. *The New England Journal of Medicine, 351*(24), 2498–2508.

Fitzpatrick, P. F. (2000). The aromatic amino acid hydroxylases. *Advances in Enzymology and Related Areas of Molecular Biology, 74*, 235–294.

Fluman, N., & Bibi, E. (2009). Bacterial multidrug transport through the lens of the major facilitator superfamily. *Biochimica et Biophysica Acta, 1794*(5), 738–747.

Fon, E. A., Pothos, E. N., Sun, B. C., Killeen, N., Sulzer, D., & Edwards, R. H. (1997). Vesicular transport regulates monoamine storage and release but is not essential for amphetamine action. *Neuron, 19*(6), 1271–1283.

Fornai, F., di Poggio, A. B., Pellegrini, A., Ruggieri, S., & Paparelli, A. (2007). Noradrenaline in Parkinson's disease: from disease progression to current therapeutics. *Current Medicinal Chemistry, 14*(22), 2330–2334.

Fornstedt, B., & Carlsson, A. (1989). A marked rise in 5-S-cysteinyl-dopamine levels in guinea-pig striatum following reserpine treatment. *Journal of Neural Transmission, 76*(2), 155–161.

Fornstedt, B., Brun, A., Rosengren, E., & Carlsson, A. (1989). The apparent autoxidation rate of catechols in dopamine-rich regions of human brains increases with the degree of depigmentation of substantia nigra. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 1*(4), 279–295.

Fuentes, P., Paris, I., Nassif, M., Caviedes, P., & Segura-Aguilar, J. (2007). Inhibition of VMAT-2 and DT-diaphorase induce cell death in a substantia nigra-derived cell line – An experimental cell model for dopamine toxicity studies. *Chemical Research in Toxicology, 20*(5), 776–783.

Fukui, M., Rodriguiz, R. M., Zhou, J., Jiang, S. X., Phillips, L. E., Caron, M. G., et al. (2007). Vmat2 heterozygous mutant mice display a depressive-like phenotype. *Journal of Neuroscience, 27*(39), 10520–10529.

Fumagalli, F., Gainetdinov, R. R., Wang, Y. M., Valenzano, K. J., Miller, G. W., & Caron, M. G. (1999). Increased methamphetamine neurotoxicity in heterozygous vesicular monoamine transporter 2 knock-out mice. *Journal of Neuroscience, 19*(7), 2424–2431.

Gainetdinov, R. R., Fumagalli, F., Wang, Y. M., Jones, S. R., Levey, A. I., Miller, G. W., et al. (1998a). Increased MPTP neurotoxicity in vesicular monoamine transporter 2 heterozygote knockout mice. *Journal of Neurochemistry, 70*, 1973–1978.

Gainetdinov, R. R., Jones, S. R., Fumagalli, F., Wightman, R. M., & Caron, M. G. (1998b). Re-evaluation of the role of the dopamine transporter in dopamine system homeostasis. *Brain Research. Brain Research Reviews, 26*(2–3), 148–153.

Gamo, N. J., & Arnsten, A. F. (2011). Molecular modulation of prefrontal cortex: Rational development of treatments for psychiatric disorders. *Behavioral Neuroscience, 125*(3), 282–296.

Glatt, C. E., Wahner, A. D., White, D. J., Ruiz-Linares, A., & Ritz, B. (2006). Gain-of-function haplotypes in the vesicular monoamine transporter promoter are protective for Parkinson disease in women. *Human Molecular Genetics, 15*(2), 299–305.

Goldstein, M., & Lieberman, A. (1992). The role of the regulatory enzymes of catecholamine synthesis in Parkinson's disease. *Neurology, 42*(Suppl 4), 8–12.

Graham, D. G. (1978). Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Molecular Pharmacology, 14*(4), 633–643.

Gubernator, N. G., Zhang, H., Staal, R. G., Mosharov, E. V., Pereira, D. B., Yue, M., et al. (2009). Fluorescent false neurotransmitters visualize dopamine release from individual presynaptic terminals. *Science, 324*(5933), 1441–1444.

Guillot, T. S., & Miller, G. W. (2009). Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons. *Molecular Neurobiology, 39*(2), 149–170.

Guillot, T. S., Richardson, J. R., Wang, M. Z., Li, Y. J., Taylor, T. N., Ciliax, B. J., et al. (2008). PACAP38 increases vesicular monoamine transporter 2 (VMAT2) expression and attenuates methamphetamine toxicity. *Neuropeptides, 42*(4), 423–434.

Hadjiconstantinou, M., & Neff, N. H. (2008). Enhancing aromatic L-amino acid decarboxylase activity: Implications for L-DOPA treatment in Parkinson's disease. *CNS Neuroscience & Therapeutics, 14*(4), 340–351.

Hastings, T. G., & Berman, S. B. (1999). Dopamine-induced toxicity and quinone modification of proteins: Implications for Parkinson's disease. In C. R. Creveling (Ed.), *Role of catechol quinone species in cellular toxicity* (pp. 69–89). Johnson City, TN: F.P. Graham Publishing.

Hetzenauer, A., Sinnegger-Brauns, M. J., Striessnig, J., & Singewald, N. (2006). Brain activation pattern induced by stimulation of L-type Ca^{2+}−channels: Contribution of Ca(V)1.3 and Ca(V) 1.2 isoforms. *Neuroscience, 139*(3), 1005–1015.

Hirsch, E., Graybiel, A. M., & Agid, Y. A. (1988). Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature, 334*(6180), 345–348.

Hnasko, T. S., Chuhma, N., Zhang, H., Goh, G. Y., Sulzer, D., Palmiter, R. D., et al. (2010). Vesicular glutamate transport promotes dopamine storage and glutamate corelease in vivo. *Neuron, 65*(5), 643–656.

Holford, N. H., Chan, P. L., Nutt, J. G., Kieburtz, K., & Shoulson, I. (2006). Disease progression and pharmacodynamics in Parkinson disease – Evidence for functional protection with levodopa and other treatments. *Journal of Pharmacokinetics and Pharmacodynamics, 33*(3), 281–311.

Hornykiewicz, O. (2010). A brief history of levodopa. *Journal of Neurology, 257*(Suppl 2), S249–S252.

Jaffe, E. H., Marty, A., Schulte, A., & Chow, R. H. (1998). Extrasynaptic vesicular transmitter release from the somata of substantia nigra neurons in rat midbrain slices. *Journal of Neuroscience, 18*(10), 3548–3553.

Jentsch, T. J., Poet, M., Fuhrmann, J. C., & Zdebik, A. A. (2005). Physiological functions of CLC Cl- channels gleaned from human genetic disease and mouse models. *Annual Review of Physiology, 67*, 779–807.

Johnson, R. G. (1988). Accumulation of biological amines into chromaffin granules: A model for hormone and neurotransmitter transport. *Physiological Reviews, 68*, 232–307.

Jones, S. R., Gainetdinov, R. R., Jaber, M., Giros, B., Wightman, R. M., & Caron, M. G. (1998). Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proceedings of the National Academy of Sciences of the United States of America, 95*(7), 4029–4034.

Kaakkola, S., Mannisto, P. T., & Nissinen, E. (1987). Striatal membrane-bound and soluble catechol-O-methyl-transferase after selective neuronal lesions in the rat. *Journal of Neural Transmission, 69*(3–4), 221–228.

Kariya, S., Takahashi, N., Hirano, M., & Ueno, S. (2005). Increased vulnerability to L-DOPA toxicity in dopaminergic neurons From VMAT2 heterozygote knockout mice. *Journal of Molecular Neuroscience, 27*(3), 277–279.

Kumer, S. C., & Vrana, K. E. (1996). Intricate regulation of tyrosine hydroxylase activity and gene expression. *Journal of Neurochemistry, 67*(2), 443–462.

Lang, A. E. (2011). A critical appraisal of the premotor symptoms of Parkinson's disease: Potential usefulness in early diagnosis and design of neuroprotective trials. *Movement Disorders, 26*(5), 775–783.

Larsen, K. E., Fon, E. A., Hastings, T. G., Edwards, R. H., & Sulzer, D. (2002). Methamphetamine-induced degeneration of dopaminergic neurons involves autophagy and upregulation of dopamine synthesis. *Journal of Neuroscience, 22*(20), 8951–8960.

LaVoie, M. J., & Hastings, T. G. (1999). Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: Evidence against a role for extracellular dopamine. *Journal of Neuroscience, 19*(4), 1484–1491.

Lee, M., Gubernator, N. G., Sulzer, D., & Sames, D. (2010). Development of pH-responsive fluorescent false neurotransmitters. *Journal of the American Chemical Society, 132*(26), 8828–8830.

Liang, C. L., Nelson, O., Yazdani, U., Pasbakhsh, P., & German, D. C. (2004). Inverse relationship between the contents of neuromelanin pigment and the vesicular monoamine transporter-2: Human midbrain dopamine neurons. *The Journal of Comparative Neurology, 473*(1), 97–106.

Liu, Y., Peter, D., Roghani, A., Schuldiner, S., Prive, G. G., Eisenberg, D., et al. (1992a). A cDNA that suppresses MPP^+ toxicity encodes a vesicular amine transporter. *Cell, 70*, 539–551.

Liu, Y., Roghani, A., & Edwards, R. H. (1992b). Gene transfer of a reserpine-sensitive mechanism of resistance to *N*-methyl-4-phenylpyridinium. *Proceedings of the National Academy of Sciences of the United States of America, 89*(19), 9074–9078.

Lotharius, J., & Brundin, P. (2002). Impaired dopamine storage resulting from alpha-synuclein mutations may contribute to the pathogenesis of Parkinson's disease. *Human Molecular Genetics, 11*(20), 2395–2407.

Marchitti, S. A., Deitrich, R. A., & Vasiliou, V. (2007). Neurotoxicity and metabolism of the catecholamine-derived 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylglycolaldehyde: The role of aldehyde dehydrogenase. *Pharmacological Reviews, 59*(2), 125–150.

Markov, D., Mosharov, E. V., Setlik, W., Gershon, M. D., & Sulzer, D. (2008). Secretory vesicle rebound hyperacidification and increased quantal size resulting from prolonged methamphetamine exposure. *Journal of Neurochemistry, 107*(6), 1709–1721.

Martin, I., Dawson, V. L., & Dawson, T. M. (2011). Recent advances in the genetics of Parkinson's disease. *Annual Review of Genomics and Human Genetics, 12*:301–325.

Martinez-Vicente, M., Talloczy, Z., Kaushik, S., Massey, A. C., Mazzulli, J., Mosharov, E. V., et al. (2008). Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *The Journal of Clinical Investigation, 118*(2), 777–788.

Miller, G. W., Gainetdinov, R. R., Levey, A. I., & Caron, M. G. (1999). Dopamine transporters and neuronal injury. *Trends in Pharmacological Sciences, 20*(10), 424–429.

Montine, T. J., Farris, D. B., & Graham, D. G. (1995). Covalent crosslinking of neurofilament proteins by oxidized catechols as a potential mechanism of Lewy body formation. *Journal of Neuropathology and Experimental Neurology, 54*(3), 311–319.

Mooslehner, K. A., Chan, P. M., Xu, W., Liu, L., Smadja, C., Humby, T., et al. (2001). Mice with very low expression of the vesicular monoamine transporter 2 gene survive into adulthood: Potential mouse model for parkinsonism. *Molecular and Cellular Biology, 21*(16), 5321–5331.

Mosharov, E. V., Gong, L.-W., Khanna, B., Lindau, M., & Sulzer, D. (2003). Intracellular patch electrochemistry: Regulation of cytosolic catecholamines in chromaffin cells. *Journal of Neuroscience, 23*(13), 5835–5845.

Mosharov, E. V., Staal, R. G., Bove, J., Prou, D., Hananiya, A., Markov, D., et al. (2006). Alpha-synuclein overexpression increases cytosolic catecholamine concentration. *Journal of Neuroscience, 26*(36), 9304–9311.

Mosharov, E. V., Larsen, K. E., Kanter, E., Phillips, K. A., Wilson, K., Schmitz, Y., et al. (2009). Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. *Neuron, 62*(2), 218–229.

Mundorf, M. L., Hochstetler, S. E., & Wightman, R. M. (1999). Amine weak bases disrupt vesicular storage and promote exocytosis in chromaffin cells. J Neurochem 73:2397–2405.

Myohanen, T. T., Schendzielorz, N., & Mannisto, P. T. (2010). Distribution of catechol-O-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. *Journal of Neurochemistry, 113*(6), 1632–1643.

Mytilineou, C., Han, S. K., & Cohen, G. (1993). Toxic and protective effects of L-DOPA on mesencephalic cell cultures. *Journal of Neurochemistry, 61*(4), 1470–1478.

Naoi, M., & Maruyama, W. (2010). Monoamine oxidase inhibitors as neuroprotective agents in age-dependent neurodegenerative disorders. *Current Pharmaceutical Design, 16*(25), 2799–2817.

Naoi, M., Dostert, P., Yoshida, M., & Nagatsu, T. (1993). *N*-methylated tetrahydroisoquinolines as dopaminergic neurotoxins. *Advances in Neurology, 60*, 212–217.

Obeso, J. A., Marin, C., Rodriguez-Oroz, C., Blesa, J., Benitez-Temino, B., Mena-Segovia, J., et al. (2008). The basal ganglia in Parkinson's disease: Current concepts and unexplained observations. *Annals of Neurology, 64*(Suppl 2), S30–S46.

Pardo, B., Mena, M. A., Casarejos, M. J., Paino, C. L., & De Yebenes, J. G. (1995). Toxic effects of L-DOPA on mesencephalic cell cultures: Protection with antioxidants. *Brain Research, 682*, 133–143.

Paris, I., Lozano, J., Perez-Pastene, C., Munoz, P., & Segura-Aguilar, J. (2009). Molecular and neurochemical mechanisms in PD pathogenesis. *Neurotoxicity Research, 16*(3), 271–279.

Peter, D., Liu, Y., Sternini, C., de Giorgio, R., Brecha, N., & Edwards, R. H. (1995). Differential expression of two vesicular monoamine transporters. *Journal of Neuroscience, 15*(9), 6179–6188.

Pothos, E. N., Davila, V., & Sulzer, D. (1998). Presynaptic recording of quanta from midbrain dopamine neurons and modulation of the quantal size. *Journal of Neuroscience, 18*(11), 4106–4118.

Pothos, E. N., Larsen, K. E., Krantz, D. E., Liu, Y., Haycock, J. W., Setlik, W., et al. (2000). Synaptic vesicle transporter expression regulates vesicle phenotype and quantal size. *Journal of Neuroscience, 20*(19), 7297–7306.

Ramsey, A. J., & Fitzpatrick, P. F. (2000). Effects of phosphorylation on binding of catecholamines to tyrosine hydroxylase: Specificity and thermodynamics. *Biochemistry, 39*(4), 773–778.

Reveron, M. E., Savelieva, K. V., Tillerson, J. L., McCormack, A. L., Di Monte, D. A., & Miller, G. W. (2002). L-DOPA does not cause neurotoxicity in VMAT2 heterozygote knockout mice. *Neurotoxicology, 23*(4–5), 611–619.

Ricaurte, G. A., Guillery, R. W., Seiden, L. S., Schuster, C. R., & Moore, R. Y. (1982). Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Research, 235*(1), 93–103.

Rice, M. E., Cragg, S. J., & Greenfield, S. A. (1997). Characteristics of electrically evoked somatodendritic dopamine release in substantia nigra and ventral tegmental area in vitro. *Journal of Neurophysiology, 77*(2), 853–862.

Rochet, J. C., Outeiro, T. F., Conway, K. A., Ding, T. T., Volles, M. J., Lashuel, H. A., et al. (2004). Interactions among alpha-synuclein, dopamine, and biomembranes: Some clues for understanding neurodegeneration in Parkinson's disease. *Journal of Molecular Neuroscience, 23*(1–2), 23–34.

Rommelfanger, K. S., & Weinshenker, D. (2007). Norepinephrine: The redheaded stepchild of Parkinson's disease. *Biochemical Pharmacology, 74*(2), 177–190.

Sang, T. K., Chang, H. Y., Lawless, G. M., Ratnaparkhi, A., Mee, L., Ackerson, L. C., et al. (2007). A Drosophila model of mutant human parkin-induced toxicity demonstrates selective loss of dopaminergic neurons and dependence on cellular dopamine. *Journal of Neuroscience, 27*(5), 981–992.

Satou, T., Anderson, A. J., Itoh, T., Tamai, Y., Hayashi, Y., & Hashimoto, S. (2001). Repetitive administration of tetrabenazine induces irreversible changes in locomotion and morphology of the substantia nigra in rats. *Experimental and Toxicologic Pathology, 53*(4), 303–308.

Schmitt, K. C., & Reith, M. E. (1187). Regulation of the dopamine transporter: Aspects relevant to psychostimulant drugs of abuse. *Annals of the New York Academy of Sciences, 1187*, 316–340.

Specht, C. G., & Schoepfer, R. (2001). Deletion of the alpha-synuclein locus in a subpopulation of C57BL/6J inbred mice. *BMC Neuroscience, 2*(11), 11.

Spencer, J. P., Whiteman, M., Jenner, P., & Halliwell, B. (2002). 5-s-Cysteinyl-conjugates of catecholamines induce cell damage, extensive DNA base modification and increases in caspase-3 activity in neurons. *Journal of Neurochemistry, 81*(1), 122–129.

Spina, M. B., & Cohen, G. (1989). Dopamine turnover and glutathione oxidation: Implications for Parkinson disease. *Proceedings of the National Academy of Sciences of the United States of America, 86*(4), 1398–1400.

Stobrawa, S. M., Breiderhoff, T., Takamori, S., Engel, D., Schweizer, M., Zdebik, A. A., et al. (2001). Disruption of ClC-3, a chloride channel expressed on synaptic vesicles, leads to a loss of the hippocampus. *Neuron, 29*(1), 185–196.

Stuber, G. D., Hnasko, T. S., Britt, J. P., Edwards, R. H., & Bonci, A. (2010). Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *Journal of Neuroscience, 30*(24), 8229–8233.

Sulzer, D. (2001). Alpha-synuclein and cytosolic dopamine: Stabilizing a bad situation. *Nature Medicine, 7*(12), 1280–1282.

Sulzer, D. (2007). Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. *Trends in Neurosciences, 30*(5), 244–250.

Sulzer, D. (2011). How addictive drugs disrupt presynaptic dopamine neurotransmission. *Neuron, 69*(4), 628–649.

Sulzer, D., & Zecca, L. (2000). Intraneuronal dopamine-quinone synthesis: A review. *Neurotoxicity Research, 1*(3), 181–195.

Sulzer D, Chen T, Lau Y, Kristensen H, Rayport S, Ewing A (1995). Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. J Neurosci 15:4102–4108.

Sulzer, D., Bogulavsky, J., Larsen, K. E., Behr, G., Karatekin, E., Kleinman, M. H., et al. (2000). Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. *Proceedings of the National Academy of Sciences of the United States of America, 97*(22), 11869–11874.

Surmeier, D. J., Guzman, J. N., Sanchez-Padilla, J., & Goldberg, J. A. (2010). What causes the death of dopaminergic neurons in Parkinson's disease? *Progress in Brain Research, 183*, 59–77.

Takahashi, N., Miner, L. L., Sora, I., Ujike, H., Revay, R. S., Kostic, V., et al. (1997). VMAT2 knockout mice: Heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 9938–9943.

Taylor, T. N., Caudle, W. M., Shepherd, K. R., Noorian, A., Jackson, C. R., Iuvone, P. M., et al. (2009). Nonmotor symptoms of Parkinson's disease revealed in an animal model with reduced monoamine storage capacity. *Journal of Neuroscience, 29*(25), 8103–8113.

Taylor, T. N., Caudle, W. M., & Miller, G. W. (2011). VMAT2-deficient mice display nigral and extranigral pathology and motor and nonmotor symptoms of Parkinson's disease. *Parkinsons Dis*, 124165.

Uhl, G. R. (1998). Hypothesis: The role of dopaminergic transporters in selective vulnerability of cells in Parkinson's disease. *Annals of Neurology, 43*(5), 555–560.

van Rossum, J. M. (1966). The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. *Archives Internationales de Pharmacodynamie et de Thérapie, 160*(2), 492–494.

Vauzour, D., Ravaioli, G., Vafeiadou, K., Rodriguez-Mateos, A., Angeloni, C., & Spencer, J. P. (2008). Peroxynitrite induced formation of the neurotoxins 5-S-cysteinyl-dopamine and DHBT-1: Implications for Parkinson's disease and protection by polyphenols. *Archives of Biochemistry and Biophysics, 476*(2), 145–151.

Vergo, S., Johansen, J. L., Leist, M., & Lotharius, J. (2007). Vesicular monoamine transporter 2 regulates the sensitivity of rat dopaminergic neurons to disturbed cytosolic dopamine levels. *Brain Research, 1185*, 18–32.

Vila, M., & Przedborski, S. (2004). Genetic clues to the pathogenesis of Parkinson's disease. *Nature Medicine, 10*(Suppl), S58–S62.

Wagner, G. C., Lucot, J. B., Schuster, C. R., & Seiden, L. S. (1983). Alpha-methyltyrosine attenuates and reserpine increases methamphetamine-induced neuronal changes. *Brain Research, 270*(2), 285–288.

Wang, Y. M., Gainetdinov, R. R., Fumagalli, F., Xu, F., Jones, S. R., Bock, C. B., et al. (1997). Knockout of the vesicular monoamine transporter 2 gene results in neonatal death and supersensitivity to cocaine and amphetamine. *Neuron, 19*(6), 1285–1296.

Weihe, E., Schafer, M. K., Erickson, J. D., & Eiden, L. E. (1994). Localization of vesicular monoamine transporter isoforms (VMAT1 and VMAT2) to endocrine cells and neurons in rat. *Journal of Molecular Neuroscience, 5*(3), 149–164.

Weinreb, O., Amit, T., Bar-Am, O., & Youdim, M. B. (2010). Rasagiline: a novel anti-Parkinsonian monoamine oxidase-B inhibitor with neuroprotective activity. *Progress in Neurobiology, 92*(3), 330–344.

Westlund, K. N., Denney, R. M., Kochersperger, L. M., Rose, R. M., & Abell, C. W. (1985). Distinct monoamine oxidase A and B populations in primate brain. *Science, 230*(4722), 181–183.

Wightman, R. M., Jankowski, J. A., Kennedy, R. T., Kawagoe, K. T., Schroeder, T. J., Leszczyszyn, D. J., et al. (1991). Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells. *Proceedings of the National Academy of Sciences of the United States of America, 88*(23), 10754–10758.

Wimalasena, K. (2011). Vesicular monoamine transporters: Structure-function, pharmacology, and medicinal chemistry. *Medicinal Research Reviews, 31*(4), 483–519.

Yamamoto, H., Kamegaya, E., Hagino, Y., Imai, K., Fujikawa, A., Tamura, K., et al. (2007). Genetic deletion of vesicular monoamine transporter-2 (VMAT2) reduces dopamine transporter activity in mesencephalic neurons in primary culture. *Neurochemistry International, 51*(2–4), 237–244.

Yelin, R., & Schuldiner, S. (1995). The pharmacological profile of the vesicular monoamine transporter resembles that of multidrug transporters. *FEBS Letters, 377*(2), 201–207.

Zecca, L., Tampellini, D., Gerlach, M., Riederer, P., Fariello, R. G., & Sulzer, D. (2001). Substantia nigra neuromelanin: Structure, synthesis, and molecular behaviour. *Molecular Pathology, 54*(6), 414–418.

Zecca, L., Fariello, R., Riederer, P., Sulzer, D., Gatti, A., & Tampellini, D. (2002). The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. *FEBS Letters, 510*(3), 216–220.

Zhang, F., & Dryhurst, G. (1994). Effects of L-cysteine on the oxidation chemistry of dopamine: New reaction pathways of potential relevance to idiopathic Parkinson's disease. *Journal of Medicinal Chemistry, 37*(8), 1084–1098.

Zhu, J., & Reith, M. E. (2008). Role of the dopamine transporter in the action of psychostimulants, nicotine, and other drugs of abuse. *CNS & Neurological Disorders Drug Targets, 7*(5), 393–409.

Zucca, F. A., Bellei, C., Giannelli, S., Terreni, M. R., Gallorini, M., Rizzio, E., et al. (2006). Neuromelanin and iron in human locus coeruleus and substantia nigra during aging: Consequences for neuronal vulnerability. *Journal of Neural Transmission, 113*(6), 757–767.

Rotenone as Preclinical Model Compound in Parkinson Disease

Jason R. Cannon and J. Timothy Greenamyre

Contents

Abstract

The motor symptoms of Parkinson's disease (PD) are characterized by a combination of bradykinesia, resting tremor, rigidity, and postural instability, which are caused by striatal dopamine depletion resulting from loss of nigral dopamine neurons and dopaminergic projections. At the time of diagnosis,

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there is already extensive loss of striatal dopamine and nigral dopamine neurons. Patients also commonly experience a host of cognitive, psychiatric, autonomic, sleep, and sensory disturbances that likely result from pathology to regions outside the substantia nigra. These nonmotor abnormalities may occur decades before diagnosis. "Preclinical" PD is the disease before motor symptom severity elicits a PD diagnosis. Currently, there is an emphasis on the creation and characterization of preclinical models to identify early-stage PD features and test early intervention strategies. The rat rotenone model tests the hypothesis that nigral dopamine neurons are selectively sensitive to systemic mitochondrial complex I inhibition. The model replicates selective loss of nigral dopamine neurons and was the first neurotoxicant model to reproduce intracellular α-synuclein accumulation, similar to Lewy bodies. Subsequent investigations have found the model to replicate many additional PD features. In this chapter, the utility of this model to examine preclinical PD endpoints is evaluated. The major emphasis is pathological and functional alterations that are known to occur in the earliest stages of PD or even be predictive of disease development. Finally, specific rotenone administration regimens to produce preclinical PD models are detailed. In summary, available data suggest that the rat rotenone model is an excellent system to examine preclinical PD.

Keywords
Parkinson's disease • Preclinical • Rotenone

List of Abbreviations

6-OHDA	6-hydroxydopamine
AD	Alzheimer's disease
DNA	Deoxyribonucleic acid
LBD	Lewy body dementia
MIBG	Metaiodobenzylguanidine
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PD	Parkinson's disease
PET	Positron emission tomography
RBD	REM behavior disorder
REM	Rapid eye movement
SPECT	Single-photon emission computed tomography

1 Introduction

Parkinson's disease (PD) is characterized behaviorally by the presence of a combination of the cardinal motor features that include bradykinesia, resting tremor, rigidity, and postural instability. These key motor deficits are caused by striatal dopamine depletion that results from the loss of nigral dopamine neurons and their striatal dopaminergic projections (Bertler et al. 1958; Carlsson et al. 1958;

Moore et al. 1971). A major pathological hallmark is the presence of cytoplasmic inclusions known as Lewy bodies in surviving dopamine neurons (Forno 1996). Aggregated α-synuclein and ubiquitin are major components of Lewy bodies, and mutations in α-synuclein that promote aggregation or in proteins that affect proteasomal activity cause PD (Morris 2005; Spillantini et al. 1997). In addition to pathology in the nigrostriatal dopamine system, pathology is evident in other brain regions, particularly in brainstem nuclei, where α-synuclein accumulation occurs and is thought to temporally precede nigral aggregation (Braak et al. 2003; Braak et al. 2004). Systemic pathology also occurs, especially in the enteric nervous system (Braak et al. 2006; Wakabayashi et al. 1988). PD patients also commonly experience a host of cognitive, psychiatric, autonomic, sleep, and sensory disorders that likely result from pathology to neuronal regions outside the substantia nigra (Adler 2005; Emre 2004; McDonald et al. 2003; Papapetropoulos and Mash 2005).

Pharmacologic and surgical approaches can effectively treat many of the motor symptoms. However, current treatment options do not slow or prevent disease progression. PD is currently thought to affect at least 5 million individuals worldwide (Dorsey et al. 2007). The most significant risk factor for PD is ageing (Collier et al. 2011). Thus, given that the average life span is rising, in part due to medical advances related to cardiovascular diseases and cancers, PD incidence is expected to increase in an ageing population.

The causes of most cases of PD remain unknown. Genetic factors continue to be identified. However, monogenetic causes likely account for less than 10 % of total PD cases. Environmental exposures have long been linked to PD, with numerous structural and use classes of compounds linked to PD and other neurodegenerative diseases. While many reports have suggested links between environmental exposures and PD, identifying specific causative risk factors has been very difficult (Cannon and Greenamyre 2011). More recently specific toxicant exposures have been identified as risk factors using specialized epidemiological techniques. Notably, exposures to the herbicide paraquat and the pesticide rotenone have been identified as PD risk factors (Tanner et al. 2011). The identification of these toxicants as risk factors is supported by the fact that both of these toxicants have been utilized extensively to model PD in animals. Unfortunately, for most PD cases there is limited understanding of etiology. The majority of cases are thought to arise from a combination of genetic predisposition and environmental exposures. There is some evidence for a role of specific interactions in PD risk; however, our understanding of these interactions remains rudimentary (Cannon and Greenamyre 2012).

Thus, there are two key challenges that PD researchers and clinicians continue to have: (1) identifying causative factors (a reduction in exposure to causative factors could, in principle, reduce PD incidence over time) and (2) the development of disease-modifying therapies (such therapies would slow or halt progression in addition to treating the primary motor symptoms). While work to accomplish these goals typically involves human studies, animal models are extensively utilized during the earliest stages and throughout the process. Unfortunately, translating positive results from neurotoxicant-based animal studies to clinical trials has been largely unsuccessful. There are several possible explanations: (1) Neuroprotective

regimens are usually administered prior to toxicant insult. This type of regimen bears little relevance to a clinical setting, where a patient would be treated after diagnosis. Thus, positive studies from pretreatment regimens may have little relevance and translational impact in humans. (2) Relevance of the model. Most neurotoxicant models do an outstanding job of lesioning the nigrostriatal dopamine system. However, a model that does so in hours or a few days does not reproduce the chronic development of pathology in human PD. Further, regimens that simply prevent the primary mechanism of action of the insult in a neurotoxicant model are unlikely to be meaningful, for example, showing that an antioxidant is protective in an acute oxidative stress model (6-hydroxydopamine, 6-OHDA) or that a drug which alters 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) metabolism or metabolite accumulation in dopamine neurons is unlikely to provide substantially useful information on whether it is a viable neuroprotective agent (Cannon and Greenamyre 2010). (3) Failure to appreciate early-stage features of PD and PD models. The earlier a neuroprotective intervention is administered in the disease process, the more likely it will have a disease-modifying impact. The use of preclinical animal models can help to test potential therapeutics for an early-stage administration both directly and indirectly, by aiding in the development of early-stage diagnosis and testing whether therapeutic regimens prevent development of preclinical endpoints or are neuroprotective. Currently, preclinical animal models are limited – most models focus on reproducing the end-stage phenotype, primarily major striatal dopamine depletion and nigral dopamine cell loss. In this brief chapter, the development and use of the rotenone model to reproduce and identify preclinical features of PD is critically evaluated.

2 The Rotenone Model of Parkinson's Disease

Rotenone is a compound that occurs naturally in the roots of a variety of plant species. It is a highly selective inhibitor of mitochondrial complex I that has been historically used as an insecticide and to kill fish (Ravanel et al. 1984). Systemic complex I inhibition occurs in PD patients (Parker et al. 1989; Schapira et al. 1989). Thus, it has been hypothesized that toxicants that produce systemic complex I inhibition may have a role in PD. It should be noted that the classic dopaminergic neurotoxicant, MPTP, also selectively inhibits complex I. However, due to its affinity for the dopamine transporter, it selectively affects catecholaminergic neurons (Javitch et al. 1985; Nicklas et al. 1985). Conversely, rotenone is a highly lipophilic molecule able to enter all cells and produce complex I inhibition.

There have been many attempts to produce a rotenone model of PD, including high-dose systemic administration and intracerebral infusions that produce nonspecific lesions (Ferrante et al. 1997; Heikkila et al. 1985; Rojas et al. 2009). A major advancement came when systemic rotenone was chronically administered to rats at much lower doses, producing complex I inhibition similar to that observed in PD patients. This regimen produced highly selective nigral dopamine neuron degeneration (Betarbet et al. 2000). Here, for the first time in an animal model,

cytoplasmic, α-synuclein-positive inclusions that have striking similarities to Lewy bodies were observed in surviving dopamine neurons. Reproduction of this key pathological feature represented a major advantage over other available neurotoxicant models. While variability in the number of animals that exhibited a lesion and variability in the magnitude and morphology of the lesion were major obstacles for using the model in neuroprotection and potentiation studies, a more recent version is much more reproducible (Cannon et al. 2009). While the rat is the most common species that rotenone has been used to model PD, it has been used in other major biological models including *Drosophila*, *C. elegans*, and zebrafish (Cannon and Greenamyre 2010). Not only has the rotenone model replicated key PD features, it has been found to predict previously unknown features of human PD. Notably, rotenone treatment in the rat produces iron accumulation in the substantia nigra through a previously unknown mechanism involving transferrin and transferrin receptor 2 (Mastroberardino et al. 2009). This study also found the same alterations in the brains of PD patients. In addition to replicating these key pathological features, the rotenone model has been found to be useful in reproducing several key preclinical features of PD, which are discussed in this chapter.

3 Preclinical Parkinson's Disease and the Relevance of the Rotenone Model

What is preclinical PD? Simply stated, "preclinical" is the disease before characteristic motor symptoms become severe enough that the patient enters a clinic and is diagnosed with PD. Certainly, there is much patient-to-patient variability in the threshold of symptoms that are required to reach "clinical" PD. However, it is worth noting that by the time a patient is diagnosed with PD, ~70–80 % of striatal dopamine and ~50–60 % of nigral dopamine neurons are lost (Bernheimer et al. 1973). Thus, it is thought that damage and cell loss may occur over several decades before the pathology becomes severe enough to elicit noticeable symptoms.

3.1 Autonomic Dysfunction

Autonomic dysfunction may precede motor deficits by decades. For example, gastrointestinal dysfunction in the form of reduced bowel movements is a risk factor for future development of PD, an average of 12 years into follow-up (Abbott et al. 2001). A second study confirmed that constipation is a risk for PD (Savica et al. 2009). Both studies conclude that gastrointestinal dysfunction can precede the PD diagnosis by 10–20 years. Interestingly, Lewy bodies and Lewy neurites are also found in the myenteric and submucosal plexuses of the gut (Braak et al. 2006; Lebouvier et al. 2008; Wakabayashi et al. 1990). While these histological examinations were performed postmortem, the temporal development of symptoms would suggest that pathology in the gut is an early event in PD.

In rats treated with low-dose rotenone, which does not elicit overt nigrostriatal toxicity (2.0 mg/kg/day, 5x/week for 6 weeks), gastrointestinal dysfunction similar to that in PD patients is observed (Drolet et al. 2009). Insoluble α-synuclein-positive aggregates strikingly similar to that which occur in the gut of PD patients were found in the small intestine myenteric plexus 6 months after treatment. Neuronal loss in the gut and increased gastric transit time were also found in this study, also 6 months after rotenone treatment. Thus, chronic, low-dose systemic rotenone produces gastrointestinal dysfunction, in the absence of overt motor deficits or brain pathology. A second rat study also showed the development of delayed gastric emptying and enteric neuron dysfunction after rotenone treatment (Greene et al. 2009). Direct gastric administration of rotenone in mice is also able to produce α-synuclein aggregation (Pan-Montojo et al. 2010). The pathological findings in this study also showed that pathology in the gut preceded that in the substantia nigra. Thus, chronic rotenone treatment in rodents is able to reproduce this key preclinical event that occurs in many PD patients. Beyond simply validating the ability of this model to replicate key PD features, it is expected that gastrointestinal endpoints in future studies will provide much useful information on the pathophysiology of gut abnormalities in PD and a useful tool for examining treatments relevant to autonomic symptoms.

3.2 Olfactory Pathology and Deficits

The olfactory system is also affected in the earliest stages of PD. α-Synuclein accumulation in the anterior nucleus of the olfactory bulb precedes nigral pathology (Braak et al. 2003). Further, at onset, olfaction is typically severely impaired in the majority of PD patients, with normal function or mild impairment in other parkinsonian disorders (Hawkes 2003). Olfactory deficits may also be an important marker for Lewy body dementia (LBD), and although Alzheimer's disease (AD) patients may exhibit olfactory loss, it is typically less severe than in LBD (Olichney et al. 2005; Postuma et al. 2010). While numerous studies have found olfactory deficits to be predictive, the time where the predictive power is significant is typically 2–4 years prior to diagnosis (Postuma et al. 2010). Specificity may also be low; while those in the lowest quartile of olfactory function in the Honolulu-Asia Ageing Study had an odds ratio of 5.2 for developing PD, only 2 % of those in this quartile developed PD (Postuma et al. 2010; Ross et al. 2008).

In the rotenone model, examination of oxidative damage in the brain found that the most affected areas were the midbrain and olfactory bulb (Sherer et al. 2003). While this data was obtained from animals exhibiting severe parkinsonism, the data suggest that this brain region is a target and should be evaluated in earlier stages of the model or under low-dose regimens. Specifically, functional (olfactory thresholds and discrimination) and detailed neuroanatomical (examination of specific cell populations) studies are needed to determine how closely effects on the olfactory system resemble that which occur in human PD.

3.3 Pathology in Brainstem Nuclei

Pathology in the brainstem occurs in PD and is thought to precede damage to nigral dopamine neurons. In particular, α-synuclein accumulation in the dorsal motor nucleus of the vagus (acetylcholinergic) and locus coeruleus (noradrenergic) occurs in the earliest stages of PD (Braak et al. 2003, 2004). Pathophysiology in these nuclei may underlie some of the nondopaminergic PD symptoms.

To date one group has reported overt neuronal loss in the locus coeruleus after systemic subcutaneous rotenone administration (Lin et al. 2008). This finding was observed along with the typical nigrostriatal dopamine neuron degeneration. A recent in vitro study found that rotenone treatment promotes α-synuclein aggregation in primary neuronal cultures from the locus coeruleus (Chaves et al. 2010). A more thorough examination is needed to determine how early the locus coeruleus is affected in the rotenone model.

Mice receiving gastric rotenone administration exhibit inflammation in the dorsal motor nucleus of the vagus and α-synuclein-positive aggregate formation in acetylcholine positive neurons (Pan-Montojo et al. 2010). Notably, in this time-course study, brainstem pathology occurred prior to nigral pathology. Thus, chronic rotenone treatment is able to replicate the temporal development of key preclinical brainstem pathology.

3.4 Cardiac Function

Cardiac function is clearly altered in PD patients. In particular, postganglionic sympathetic innervation is affected in PD and DLB patients (Postuma et al. 2010). Although it requires cardiac metaiodobenzylguanidine (MIBG) scintigraphy to measure, which is costly, PD patients typically exhibit abnormal MIBG scintigraphy in the earliest disease stages (Spiegel et al. 2007).

Rotenone-treated rats that exhibit nigrostriatal dopamine lesions have decreased baroreflex sensitivity similar to that which occurs in PD patients (Yu et al. 2008). In this study, cardiac function was assessed at the end of rotenone administration. Given that in human PD patients, attenuated baroreflex function may precede motor symptoms, it is possible that rotenone treatment may recapitulate this preclinical PD symptom (Goldstein 2011; Goldstein et al. 2007). Evaluation of cardiac function in rotenone-treated rats, before nigrostriatal lesions develop or at subthreshold doses, will help to determine if this is a preclinical feature of the model.

3.5 Sleep Disorders

Sleep disorders are also common in PD patients and may precede motor deficits. Most notably is REM behavior disorder (RBD), where patients do not exhibit the normal atonia that occurs in REM sleep. The result is ultimately “acting out” of dreams, which may lead to crying out, kicking, or thrashing (Gagnon et al. 2006).

RBD occurs in roughly one third of PD patients, with about another one third exhibiting asymptomatic loss of atonia (Gagnon et al. 2002). RBD is also common in other neurodegenerative disorders. It is thought to stem from damage to pontine nuclei, which are affected in the early stages of PD. Further, those with RBD have a high risk for developing PD, with 40–60 % developing a neurodegenerative disorder (1/2 develop PD) 10 years after follow-up (Postuma et al. 2010). Thus, the long-lag period indicates that RBD/reduced REM atonia is a key preclinical feature of many PD cases. PD patients also exhibit insomnia and excessive daytime somnolence, also thought to arise in part due to degeneration of pontine nuclei (Braak et al. 2003; Chaudhuri et al. 2006; Postuma et al. 2010).

A few sleep-related studies have been conducted on rotenone-treated rats. The first report found that both vehicle (dimethyl sulfoxide and polyethylene glycol; 1:1) and rotenone-treated animals exhibited sleep disturbances, rendering interpretation of the direct effect of rotenone on sleep architecture difficult to discern (Garcia-Garcia et al. 2005). A later study in rotenone-treated rats found that slow-wave sleep was increased during the active time (dark) and decreased during the inactive period (light) (Yi et al. 2007). Further, REM sleep was enhanced during the dark period. Interestingly, this study found that alterations to sleep architecture were not mediated by dopamine. More detailed studies are needed to assess the temporal development of sleep disturbances in rotenone-treated rats. Muscle activity will also need to be assessed to determine if atonia occurs during REM, following rotenone treatment.

3.6 Oxidative Stress and Damage to Biomolecules

Oxidative stress has long been known to occur in human PD. Proving a direct causative role for oxidative stress in human PD is difficult because brain samples are obtained at autopsy. It should be noted that many of the prominent models of PD (6-OHDA, MPTP, and rotenone) utilize oxidative stress (through different mechanisms of action) to effectively lesion the nigrostriatal dopamine system and replicate the key pathogenic features of clinical PD (Betarbet et al. 2006; Przedborski and Vila 2001; Schwarting and Huston 1996). Such data would suggest that oxidative stress is important in the pathogenesis of the disease, not simply a by-product of end-stage pathology.

Diverse examples of oxidative damage in the human PD brain include increases in total iron content in the substantia nigra (thought to be a contributor to oxidative stress), superoxide dismutase (involved in cellular response to oxidative stress), lipid peroxidation (a marker for oxidative damage to lipids), protein oxidation, and DNA oxidation versus age-matched controls (Alam et al. 1997; Dexter et al. 1989; Floor and Wetzel 1998; Saggu et al. 1989; Sofic et al. 1988). However, brain oxidative stress and damage are difficult to utilize as preclinical features in humans due to sampling availability of relevant endpoints until after autopsy.

Oxidative stress and damage in PD is not limited to the brain, with evidence in serum and systemic tissues. For example, an overall index of serum oxidant status is

increased in PD patients and the antioxidant status is decreased (Forte et al. 2004). More specifically, plasma lipid peroxide levels are increased in PD patients (Kilinc et al. 1988), indicating a general systemic oxidative damage in PD. Serum levels of superoxide dismutase and glutathione peroxidase have been found in PD patients, indicating a systemic response to oxidative stress (Kalra et al. 1992). Oxidation of plasma nucleic acid is also increased in PD patients (Abe et al. 2003; Kikuchi et al. 2002). Additionally, several cell types undergo oxidative damage in PD, including platelets (Gotz et al. 2000; Krige et al. 1992; Yoshino et al. 1992), erythrocytes (Kilinc et al. 1988; Younes-Mhenni et al. 2007), lymphocytes (Migliore et al. 2001, 2002; Petrozzi et al. 2001), and neutrophils (Gatto et al. 1996; Vitte et al. 2004).

Rotenone has been found to selectively lesion nigral dopamine neurons through complex I inhibition, resulting in oxidative stress, to which these neurons are particularly susceptible (Sherer et al. 2003). Given the number of clinical PD parameters that the rotenone model replicates, it is highly likely that oxidative stress in the earliest disease stages is important in human PD pathogenesis. In vitro studies have shown that rotenone treatment leads to progressive oxidative stress in neuroblastoma cells (Sherer et al. 2002). Further, work in organotypic slice cultures showed the development of oxidative stress along with loss of nigral dopamine neurons. Both oxidative stress and neuronal loss were prevented with antioxidant treatment (Testa et al. 2005). Later work showed that DJ-1, an important oxidative stress protein, is itself oxidatively modified in nigral neurons from both lesioned and non-lesioned rotenone-treated rats (Betarbet et al. 2006). This finding suggests that oxidative stress and damage are early events occurring prior to overt neuronal loss. Specialized redox imaging performed as a time course in zebrafish larvae also shows that oxidative stress is an early event after rotenone treatment (Horowitz et al. 2011).

Systemic studies on the temporal development of oxidative stress are limited in the rotenone model. Studies in progress in the Greenamyre lab (unpublished) show that DNA damage in the blood is profound and occurs within hours of the first rotenone administration. Studies in the future on the temporal development of damage to specific cell types are expected to be highly informative.

4 Practical Considerations: Executing and Utilizing the Rat Rotenone Model to Assess Preclinical Endpoints

In the rat rotenone model, there are two basic regimens to assess preclinical endpoints:

1. Standard dose, with early sampling. While there are certainly lab-to-lab differences, dependent on age and strain, in our experience, administration of systemic rotenone at 3.0 mg/kg/day will typically produce debilitating motor deficits ~7–14 days after initial administration (Cannon et al. 2009). Sampling at 5 days or less, before motor deficits or nigrostriatal pathology arises, allows for the assessment of preclinical endpoints. A major advantage of this approach is that it is known that at this dose, animals will eventually develop the key motor and pathological features

of end-stage PD. However, sampling after an acute administration may result in the inability to detect preclinical endpoints that develop chronically.

2. Low-dose, long-term treatments. Animals treated at rotenone doses 2.0–2.5 mg/kg/day do not typically develop severe motor deficits or nigrostriatal pathology (Cannon et al. 2009; Drolet et al. 2009). A major advantage to this approach is that the development of long-term preclinical endpoints can be assessed. For example, after 2.0 mg/kg/day rotenone, 5 days/week for 6 weeks, rotenone treatment was discontinued and rats were found to develop gastrointestinal pathology and transit deficits 6 months later (Drolet et al. 2009). A dose of 2.5 mg/kg/day was found to elicit motor symptoms in few wild-type animals, but potentiation occurred in transgenic animals expressing disease-causing mutations in α-synuclein (Cannon et al. 2013). Thus, low-dose, long-term rotenone can be used to assess the development of preclinical endpoints and potentiation (especially, gene-environment interactions). Potential disadvantages are that animals likely will not eventually develop a phenotype characteristic end-stage PD and a lengthy dosing period may be required to elicit the desired preclinical features. Rats treated at 2.0–2.5 mg/kg/day, as long as 60 days, may not develop motor deficits (Cannon et al. 2009). Thus, it may be somewhat more difficult to determine the importance of potential preclinical features in a model that does not develop the key features of end-stage pathology. Further, evaluation at up to 6 months of age can be costly and time consuming. However, it should be noted that low-dose neurotoxicant exposures and chronic development are likely more relevant to human exposures and the temporal development of the disease.

5 Future Directions: The Need for Better Preclinical Models and Suggested Uses

Modeling preclinical features is of major importance to advancing PD research. First understanding the key preclinical features of the disease will aid in earlier diagnosis. This will be particularly important as neuroprotective agents become available in the future. The earlier a neuroprotective agent could be administered, the more likely it would be effective. Second, a better understanding of preclinical features will increase our understanding of the pathophysiology of PD and perhaps lead to new therapeutic targets.

5.1 How Can the Rotenone Model be Further Validated for Modeling Preclinical Use?

While it is now over a decade old, the rotenone model continues to evolve. In its most recent form, it is much more reproducible and amenable to neuroprotection and potentiation studies (Cannon et al. 2009, 2013; Tapias et al. 2010). Current data suggest that the rotenone model can replicate many of the key preclinical features. However, much more data is needed. In particular, alterations in olfactory function,

sleep architecture, and cardiac function need to be further assessed to determine when they may occur, relative to nigrostriatal dopamine system degeneration. Advanced olfactory tests able to measure discrimination are now available for use in rats and could be tested in the rotenone model (Tillerson et al. 2006). More detailed sleep studies need to be performed on rats. In particular, given the risk of developing PD in those with RBD, particular focus should be on REM and muscle activity to assess atonia during REM. Evaluation should be repeated in conjunction with other behavioral assays to determine when sleep disturbances arise relative to motor deficits.

5.2 What Are Some Potential Future Uses of Preclinical Rotenone Model?

The identification of preclinical features in the rotenone model provides further validation of model utility. The key question is as follows: how can modeling preclinical features of PD be used to advance our understanding of the disease? It is important to note that although it is now over a decade in use, the rotenone model is still several decades younger than other prominent neurotoxicant modes (6-OHDA, MPTP). Thus, we are still learning much about the model.

In humans, imaging can identify neurological dysfunction prior to diagnosis. Dopaminergic PET and SPECT have high sensitivity and specificity for parkinsonism, with studies estimating that measurable abnormalities may occur 4–7 years before clinical PD (Hilker et al. 2005; Morrish et al. 1998; Postuma et al. 2010; Ravina et al. 2005; Vingerhoets et al. 1994). As these imaging techniques become readily available for rodents with high enough resolution, it should be possible to noninvasively determine temporally, when dopaminergic dysfunction begins in live animals. This would be particularly useful in low-dose, long-term studies, where sampling for biomarkers and nondopaminergic effects can be obtained. Here, an emphasis on what occurs prior to or near the beginning of nigrostriatal dopamine system dysfunction will be important.

Most PD cases likely arise from multiple insults. Thus, when studying etiological factors, exposure to environmentally relevant mixtures at different times in development/life span and exploring the effects of gene-environment interactions are important. Practically, in a laboratory setting, this is expensive and difficult, because even studying a single insult in an animal model is costly and time consuming. Nonetheless, combination and multiple "hit" approaches are being explored in animal models and are becoming more common as a way to address exposures relevant to human health. Given its environmental relevance and now being implicated as a bona fide risk factor, rotenone exposure is particularly relevant to such studies. For example, low-dose rotenone (2.0–2.5 mg/kg/day) reproduces many preclinical features as described above, yet in the absence of overt nigrostriatal toxicity. This regimen is ideal for examining potentiation and synergistic effects from other insults. A recent study where striatal dopamine terminal loss and severe parkinsonian behavioral features were observed in rats

expressing disease-causing mutations in α-synuclein, but not in wild type, shows how the model can be used to assess potentiation (Cannon et al. 2013). It is expected that such forms of the rotenone model will continue to be used in transgenic animals or along with other toxicants of relevance to continue to identify etiological factors.

6 Conclusion

The rotenone model replicates the key behavioral, neurochemical, and pathological features of PD. Many models, both neurotoxicant and genetic based, have shown promise in replicating at least some of the key preclinical features of PD. Here, we have presented specific evidence that the rotenone model replicates many key preclinical features of human PD. It has the major advantages of environmental relevance, chronic development, and reproduction of important pathological hallmarks. Future studies are required, but it is expected that findings from the rotenone model will confirm its relevance to model preclinical PD. Finally, exploratory studies in the rotenone model may identify preclinical features that are not yet known to occur in human PD patients.

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References

Abbott, R. D., Petrovitch, H., White, L. R., Masaki, K. H., Tanner, C. M., Curb, J. D., & Ross, G. W. (2001). Frequency of bowel movements and the future risk of Parkinson's disease. *Neurology, 57*(3), 456–462 (Research Support, U.S. Gov't, Non-P.H.S., Research Support, U.S. Gov't, P.H.S.).

Abe, T., Isobe, C., Murata, T., Sato, C., & Tohgi, H. (2003). Alteration of 8-hydroxyguanosine concentrations in the cerebrospinal fluid and serum from patients with Parkinson's disease. *Neuroscience Letters, 336*(2), 105–108.

Adler, C. H. (2005). Nonmotor complications in Parkinson's disease. *Movement Disorders, 20* (Suppl 11), S23–S29. doi:10.1002/mds.20460 (Review).

Alam, Z. I., Jenner, A., Daniel, S. E., Lees, A. J., Cairns, N., Marsden, C. D., & Halliwell, B. (1997). Oxidative DNA damage in the parkinsonian brain: An apparent selective increase in 8-hydroxyguanine levels in substantia nigra. *Journal of Neurochemistry, 69*(3), 1196–1203.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndromes of parkinson and huntington. Clinical, morphological and neurochemical correlations. *Journal of Neurological Sciences, 20*(4), 415–455.

Bertler, A., Carlsson, A., & Rosengren, E. (1958). A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta Physiologica Scandinavica, 44*(3–4), 273–292.

Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., & Greenamyre, J. T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neuroscience, 3*(12), 1301–1306. doi:10.1038/81834 (Research Support, U.S. Gov't, P.H.S.).

Betarbet, R., Canet-Aviles, R. M., Sherer, T. B., Mastroberardino, P. G., McLendon, C., Kim, J. H., Lund, S., Na, H. M., Taylor, G., Bence, N. F., Kopito, R., Seo, B. B., Yagi, T., Yagi, A., Klinefelter, G., Cookson, M. R., & Greenamyre, J. T. (2006). Intersecting pathways to neurodegeneration in Parkinson's disease: Effects of the pesticide rotenone on dj-1, alpha-synuclein, and the ubiquitin-proteasome system. *Neurobiology of Disease, 22*(2), 404–420. doi:10.1016/j.nbd.2005.12.003 (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't).

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging, 24*(2), 197–211 (Research Support, Non-U.S. Gov't).

Braak, H., Ghebremedhin, E., Rub, U., Bratzke, H., & Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell and Tissue Research, 318*(1), 121–134. doi:10.1007/s00441-004-0956-9 (Research Support, Non-U.S. Gov't Review).

Braak, H., de Vos, R. A., Bohl, J., & Del Tredici, K. (2006). Gastric alpha-synuclein immunoreactive inclusions in meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neuroscience Letters, 396*(1), 67–72. doi:10.1016/j.neulet.2005.11.012 (Research Support, Non-U.S. Gov't).

Cannon, J. R., & Greenamyre, J. T. (2010). Neurotoxic in vivo models of Parkinson's disease recent advances. *Progress in Brain Research, 184*, 17–33. doi:10.1016/S0079-6123(10)84002-6 (Review).

Cannon, J. R., & Greenamyre, J. T. (2011). The role of environmental exposures in neurodegeneration and neurodegenerative diseases. *Toxicological Sciences, 124*(2), 225–250. doi:10.1093/toxsci/kfr239 (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't).

Cannon, J. R., & Greenamyre, J. T. (2012). Gene-environment interactions in Parkinson's disease: Specific evidence in humans and mammalian models. *Neurobiology of Disease*. doi:10.1016/j.nbd.2012.06.025.

Cannon, J. R., Tapias, V., Na, H. M., Honick, A. S., Drolet, R. E., & Greenamyre, J. T. (2009). A highly reproducible rotenone model of Parkinson's disease. *Neurobiology of Disease, 34*(2), 279–290 (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't).

Cannon, J. R., Geghman, K. D., Tapias, V., Sew, T., Dail, M. K., Li, C., & Greenamyre, J. T. (2013). Expression of human e46k-mutated alpha-synuclein in bac-transgenic rats replicates early-stage Parkinson's disease features and enhances vulnerability to mitochondrial impairment. *Exp Neurol, 240,* 44–56. doi: 10.1016/j.expneurol.2012.11.007.

Carlsson, A., Lindqvist, M., Magnusson, T., & Waldeck, B. (1958). On the presence of 3-hydroxytyramine in brain. *Science, 127*(3296), 471.

Chaudhuri, K. R., Healy, D. G., & Schapira, A. H. (2006). Non-motor symptoms of Parkinson's disease: Diagnosis and management. *Lancet Neurology, 5*(3), 235–245. doi:10.1016/S1474-4422(06)70373-8 (Review).

Chaves, R. S., Melo, T. Q., Martins, S. A., & Ferrari, M. F. (2010). Protein aggregation containing beta-amyloid, alpha-synuclein and hyperphosphorylated tau in cultured cells of hippocampus, substantia nigra and locus coeruleus after rotenone exposure. *BMC Neuroscience, 11*, 144. doi:10.1186/1471-2202-11-144 (Research Support, Non-U.S. Gov't).

Collier, T. J., Kanaan, N. M., & Kordower, J. H. (2011). Ageing as a primary risk factor for Parkinson's disease: Evidence from studies of non-human primates. *Nature Reviews Neuroscience, 12*(6), 359–366. doi:10.1038/nrn3039.

Dexter, D. T., Carter, C. J., Wells, F. R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P., & Marsden, C. D. (1989). Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *Journal of Neurochemistry, 52*(2), 381–389.

Dorsey, E. R., Constantinescu, R., Thompson, J. P., Biglan, K. M., Holloway, R. G., Marshall, F. J., Ravina, B. M., Schifitto, G., Siderowf, A., & Tanner, C. M. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology, 68*(5), 384–386. doi:10.1212/01.wnl.0000247740.47667.03 (Meta-Analysis).

Drolet, R. E., Cannon, J. R., Montero, L., & Greenamyre, J. T. (2009). Chronic rotenone exposure reproduces Parkinson's disease gastrointestinal neuropathology. *Neurobiology of Disease, 36*(1), 96–102. doi:10.1016/j.nbd.2009.06.017 (Research Support, Non-U.S. Gov't).

Emre, M. (2004). Dementia in Parkinson's disease: Cause and treatment. *Current Opinion in Neurology, 17*(4), 399–404 (Review).

Ferrante, R. J., Schulz, J. B., Kowall, N. W., & Beal, M. F. (1997). Systemic administration of rotenone produces selective damage in the striatum and globus pallidus, but not in the substantia nigra. *Brain Research, 753*(1), 157–162 (Comparative Study Research Support, Non-U.S. Gov't, Research Support, U.S. Gov't, Non-P.H.S., Research Support, U.S. Gov't, P.H.S.).

Floor, E., & Wetzel, M. G. (1998). Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay. *Journal of Neurochemistry, 70*(1), 268–275.

Forno, L. S. (1996). Neuropathology of Parkinson's disease. *Journal of Neuropathology and Experimental Neurology, 55*(3), 259–272 (Review).

Forte, G., Bocca, B., Senofonte, O., Petrucci, F., Brusa, L., Stanzione, P., Zannino, S., Violante, N., Alimonti, A., & Sancesario, G. (2004). Trace and major elements in whole blood, serum, cerebrospinal fluid and urine of patients with Parkinson's disease. *Journal of Neural Transmission, 111*(8), 1031–1040.

Gagnon, J. F., Bedard, M. A., Fantini, M. L., Petit, D., Panisset, M., Rompre, S., Carrier, J., & Montplaisir, J. (2002). Rem sleep behavior disorder and rem sleep without atonia in Parkinson's disease. *Neurology, 59*(4), 585–589 (Clinical Trial Controlled Clinical TrialResearch Support, Non-U.S. Gov't).

Gagnon, J. F., Postuma, R. B., Mazza, S., Doyon, J., & Montplaisir, J. (2006). Rapid-eye-movement sleep behaviour disorder and neurodegenerative diseases. *Lancet Neurology, 5*(5), 424–432. doi:10.1016/S1474-4422(06)70441-0 (Research Support, Non-U.S. Gov't Review).

Garcia-Garcia, F., Ponce, S., Brown, R., Cussen, V., & Krueger, J. M. (2005). Sleep disturbances in the rotenone animal model of Parkinson disease. *Brain Research, 1042*(2), 160–168. doi:10.1016/j.brainres.2005.02.036 (Comparative Study Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S.).

Gatto, E. M., Carreras, M. C., Pargament, G. A., Riobo, N. A., Reides, C., Pardal, M. M. F., Llesuy, S., & Poderoso, J. J. (1996). Neutrophil function, nitric oxide, and blood oxidative stress in Parkinson's disease. *Movement Disorders, 11*(3), 261–267. doi:10.1002/mds.870110308 (Research Support, Non-U.S. Gov't).

Goldstein, D. S. (2011). Stress, allostatic load, catecholamines, and other neurotransmitters in neurodegenerative diseases. *Endocrine Regulations, 45*(2), 91–98 (Research Support, N.I.H., Intramural Review).

Goldstein, D. S., Sharabi, Y., Karp, B. I., Bentho, O., Saleem, A., Pacak, K., & Eisenhofer, G. (2007). Cardiac sympathetic denervation preceding motor signs in Parkinson disease. *Clinical Autonomic Research, 17*(2), 118–121. doi:10.1007/s10286-007-0396-1 (Case Reports Research Support, N.I.H., Intramural).

Gotz, M. E., Gerstner, A., Harth, R., Dirr, A., Janetzky, B., Kuhn, W., Riederer, P., & Gerlach, M. (2000). Altered redox state of platelet coenzyme q10 in Parkinson's disease. *Journal of Neural Transmission, 107*(1), 41–48 (Research Support, Non-U.S. Gov't).

Greene, J. G., Noorian, A. R., & Srinivasan, S. (2009). Delayed gastric emptying and enteric nervous system dysfunction in the rotenone model of Parkinson's disease. *Experimental Neurology, 218*(1), 154–161. doi:10.1016/j.expneurol.2009.04.023 (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't).

Hawkes, C. (2003). Olfaction in neurodegenerative disorder. *Movement Disorders, 18*(4), 364–372. doi:10.1002/mds.10379 (Review).

Heikkila, R. E., Nicklas, W. J., Vyas, I., & Duvoisin, R. C. (1985). Dopaminergic toxicity of rotenone and the 1-methyl-4-phenylpyridinium ion after their stereotaxic administration to rats: Implication

for the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity. *Neuroscience Letters, 62*(3), 389–394 (Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.).

Hilker, R., Schweitzer, K., Coburger, S., Ghaemi, M., Weisenbach, S., Jacobs, A. H., & Heiss, W. D. (2005). Nonlinear progression of Parkinson disease as determined by serial positron emission tomographic imaging of striatal fluorodopa f 18 activity. *Archives of Neurology, 62*(3), 378–382. doi:10.1001/archneur.62.3.378 (Comparative Study).

Horowitz, M. P., Milanese, C., Di Maio, R., Hu, X., Montero, L. M., Sanders, L. H., & Mastroberardino, P. G. (2011). Single-cell redox imaging demonstrates a distinctive response of dopaminergic neurons to oxidative insults. *Antioxidants & Redox Signaling, 15*(4), 855–871. doi:10.1089/ars.2010.3629 (Research Support, N.I.H., Extramural Research Support, Non-U. S. Gov't).

Javitch, J. A., D'Amato, R. J., Strittmatter, S. M., & Snyder, S. H. (1985). Parkinsonism-inducing neurotoxin, n-methyl-4-phenyl-1,2,3,6 -tetrahydropyridine: Uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proceedings of the National Academy of Sciences of the United States of America, 82*(7), 2173–2177 (In Vitro Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.).

Kalra, J., Rajput, A. H., Mantha, S. V., & Prasad, K. (1992). Serum antioxidant enzyme activity in Parkinson's disease. *Molecular and Cellular Biochemistry, 110*(2), 165–168.

Kikuchi, A., Takeda, A., Onodera, H., Kimpara, T., Hisanaga, K., Sato, N., Nunomura, A., Castellani, R. J., Perry, G., Smith, M. A., & Itoyama, Y. (2002). Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiology of Disease, 9*(2), 244–248.

Kilinc, A., Yalcin, A. S., Yalcin, D., Taga, Y., & Emerk, K. (1988). Increased erythrocyte susceptibility to lipid peroxidation in human Parkinson's disease. *Neuroscience Letters, 87*(3), 307–310.

Krige, D., Carroll, M. T., Cooper, J. M., Marsden, C. D., & Schapira, A. H. (1992). Platelet mitochondrial function in Parkinson's disease. The royal kings and queens Parkinson disease research group. *Annals of Neurology, 32*(6), 782–788.

Lebouvier, T., Chaumette, T., Damier, P., Coron, E., Touchefeu, Y., Vrignaud, S., Naveilhan, P., Galmiche, J. P., Bruley des Varannes, S., Derkinderen, P., & Neunlist, M. (2008). Pathological lesions in colonic biopsies during Parkinson's disease. *Gut, 57*(12), 1741–1743. doi:10.1136/gut.2008.162503 (Letter).

Lin, C. H., Huang, J. Y., Ching, C. H., & Chuang, J. I. (2008). Melatonin reduces the neuronal loss, downregulation of dopamine transporter, and upregulation of d2 receptor in rotenone-induced parkinsonian rats. *Journal of Pineal Research, 44*(2), 205–213. doi:10.1111/j.1600-079X.2007.00510.x (Research Support, Non-U.S. Gov't).

Mastroberardino, P. G., Hoffman, E. K., Horowitz, M. P., Betarbet, R., Taylor, G., Cheng, D., Na, H. M., Gutekunst, C. A., Gearing, M., Trojanowski, J. Q., Anderson, M., Chu, C. T., Peng, J., & Greenamyre, J. T. (2009). A novel transferrin/tfr2-mediated mitochondrial iron transport system is disrupted in Parkinson's disease. *Neurobiology of Disease, 34*(3), 417–431. doi:10.1016/j.nbd.2009.02.009 (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't).

McDonald, W. M., Richard, I. H., & DeLong, M. R. (2003). Prevalence, etiology, and treatment of depression in Parkinson's disease. *Biological Psychiatry, 54*(3), 363–375 (Review).

Migliore, L., Scarpato, R., Coppede, F., Petrozzi, L., Bonuccelli, U., & Rodilla, V. (2001). Chromosome and oxidative damage biomarkers in lymphocytes of Parkinson's disease patients. *International Journal of Hygiene and Environmental Health, 204*(1), 61–66 (Research Support, Non-U.S. Gov't).

Migliore, L., Petrozzi, L., Lucetti, C., Gambaccini, G., Bernardini, S., Scarpato, R., Trippi, F., Barale, R., Frenzilli, G., Rodilla, V., & Bonuccelli, U. (2002). Oxidative damage and cytogenetic analysis in leukocytes of Parkinson's disease patients. *Neurology, 58*(12), 1809–1815 (Comparative Study Research Support, Non-U.S. Gov't).

Moore, R. Y., Bhatnagar, R. K., & Heller, A. (1971). Anatomical and chemical studies of a nigroneostriatal projection in the cat. *Brain Research, 30*(1), 119–135.

Morris, H. R. (2005). Genetics of Parkinson's disease. *Annals of Medicine, 37*(2), 86–96 (Review).

Morrish, P. K., Rakshi, J. S., Bailey, D. L., Sawle, G. V., & Brooks, D. J. (1998). Measuring the rate of progression and estimating the preclinical period of Parkinson's disease with [^{18}F]dopa PET. *Journal of Neurology, Neurosurgery, and Psychiatry, 64*(3), 314–319 (Research Support, Non-U.S. Gov't).

Nicklas, W. J., Vyas, I., & Heikkila, R. E. (1985). Inhibition of nadh-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *Life Sciences, 36*(26), 2503–2508 (Research Support, U.S. Gov't, P.H.S.).

Olichney, J. M., Murphy, C., Hofstetter, C. R., Foster, K., Hansen, L. A., Thal, L. J., & Katzman, R. (2005). Anosmia is very common in the Lewy body variant of Alzheimer's disease. *Journal of Neurology, Neurosurgery, and Psychiatry, 76*(10), 1342–1347. doi:10.1136/jnnp.2003.032003 (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't, Research Support, U.S. Gov't, P.H.S.).

Pan-Montojo, F., Anichtchik, O., Dening, Y., Knels, L., Pursche, S., Jung, R., Jackson, S., Gille, G., Spillantini, M. G., Reichmann, H., & Funk, R. H. (2010). Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. *PLoS One, 5*(1), e8762. doi:10.1371/journal.pone.0008762.

Papapetropoulos, S., & Mash, D. C. (2005). Psychotic symptoms in Parkinson's disease. From description to etiology. *Journal of Neurology, 252*(7), 753–764. doi:10.1007/s00415-005-0918-5 (Review).

Parker, W. D., Jr., Boyson, S. J., & Parks, J. K. (1989). Abnormalities of the electron transport chain in idiopathic Parkinson's disease. *Annals of Neurology, 26*(6), 719–723. doi:10.1002/ana.410260606 (Research Support, U.S. Gov't, P.H.S.).

Petrozzi, L., Lucetti, C., Gambaccini, G., Bernardini, S., Del Dotto, P., Migliore, L., Scarpato, R., & Bonuccelli, U. (2001). Cytogenetic analysis oxidative damage in lymphocytes of Parkinson's disease patients. *Neurological Science, 22*(1), 83–84.

Postuma, R. B., Gagnon, J. F., & Montplaisir, J. (2010). Clinical prediction of Parkinson's disease: Planning for the age of neuroprotection. *Journal of Neurology, Neurosurgery, and Psychiatry, 81*(9), 1008–1013. doi:10.1136/jnnp.2009.174748 (Research Support, Non-U.S. Gov't).

Przedborski, S., & Vila, M. (2001). MPTP: A review of its mechanisms of neurotoxicity. *Clinical Neuroscience Research, 1*, 407–418.

Ravanel, P., Tissut, M., & Douce, R. (1984). Effects of rotenoids on isolated plant mitochondria. *Plant Physiology, 75*(2), 414–420.

Ravina, B., Eidelberg, D., Ahlskog, J. E., Albin, R. L., Brooks, D. J., Carbon, M., Dhawan, V., Feigin, A., Fahn, S., Guttman, M., Gwinn-Hardy, K., McFarland, H., Innis, R., Katz, R. G., Kieburtz, K., Kish, S. J., Lange, N., Langston, J. W., Marek, K., Morin, L., Moy, C., Murphy, D., Oertel, W. H., Oliver, G., Palesch, Y., Powers, W., Seibyl, J., Sethi, K. D., Shults, C. W., Sheehy, P., Stoessl, A. J., & Holloway, R. (2005). The role of radiotracer imaging in Parkinson disease. *Neurology, 64*(2), 208–215. doi:10.1212/01.WNL.0000149403.14458.7F (Research Support, U.S. Gov't, P.H.S. Review).

Rojas, J. C., Simola, N., Kermath, B. A., Kane, J. R., Schallert, T., & Gonzalez-Lima, F. (2009). Striatal neuroprotection with methylene blue. *Neuroscience, 163*(3), 877–889. doi:10.1016/j.neuroscience.2009.07.012 (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't).

Ross, G. W., Petrovitch, H., Abbott, R. D., Tanner, C. M., Popper, J., Masaki, K., Launer, L., & White, L. R. (2008). Association of olfactory dysfunction with risk for future Parkinson's disease. *Annals of Neurology, 63*(2), 167–173. doi:10.1002/ana.21291 (Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S.).

Saggu, H., Cooksey, J., Dexter, D., Wells, F. R., Lees, A., Jenner, P., & Marsden, C. D. (1989). A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. *Journal of Neurochemistry, 53*(3), 692–697.

Savica, R., Carlin, J. M., Grossardt, B. R., Bower, J. H., Ahlskog, J. E., Maraganore, D. M., Bharucha, A. E., & Rocca, W. A. (2009). Medical records documentation of constipation preceding Parkinson disease: A case–control study. *Neurology, 73*(21), 1752–1758.

doi:10.1212/WNL.0b013e3181c34af5 (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't).

Schapira, A. H., Cooper, J. M., Dexter, D., Jenner, P., Clark, J. B., & Marsden, C. D. (1989). Mitochondrial complex i deficiency in Parkinson's disease. *Lancet, 1*(8649), 1269 (Letter Research Support, Non-U.S. Gov't).

Schwarting, R. K., & Huston, J. P. (1996). Unilateral 6-hydroxydopamine lesions of meso-striatal dopamine neurons and their physiological sequelae. *Progress in Neurobiology, 49*(3), 215–266 (Research Support, Non-U.S. Gov't Review).

Sherer, T. B., Betarbet, R., Stout, A. K., Lund, S., Baptista, M., Panov, A. V., Cookson, M. R., & Greenamyre, J. T. (2002). An in vitro model of Parkinson's disease: Linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. *Journal of Neuroscience, 22*(16), 7006–7015. doi:20026721 (Research Support, U.S. Gov't, P.H.S.).

Sherer, T. B., Betarbet, R., Testa, C. M., Seo, B. B., Richardson, J. R., Kim, J. H., Miller, G. W., Yagi, T., Matsuno-Yagi, A., & Greenamyre, J. T. (2003). Mechanism of toxicity in rotenone models of Parkinson's disease. *Journal of Neuroscience, 23*(34), 10756–10764 (In Vitro Research Support, Non-U.S. Gov't, Research Support, U.S. Gov't, P.H.S.).

Sofic, E., Riederer, P., Heinsen, H., Beckmann, H., Reynolds, G. P., Hebenstreit, G., & Youdim, M. B. (1988). Increased iron (iii) and total iron content in post mortem substantia nigra of parkinsonian brain. *Journal of Neural Transmission, 74*(3), 199–205.

Spiegel, J., Hellwig, D., Farmakis, G., Jost, W. H., Samnick, S., Fassbender, K., Kirsch, C. M., & Dillmann, U. (2007). Myocardial sympathetic degeneration correlates with clinical phenotype of Parkinson's disease. *Movement Disorders, 22*(7), 1004–1008. doi:10.1002/mds.21499 (Research Support, Non-U.S. Gov't).

Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). Alpha-synuclein in Lewy bodies. *Nature, 388*(6645), 839–840. doi:10.1038/42166 (Letter).

Tanner, C. M., Kamel, F., Ross, G. W., Hoppin, J. A., Goldman, S. M., Korell, M., Marras, C., Bhudhikanok, G. S., Kasten, M., Chade, A. R., Comyns, K., Richards, M. B., Meng, C., Priestley, B., Fernandez, H. H., Cambi, F., Umbach, D. M., Blair, A., Sandler, D. P., & Langston, J. W. (2011). Rotenone, paraquat, and Parkinson's disease. *Environmental Health Perspectives, 119*(6), 866–872. doi:10.1289/ehp.1002839 (Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't).

Tapias, V., Cannon, J. R., & Greenamyre, J. T. (2010). Melatonin treatment potentiates neurodegeneration in a rat rotenone Parkinson's disease model. *Journal of Neuroscience Research, 88*(2), 420–427. doi:10.1002/jnr.22201 (Research Support, Non-U.S. Gov't).

Testa, C. M., Sherer, T. B., Greenamyre, J. T. (2005). Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures [Comparative Study In Vitro Research Support, N.I.H., Extramural, Research Support, Non-U.S. Gov't, Research Support, U.S. Gov't, P.H.S.]. *Brain Res Mol Brain Res*, *134*(1), 109–118. doi: 10.1016/j.molbrainres.2004.11.007

Tillerson, J. L., Caudle, W. M., Parent, J. M., Gong, C., Schallert, T., & Miller, G. W. (2006). Olfactory discrimination deficits in mice lacking the dopamine transporter or the D_2 dopamine receptor. *Behavioural Brain Research, 172*(1), 97–105. doi:10.1016/j.bbr.2006.04.025 (Research Support, N.I.H., Extramural).

Vingerhoets, F. J., Snow, B. J., Lee, C. S., Schulzer, M., Mak, E., & Calne, D. B. (1994). Longitudinal fluorodopa positron emission tomographic studies of the evolution of idiopathic parkinsonism. *Annals of Neurology, 36*(5), 759–764. doi:10.1002/ana.410360512 (Research Support, Non-U.S. Gov't).

Vitte, J., Michel, B. F., Bongrand, P., & Gastaut, J. L. (2004). Oxidative stress level in circulating neutrophils is linked to neurodegenerative diseases. *Journal of Clinical Immunology, 24*(6), 683–692.

Wakabayashi, K., Takahashi, H., Takeda, S., Ohama, E., & Ikuta, F. (1988). Parkinson's disease: The presence of Lewy bodies in Auerbach's and Meissner's plexuses. *Acta Neuropathologica, 76*(3), 217–221.

Wakabayashi, K., Takahashi, H., Ohama, E., & Ikuta, F. (1990). Parkinson's disease: An immunohistochemical study of lewy body-containing neurons in the enteric nervous system. *Acta Neuropathologica, 79*(6), 581–583 (Research Support, Non-U.S. Gov't).

Yi, P. L., Tsai, C. H., Lu, M. K., Liu, H. J., Chen, Y. C., & Chang, F. C. (2007). Interleukin-1beta mediates sleep alteration in rats with rotenone-induced parkinsonism. *Sleep, 30*(4), 413–425 (Research Support, Non-U.S. Gov't).

Yoshino, H., Nakagawa-Hattori, Y., Kondo, T., & Mizuno, Y. (1992). Mitochondrial complex I and II activities of lymphocytes and platelets in Parkinson's disease. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 4*(1), 27–34.

Younes-Mhenni, S., Frih-Ayed, M., Kerkeni, A., Bost, M., & Chazot, G. (2007). Peripheral blood markers of oxidative stress in Parkinson's disease. *European Neurology, 58*(2), 78–83. doi:10.1159/000103641.

Yu, J. G., Wu, J., Shen, F. M., Cai, G. J., Liu, J. G., & Su, D. F. (2008). Arterial b aroreflex dysfunction fails to mimic Parkinson's disease in rats. *Journal of Pharmacological Sciences, 108*(1), 56–62 (Research Support, Non-U.S. Gov't).

Part III

Glutamate Receptor Agonists and Antagonists

Concept of Excitotoxicity via Glutamate Receptors

Juan C. Piña-Crespo, Sara Sanz-Blasco, and Stuart A. Lipton

Contents

Abstract

Since its inception, the concept of glutamate excitotoxicity has provided a foundational framework for understanding the role played by excitatory amino acids in disease states of the brain. At the same time, it has served as a guiding principle in the development and evaluation of new anti-excitotoxic drugs, many of which show promise as neuroprotective therapies in a number of neurological conditions. The discovery that glutamate receptors on the cell surface can engage, through second messengers such as calcium (Ca^{2+}), nitric oxide (NO), and inositol

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phospholipids, downstream intracellular signaling cascades involved in cell death helped uncover the complexity of the excitotoxic cascade. The identification of numerous intracellular effectors of excitotoxicity has provided a physiological and pharmacological basis for understanding the cellular and molecular mechanisms behind glutamate-mediated nerve cell injury and its role in neuropsychiatric diseases. More recently, knowledge of the molecular biology of glutamate receptors has allowed, for the first time, the identification of differences in the pattern of expression of glutamate receptors in human populations afflicted by neuropsychiatric diseases. This knowledge will be useful in uncovering genes that may confer individual susceptibility to excitotoxic damage and, as a result, predisposition to the development of certain mental and neurological diseases. In this chapter, the role of glutamate receptor overactivation in excitotoxic cell injury as well as potential neuroprotective therapies for limiting glutamate-mediated neurotoxicity in disease states of the central nervous system will be discussed.

List of Abbreviations

AMPA	2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid)
ATP	Adenosine-5′-triphosphate
BAD	Bcl-2-associated death promoter
BAX	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2 protein
Bcl-X_L	B-cell lymphoma – extra large protein
Ca^{2+}	Calcium
DA	Domoic acid
DCD	Delayed calcium deregulation
DNA	Deoxyribonucleic acid
EAAs	Excitatory amino acids
ER	Endoplasmic reticulum
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Glu	L-glutamate
GluRs	Glutamate receptors
KA	Kainic acid
MCU	Mitochondrial calcium uniporter
mNCX	Mitochondrial Na^+/Ca^{2+} transporter
mPTP	Mitochondrial permeability transition pore
NCX	Na^+/Ca^{2+} exchanger
NMDA	*N*-methyl-D-aspartic acid
NO	Nitric oxide
PMCA	Plasma membrane Ca^{2+} ATPase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SIAH1	Seven in absentia homolog E3 ubiquitin-protein ligase
VDAC	Voltage-dependent anion-selective channel
$\Delta\psi m$	Inner mitochondrial membrane potential

1 Introduction

L-Glutamate is a major excitatory amino acid neurotransmitter in the brain (Nicholls et al. 2012; Watkins and Evans 1981; Watkins 2000). Under physiological conditions, glutamate plays fundamental roles in nerve cell function and brain plasticity (Bliss and Collingridge 1993; Cooke and Bliss 2006) through activation of glutamate receptors (GluRs) (Hollmann and Heinemann 1994; Traynelis et al. 2010). In contrast, excessive activation of GluRs during neurological conditions can lead to loss of nerve cell function and cell death through a process known as excitotoxicity (Olney 1978; Olney 2003). Glutamate-mediated excitotoxicity is initiated by buildup of excess glutamate in the extracellular space that leads to overactivation of GluRs, disruption in cell membrane permeability, and downstream activation of signaling cascades involved in loss of nerve cell function, cell damage, and death (Farooqui et al. 2008; Gillessen et al. 2002; Lau and Tymianski 2010). Significant experimental and clinical evidence implicates excitotoxicity in the loss of neuronal cell function and cell death associated with acute neurological conditions such as traumatic brain injury (Arundine and Tymianski 2004; Yi and Hazell 2006), stroke (Besancon et al. 2008; Brouns and De Deyn 2009; Doyle et al. 2008), hypoxia-ischemia (Jensen 2005), amnesic shellfish poisoning (Jeffery et al. 2004; Lefebvre and Robertson 2010; Perl et al. 1990; Teitelbaum et al. 1990), and epileptic seizures (Meldrum 1993), as well as during chronic neurodegenerative diseases (Lipton and Rosenberg 1994; Mattson 2003; Mehta et al. 2013) such as Alzheimer's disease (Hu et al. 2012; Mucke and Selkoe 2012), Parkinson's disease (Beal 1998; Surmeier et al. 2011), Huntington's disease (Raymond et al. 2011), AIDS encephalopathy and dementia complex (more recently termed HIV-associated neurocognitive disorders) (Kaul et al. 2001; Mattson et al. 2005), multiple sclerosis (Trapp and Stys 2009), and neuropathic pain syndromes (Latremoliere and Woolf 2009).

Neuronal function, as well as susceptibility to excitotoxic cell damage, depend on the expression of specific repertoires of receptors and ion channels. Since abnormal patterns of expression of genes encoding glutamate receptors have been described in a number of brain disorders, current research efforts may unveil how dysregulated expression of glutamate receptors affects neurotransmission and nerve cell susceptibility to excitotoxic cell death.

2 Historical Background

Although interest into the role of glutamate in the nervous system dates back to the early twentieth century (Weil-Malherbe 1950), it was not until the 1950s that evidence for a direct excitatory action of glutamate on the brain was first described (Hayashi 1952; Okamoto 1951) and insights into its mechanism of action were first inferred (Hayashi 1954). These findings were soon followed by reports of glutamate-induced neurotoxicity after observation of acute degenerative lesions in the inner retina of newborn mice (Lucas and Newhouse 1957) and depolarization of neurons in the spinal cord (Curtis et al. 1959).

During the 1960s, research into the structure-function relationship of a large number of neutral and acidic amino acids led to the concept an "amino-acid-receptor complex" present in the extracellular surface of neurons (Curtis et al. 1960). In an effort to elucidate the chemical basis of the activity and specificity of glutamate and aspartate on the "amino acid receptor," Watkins and colleagues synthesized *N*-methyl-D-aspartic acid (NMDA) (Watkins 1962), a non-metabolizable agonist with remarkable selectivity for the "amino-acid-receptor complex." Sensitivity to NMDA became a key criterion for the initial pharmacological classification of glutamate receptors into NMDA and non-NMDA receptors (Lodge 2009).

Further evidence supporting a neurotoxic role for glutamate appeared in 1969, when Olney and collaborators described neuropathological lesions in the brain of rodents and primates after systemic administration of glutamate and structurally related analogues (Olney 1969; Olney and Sharpe 1969). During the ensuing decade, Olney and colleagues proposed the concept of excitotoxicity to describe the acute neurotoxic effects of glutamate in the brain (Olney and Ho 1970; Olney 1978) and provided further experimental support for its potential role in brain pathology particularly in the newborn brain.

During the 1980s, preclinical studies reported evidence of significantly high levels of extracellular concentrations of brain glutamate in experimental models of hypoxia-ischemia in vivo (Benveniste et al. 1984; Hagberg et al. 1985), a finding later confirmed in humans (Bullock et al. 1995). At the same time, preclinical studies confirmed the neuroprotective action of glutamate receptor antagonists against hypoxic-ischemic neuronal cell death in vitro (Rothman 1984) and in vivo (Simon et al. 1984). However, successful translation of these early preclinical findings into clinically effective therapies has been an elusive target (O'Collins et al. 2006; Sutherland et al. 2012; Tymianski 2010), mainly because of lack of therapeutic strategies that can selectively target pathologically active GluRs without affecting normal glutamatergic neurotransmission (Lipton 2007).

A need for understanding the intracellular signaling cascades activated by neurotoxic glutamate led to the identification of calcium (Ca^{2+}) (Choi 1985; Choi 1987), inositol phospholipids (Sladeczek et al. 1985; Sugiyama et al. 1987), and nitric oxide (NO) (Garthwaite et al. 1988) as key intracellular effectors of glutamate signaling. This set of key insights provided the physiological and pharmacological frameworks for a greater understanding of the cellular and molecular mechanisms responsible for glutamate-mediated nerve cell injury (Choi 1988; Rothman and Olney 1986) and its potential role in neurological diseases (Greenamyre 1986). In 1987, a fatal incident due to consumption of shellfish contaminated with domoic acid (DA), a naturally occurring glutamate analogue, provided direct evidence for glutamate-mediated neurotoxicity in humans (Perl et al. 1990; Teitelbaum et al. 1990).

During the 1990s, glutamate research experienced one of its most important and significant periods with the cloning and functional expression of 26 different glutamate receptor genes (Table 1) (Alexander et al. 2011; Boulter et al. 1990; Hollmann et al. 1989; Hollmann and Heinemann 1994). This unveiled the molecular basis for the functional and pharmacological diversity of glutamate receptors in the central nervous system (Gereau and Swanson 2008; Traynelis et al. 2010).

Table 1 IUPHAR* classification of cloned glutamate receptors (GluRs)

	Receptor	Pharmacology	Gene		Previous nomenclature	
Ionotropic receptors			*Human*	*Mouse/rat*	*Rat*	*Mouse*
1	GluA1	AMPA	GRIA1	Gria1	GluR1, GluRA	α1
2	GluA2	AMPA	GRIA2	Gria2	GluR2, GluRB	α2
3	GluA3	AMPA	GRIA3	Gria3	GluR3, GluRC	α3
4	GluA4	AMPA	GRIA4	Gria4	GluR4, GluRD	α4
5	GluD1	Glutamate	GRID1	Grid1	Delta1	δ1
6	GluD2	Glutamate	GRID2	Grid2	Delta2	δ2
7	GluK1	Kainate	GRIK1	Grik1	GluR5, GluK5	β1
8	GluK2	Kainate	GRIK2	Grik2	GkuR6, GluK6	β2
9	GluK3	Kainate	GRIK3	Grik3	GluR7, GluK7	β3
10	GluK4	Kainate	GRIK4	Grik4	KA1, GluK1	γ1
11	GluK5	Kainate	GRIK5	Grik5	KA2, GluK2	γ2
12	GluN1	NMDA	GRIN1	Grin1	NR1	ζ1
13	GluN2A	NMDA	GRIN2A	Grin2a	NR2A	ε1
14	GluN2B	NMDA	GRIN2B	Grin2b	NR2B	ε2
15	GluN2C	NMDA	GRIN2C	Grin2c	NR2C	ε3
16	GluN2D	NMDA	GRIN2D	Grin2d	NR2D	ε4
17	GluN3A	NMDA	GRIN3A	Grin3a	NR3A	χ1
18	GluN3B	NMDA	GRIN3B	Grin3b	NR3B	χ2
Metabotropic receptors						
19	$mGlu_1$	Glutamate	GRM1	Grm1	mGluR1	mGluR1
20	$mGlu_2$	Glutamate	GRM2	Grm2	mGluR2	mGluR2
21	$mGlu_3$	Glutamate	GRM3	Grm3	mGluR3	mGluR3
22	$mGlu_4$	Glutamate	GRM4	Grm4	mGluR4	mGluR4
23	$mGlu_5$	Glutamate	GRM5	Grm5	mGluR5	mGluR5
24	$mGlu_6$	Glutamate	GRM6	Grm6	mGluR6	mGluR6
25	$mGlu_7$	Glutamate	GRM7	Grm7	mGluR7	mGluR7
26	$mGlu_8$	Glutamate	GRM8	Grm8	mGluR8	mGluR8

**IUPHAR Committee on Receptor Nomenclature and Drug Classification* (http://www.iuphar-db.org/index.jsp)

The molecular biology of glutamate receptors laid down the foundations for detailed cellular, molecular, and genetic studies into the role of glutamate dysfunction in diseases of the brain (Lipton and Rosenberg 1994; Nakanishi 1992).

3 Glutamate Receptors

Glutamate receptor-mediated signaling occurs through activation of two different types of receptors: ion channel-coupled (ionotropic) and G-protein-coupled (metabotropic) receptors. Ionotropic glutamate receptors (iGluRs) are coupled to ion channels and are responsible for fast changes in membrane permeability

(Traynelis et al. 2010; VanDongen 2009); metabotropic glutamate receptors (mGluRs) are coupled to intracellular effector systems via G-proteins (Nicoletti et al. 2011; Niswender and Conn 2010).

3.1 Ionotropic Glutamate Receptors

iGluRs are essential mediators of fast synaptic transmission due to their ability to generate a response within milliseconds of glutamate binding. Based on their agonist selectivity, iGluRs have been classified into three major subclasses: AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), kainate, and NMDA receptors (Table 1). iGluRs are cation-selective glutamate-gated ion channels composed of four glycosylated transmembrane subunits assembled around a central aqueous pore that spans the cell membrane. Each receptor contains three major domains: an extracellular glutamate-binding domain related to amino acid-binding bacterial periplasmic proteins, a transmembrane pore-lining domain related to the pore-forming region or "P-loop" of K^+ channels, and an intracellular regulatory domain (Gereau and Swanson 2008; Kumar and Mayer 2012; Traynelis et al. 2010).

3.1.1 AMPA Receptors

Upon activation by L-glutamate or AMPA, AMPARs mediate responses that are characterized by rapid onset, offset, and desensitization kinetics, but when they are activated by the neurotoxin kainate or domoic acid (DA), they produce large non-desensitizing responses. Four AMPAR subunits have been cloned and termed: GluA1, GluA2, GluA3, and GluA4 (Alexander et al. 2011; Boulter et al. 1990; Keinanen et al. 1990); they can form homomeric receptors as well as heteromeric receptors when expressed with other subunits. Functionally, they show rapid kinetics and low Ca^{2+} permeability in the presence of the GluA2 subunit (although the RNA-unedited form of GluA2 is permeable to Ca^{2+}) (Gereau and Swanson 2008; Traynelis et al. 2010).

3.1.2 Kainate Receptors

So far, five different sequence-related genes encoding kainate receptors (KARs) have been identified and termed: GluK1, GluK2, GluK3, GluK4, and GluK5. KARs bind kainate with intermediate to high affinity with GluK1 and GluK2 being the only kainate receptors capable of forming functional receptors upon in vitro expression. The others kainate receptor subunits can form functional receptors only when co-expressed in combination with GluK1 or GluK2 receptors. In contrast with AMPARs, KARs strongly desensitize upon activation by the neurotoxin kainate.

3.1.3 NMDA Receptors

NMDA receptors are characterized by high Ca^{2+} permeability, voltage-dependent block by Mg^{2+}, high single-channel conductance, slow gating kinetics, and activation by the concerted action of glutamate and glycine (VanDongen 2009).

During glutamatergic synaptic transmission, NMDA receptors mediate the slow component of synaptic currents. NMDA receptors result from the assembly of three different subfamilies of subunits: NR1, NR2, and NR3 (Table 1).

3.2 Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs) play an important role in the transduction of glutamate-mediated signaling via coupling through G-proteins to intracellular second messenger cascades. They have been classified into groups 1, 2, and 3. Group 1 (mGluR1 and mGluR5) receptors are coupled to phospholipase C, mediating increases in intracellular calcium. Group 2 (mGluR2 and mGluR3) and group 3 (mGluR4, mGluR6, mGluR7, mGluR8) are negatively coupled to adenylate cyclase, producing a decrease in forskolin-induced cAMP (Nicoletti et al. 2011; Niswender and Conn 2010).

4 Mechanisms of Excitotoxicity

Glutamate neurotoxicity results from a buildup of either endogenous excitatory amino acids (EAAs), exogenous glutamate-like substances in the brain, or excessive glutamate receptor activity (often NMDA receptors) in the presence of normal agonists due to loss of normal Mg^{2+} block. This form of neurotoxicity is often due to malfunctioning of cellular processes responsible for regulating endogenous EAA levels in the brain or to exposure to toxic levels of exogenous EAA analogues known as excitotoxins. These events produce intense activation of glutamate receptors on the cell surface that generates abnormally high levels of intracellular Ca^{2+}, which activates catalytic enzymes, generates highly toxic free radicals, and disrupts cell energy production. Eventual depletion of ATP stores leads to the collapse of transmembrane electrochemical gradients, loss of nerve cell excitability, neurotransmission failure, and cell death by either necrosis or apoptosis (Choi 1988; Gillessen et al. 2002; Rothman and Olney 1986).

5 Intracellular Effectors of Glutamate Neurotoxicity

5.1 Calcium

Ca^{2+} ions are powerful activators of nerve cell excitability and metabolism. Intracellular Ca^{2+} concentrations are kept relatively low by high-energy-requiring processes of active Ca^{2+}extrusion and Ca^{2+} sequestration in intracellular stores. Active Ca^{2+} efflux is mostly carried out by the Na^{+}/Ca^{2+} exchanger (NCX) and plasma membrane Ca^{2+} ATPase (PMCA), while storage of intracellular Ca^{2+} localizes mainly to the endoplasmic reticulum (ER) and mitochondria. Extracellular Ca^{2+} concentrations (1–2 mM) are over one order of magnitude larger than inside nerve

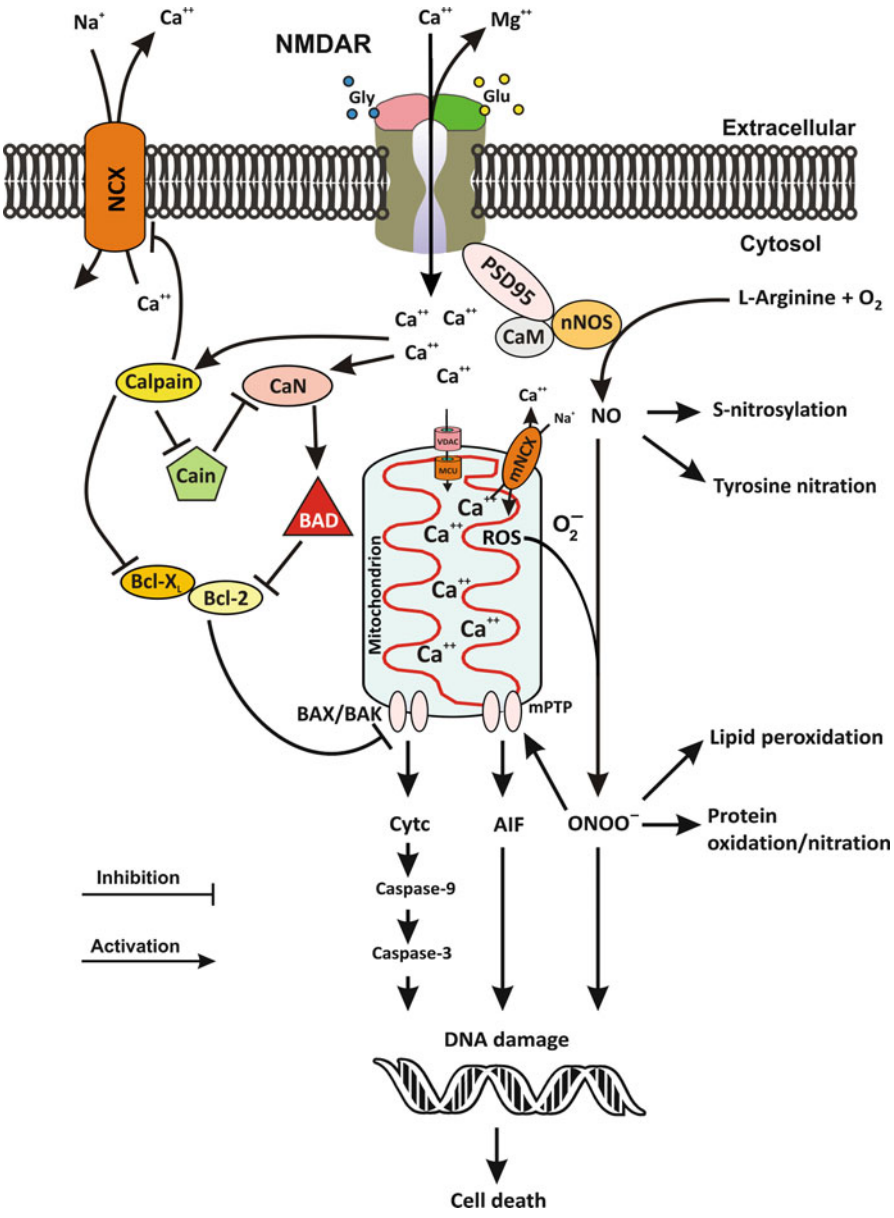

Fig. 1 Intracellular effectors of excitotoxicity. The events leading to excitotoxic cell death include overactivation of Ca^{2+}-permeable glutamate receptors (GluRs), excessive Ca^{2+} influx, and Ca^{2+}-dependent activation of intracellular effector enzymes such neuronal nitric oxide synthase (nNOS), calcineurin (CaN), and calpain together with Ca^{2+} uptake into mitochondria. Subsequent mitochondrial dysfunction due to excessive Ca^{2+} accumulation, and generation and release of toxic amounts of free radicals such as nitric oxide (NO) and reactive oxygen species (ROS) contribute to the production of peroxynitrite ($ONOO^{-}$) that together with depletion of

cells (50–200 nM). During overactivation of Ca^{2+}-permeable GluRs (Brorson et al. 1994; Choi 1985; Lu et al. 1996), this large electrochemical gradient drives the flow of extracellular Ca^{2+} into the cytoplasm where it generates an over tenfold increase in intracellular Ca^{2+}. In vitro, even a brief, few seconds long, exposure to neurotoxic levels of glutamate can trigger loss of intracellular Ca^{2+} homeostasis and cell death. Glutamate-induced intracellular loading of Ca^{2+} is characterized by an immediate but transient GluR-mediated Ca^{2+} influx that is followed by a transient return to baseline Ca^{2+} levels that can last hours until a secondary wave of sustained intracellular Ca^{2+} develops that is irreversibly followed by cell death (Randall and Thayer 1992). This dynamic demonstrates how GluR-mediated calcium influx activates specific effector pathways involved in cell death. Intense excitotoxic injury can result in necrosis (Syntichaki and Tavernarakis 2003), while less fulminant or chronic insults can cause synaptic damage followed by apoptosis (Ankarcrona et al. 1995; Bonfoco et al. 1995).

Although there is general consensus regarding the important role of intracellular Ca^{2+} in excitotoxic nerve cell death (Choi 1985; Choi 1988), many of the intracellular signaling events underlying glutamate-induced cell death are less well characterized. Reactive oxygen species (ROS), reactive nitrogen species (RNS), caspases, and lipases are among the most important Ca^{2+}-dependent effectors thought to be involved in glutamate-induced neurotoxicity. These processes are detailed below and summarized in Fig. 1.

5.2 Reactive Oxygen Species (ROS)

GluR-mediated excitotoxicity generates reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl (OH^-) radicals (Dykens et al. 1987; Dykens 1994; Lafon-Cazal et al. 1993). ROS cause cell damage through lipid peroxidation and oxidative damage of proteins and DNA (Chinopoulos and Adam-Vizi 2006; Rigoulet et al. 2011; Van Houten et al. 2006). Numerous neuroprotective strategies, including trapping, scavenging, and inhibition of ROS synthesis, have been shown to be effective under in vitro conditions (Dykens et al. 1987; Majewska and Bell 1990; Puttfarcken et al. 1992); however, evidence of their

Fig. 1 (continued) mitochondrial ATP levels promotes opening of the BAX/BAK channel, mitochondrial permeability transition pore (mPTP), or breakdown of the mitochondrial membrane to release cytochrome c (Cyt c) and the apoptosis-inducing factor (AIF). While Cyt c mediates caspase-dependent apoptosis, AIF translocates into the nucleus where it produces DNA fragmentation, chromatin condensation, and apoptosis through a caspase-independent mechanism. Simultaneously, Ca^{2+}-dependent activation of calcineurin (CaN) and calpain promotes calpain-dependent inhibition of the anti-apoptotic Bcl-X_L/Bcl-2 complex and Cain, an inhibitor of calcineurin-mediated cell death. Once calcineurin (CaN) is freed from Cain-dependent inhibition, it can activate the Bcl-2-associated death promoter (BAD), which produces further inhibition of Bcl-X_L/Bcl-2 complex. This allows the formation of a BAX/BAK mitochondrial channel permeable to pro-apoptotic molecules such as Cyt c

neuroprotective activity in vivo is still lacking. Since mitochondria are both a source of ROS (Dugan et al. 1995; Reynolds and Hastings 1995) and a target for ROS-induced damage, protection and rescue of mitochondrial function is key to reducing glutamate-mediated neurotoxicity (Cho et al. 2010; Perez-Pinzon et al. 2012).

5.3 Reactive Nitrogen Species (RNS)

Nitric oxide (NO) can react with superoxide radicals (O_2^-) to generate peroxynitrite ($ONOO^-$), a highly toxic and strongly oxidizing and nitrating agent that causes lipid peroxidation, protein oxidation, and DNA damage (Brown 2010). Inhibition of NO synthesis or genetic deletion of its synthesizing enzyme, nitric oxide synthetase (nNOS), can protect neurons from glutamate neurotoxicity (Dawson et al. 1991; Dawson et al. 1996; Lipton et al. 1993). Because of its close physical association to intracellular scaffoldings such as postsynaptic densities (PSDs), which anchor GluRs to postsynaptic sites (Sattler et al. 1999), nNOS is one of the first Ca^{2+}-dependent intracellular effectors activated by Ca^{2+} influx through GluRs, namely, NMDARs. Through this pathway, neurotoxic glutamate levels generate large amounts of RNS, especially NO, which causes misfolding and malfunction of intracellular signaling proteins through abnormal S-nitrosylation and tyrosine nitration (Martinez-Ruiz et al. 2011; Nakamura and Lipton 2011). Among the most important deleterious effects of NO accumulation is inhibition of energy-generating intracellular systems that leads to the collapse of intracellular ATP levels and cell death through either necrosis or apoptosis. During this process, NO causes further release of glutamate through inhibition of mitochondrial function, reverse glutamate uptake, and glutamate exocytosis (Brown 2010). In addition, neuronal cell death can be triggered by NO-dependent nitrosylation of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which promotes its association with the E3 ubiquitin-protein ligase SIAH1 and translocation into the nucleus (Hara et al. 2005). There is currently considerable need for effective cell-permeable inhibitors to protect nerve cells from the abnormally high levels of protein nitrosylation observed during pathological overactivation of brain GluRs (Nakamura and Lipton 2010).

5.4 Caspases

Caspases are intracellular aspartate-specific proteases that play a central role in apoptotic nerve cell death (Troy et al. 2011). They are strongly associated with glutamate-induced excitotoxic apoptosis (Tenneti et al. 1998; Tenneti and Lipton 2000), where their activation occurs via the mitochondrial or intrinsic apoptotic pathway (Budd et al. 2000). Overactive glutamate receptors produce excessive calcium influx and ROS, which causes mitochondrial calcium overload and energy depletion that triggers mitochondrial permeabilization and release of

cytochrome c (Cyt *c*) into the cytoplasm. Cyt *c* relieves calcium-induced inhibition of inositol trisphosphate receptors (IP_3R) and initiates the formation of the apoptosome which activates the apoptotic caspase cascade (Kroemer et al. 2007). The interplay between activated caspases and calpains is thought to underlie the neurotoxic effect of glutamate through excitotoxic apoptosis (Hara and Snyder 2007).

5.5 Calpains

Calpains are a group of intracellular Ca^{2+}-dependent cysteine proteases activated during excitotoxic apoptosis (Ankarcrona et al. 1995; D'Orsi et al. 2012; Vosler et al. 2008). Calcium overload due to GluR overactivation causes calpain activation and subsequently cell death through cleavage and inactivation of signaling and structural intracellular proteins (Brorson et al. 1995; Liu et al. 2008; Siman et al. 1989). Although the exact sequence of events is not clearly understood, the interplay between caspases and calpains (Wu et al. 2007) involves Ca^{2+}-dependent activation of calpain which causes cleavage and internalization of the membrane-bound Na^+/Ca^{2+} exchanger 3 (NCX3) and leads to an increase in intracellular calcium and cell death in a form of active necrosis (Bano et al. 2005). Activated calpains also target the protein Cain/Cabin-1, an intracellular inhibitor of calcineurin-mediated cell death (Kim et al. 2002; Lai et al. 1998). Calpain-mediated Cain/Cabin-1 inactivation removes a key restrictive control over calcineurin dephosphorylation of the pro-apoptotic protein Bcl-2-associated death promoter (BAD). Dephosphorylated BAD binds and inactivates the antiapoptotic proteins Bcl-2 and Bcl-X_L (Wang et al. 1999). In addition, activated calpains can directly cleave and inactivate Bcl-2 and Bcl-X_L (Vandenabeele et al. 2005), which are responsible for preventing activation of the pro-apoptotic mitochondrial membrane permeabilization complex formed by Bcl-2-associated X protein (BAX)/Bcl-2-antagonistic/killer (BAK) (Yang et al. 1997). Activation of the BAX/BAK complex forms a pore on the mitochondrial outer membrane that is responsible for the release of cytochrome c that initiates caspase-mediated apoptosis (Liu et al. 1996; Ow et al. 2008). An alternative pathway activated by calpains during excitotoxicity involves activation and release of the apoptosis-inducing factor (AIF) (Cao et al. 2007; Landshamer et al. 2008; Polster et al. 2005; Yu et al. 2002), a mitochondrial flavoprotein involved in caspase-independent apoptosis (Susin et al. 1996). The importance of calpain inhibitors as a potential neuroprotective agents is highlighted by their significant neuroprotective effect against excitotoxic brain damage induced by cerebral ischemia and traumatic brain injury (Koumura et al. 2008; Kupina et al. 2001; Lubisch et al. 2003; Nimmrich et al. 2010; Wang et al. 1996).

5.6 Lipases

NMDAR-mediated glutamate neurotoxicity activates Ca^{2+}-dependent phospholipase A2 (PLA2), which triggers release of arachidonic acid (AA) from membrane

phospholipids (Dumuis et al. 1988; Lazarewicz et al. 1988). Extracellular release of arachidonic acid can inhibit transporter-mediated glutamate (Volterra et al. 1992), while intracellular metabolism of AA by lipoxygenases (LOX), cytochrome P450 (CYP), and cyclooxygenases (COX) generates bioactive eicosanoids that trigger neuroinflammatory cell damage.

6 Role of Mitochondria in Glutamate Excitotoxicity

Calcium accumulation in mitochondria is a key event in glutamate excitotoxicity (Duchen 2012; Orrenius et al. 2003). It has been shown in many neurodegenerative diseases that overactivation of calcium-permeable GluRs causes an increase of intracellular calcium that induces overloading of calcium in the mitochondria, followed by an increase in free radicals and a decrease in ATP levels (Thayer and Wang 1995). In fact, the amount of calcium taken up by the mitochondria is decisive in cell death by glutamate excitotoxicity. Once the mitochondrial capacity for Ca^{2+} sequestration is exceeded due to depletion of mitochondrial energy resources, mitochondrial failure occurs (Rizzuto et al. 2012) (Fig. 2).

Physiologically, Ca^{2+} transport to the mitochondria through a complex formed between the voltage-dependent anion-selective channel (VDAC) and the mitochondrial calcium uniporter (MCU) contributes to the maintenance of low levels of Ca^{2+} in the cytosol (Rizzuto et al. 2012). The main route of Ca^{2+} entry into the mitochondria is through the Ca^{2+} uniporter (MCU), which is activated by Ca^{2+} in an allosteric manner (Kirichok et al. 2004). During pathological increases in cytosolic Ca^{2+} concentration, mitochondria accumulate Ca^{2+} ions and sometimes Ca^{2+}-phosphate complexes form as reversible calcium deposits (David et al. 2003; Pivovarova et al. 1999). In excitable cells, the primary method of calcium extrusion out of the mitochondria is the Na^{+}/Ca^{2+} transporter (mNCX), responsible of the low basal $[Ca^{2+}]_{mit}$. Because of its low carrier capacity, the Na^{+}/Ca^{2+} transporter (mNCX) can be easily overtaken by Ca^{2+} influx through the Ca^{2+} uniporter (MCU), generating a net accumulation of Ca^{2+} in mitochondria. An alternative pathway for Ca^{2+} extrusion is the H^{+}/Ca^{2+} exchanger, but it is slow and saturates at a lower $[Ca^{2+}]_{Cit}$ than the Na^{+}/Ca^{2+} transporter; so its contribution to fast calcium exchange is limited (Bernardi 1999; Gunter et al. 1991).

Still another route for Ca^{2+} extrusion is via the transitory opening of the mitochondrial permeability transition pore (mPTP) complex, which controls excessive influx of toxic levels of Ca^{2+} (Bernardi and von Stockum 2012; Ichas et al. 1997; Zoratti and Szabo 1995). It is known that factors such as increased oxidative stress (ROS), mitochondrial Ca^{2+} overload, and metabolic stress promote mPTP opening (Duchen 2000a; Duchen 2000b). mPTP opening determines whether a cell dies or survives. Although its opening mechanism is not very well understood, it is known that mitochondrial Ca^{2+} overload is enough to promote its opening. It has been proposed that partial mitochondrial depolarization can prevent Ca^{2+} overload in mitochondria (Budd and Nicholls 1996; Sanz-Blasco et al. 2008). Simultaneous measurements of $[Ca^{2+}]$ and inner mitochondrial

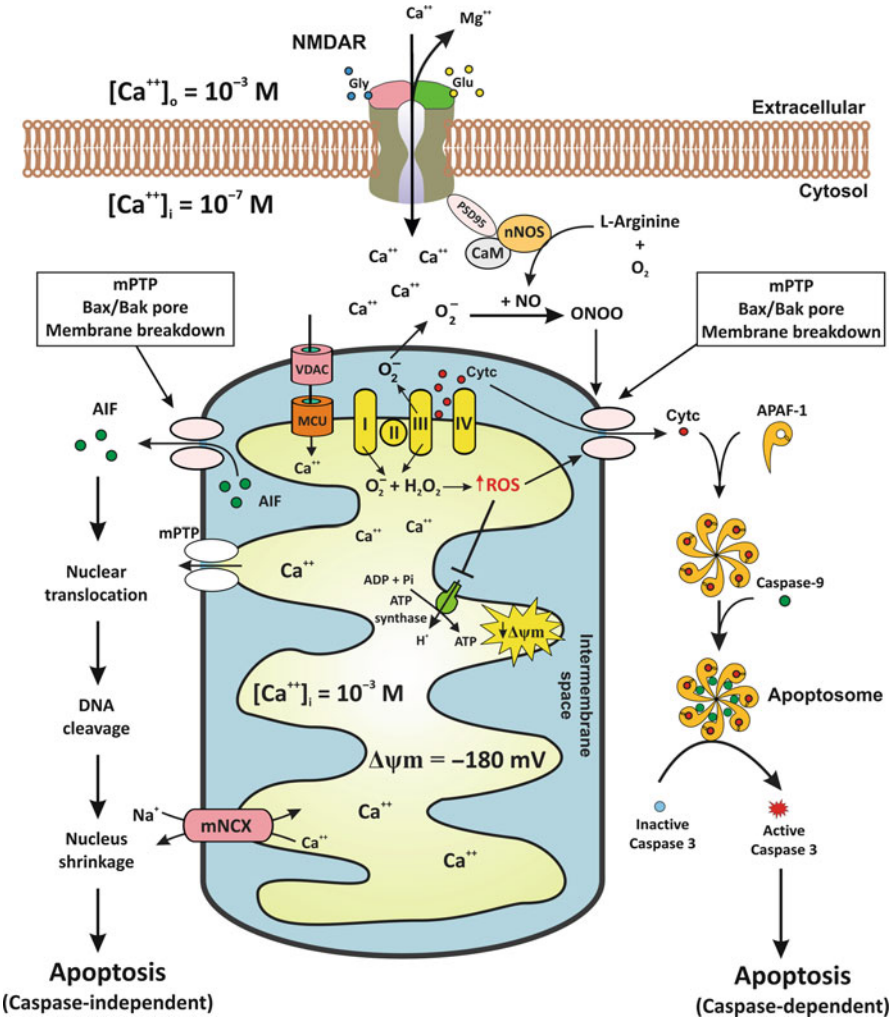

Fig. 2 Mitochondrial contribution to excitotoxicity. During GluR-mediated excitotoxicity, excess Ca^{2+} is transported into mitochondria by a complex formed between the voltage-sensitive anion channel (VDAC) and the mitochondrial Ca^{2+} uniporter (MCU). Although Ca^{2+} concentrations are higher inside the mitochondria compared with the cytosol, Ca^{2+} is normally transported inside mitochondria due to a large electrical gradient across the mitochondrial membrane, known as the inner mitochondrial membrane potential ($\Delta\psi m = -180$ mV). As excess Ca^{2+} accumulates, it produces a persistent depolarization of the inner mitochondrial membrane potential that triggers the synthesis of ATP via the ATP synthase and increases the activity of the electron transport chain with the subsequent production of reactive oxygen species (ROS). Persistent accumulation of ROS in mitochondria may inhibit ATP synthesis and the activity of the respiratory chain, leading to energy failure and loss of mitochondrial homeostasis. As the integrity of the mitochondria deteriorates, key internal components of the mitochondria such as cytochrome c (Cyt c) and apoptosis-inducing factor (AIF) are released into the cytosol where they act as powerful triggers of apoptosis. Cyt c interacts with apoptotic protease-activating factor 1 (APAF-1) and

membrane potential ($\Delta\psi m$) in hippocampal neurons during exposure to toxic glutamate levels show that $[Ca^{2+}]c$ increases transiently followed by a variable degree of recovery. This is then followed by a failure of the cell to maintain a low cytoplasmic free Ca^{2+} concentration, called delayed calcium deregulation (DCD). During this process, $\Delta\psi m$ changes only slightly during the initial phase, showing a progressive and complete depolarization later on (Abramov and Duchen 2008) that causes ATP depletion and cell death. GluR-dependent transient mitochondrial calcium loading can also initiate oxidative damage and/or inhibit mitochondrial respiration (Ward et al. 2000) observed as a reduction in oxygen consumption due to inhibition of mitochondrial complexes I, II/III, and IV (Rego et al. 2000).

7 Clinical Relevance of GluR-Mediated Excitotoxicity

Although the original concept behind the mechanism of excitotoxicity originated in strong preclinical evidence suggesting that acute and chronic neurotoxicity can use the excitatory amino acid glutamate to produce nerve cell injury and cell death (Olney 1969; Olney 1978), it was not until 20 years later that a direct link between glutamate excitotoxicity and clinical disease in humans could be identified (Perl et al. 1990; Teitelbaum et al. 1990). Although equally strong preclinical (Benveniste et al. 1984; Cook et al. 2012a; Cook et al. 2012b; Hagberg et al. 1985) and clinical (Bullock et al. 1995) evidence supports a significant role for GluR-mediated excitotoxicity in hypoxia-ischemia and stroke, the initial pace of discovery of anti-excitotoxic drugs based in GluR antagonists has been slower than expected (O'Collins et al. 2006; Savitz and Fisher 2007). One of the reasons for the slow progress of discovery of anti-excitotoxic therapies based in GluR antagonists has been the fact that most inhibitors of GluRs cannot discriminate between GluRs engaged in physiologically relevant glutamatergic neurotransmission and pathologically active GluRs associated with disease states (Lipton 2004; Lipton 2007). The search for clinically tolerated GluR antagonists that block excitotoxicity by preferentially targeting pathologically active GluRs led to the identification of memantine, an uncompetitive NMDA receptor antagonist (Chen et al. 1992; Chen and Lipton 2006) that preferentially blocks pathologically active GluRs and protects neurons against excitotoxic injury (Fig. 3) (Lipton 2007). Memantine's neuroprotective activity has made it a drug of choice in the treatment of chronic neurological diseases such as Parkinson's (Olney et al. 1987; Rabey et al. 1992), dementia with Lewy bodies (Sabbagh et al. 2005), and Alzheimer's disease, for which it is approved by the FDA for clinical use (Fleischhacker et al. 1986; McShane et al. 2006; Reisberg et al. 2003).

Fig. 2 (continued) caspase 9 to form the apoptosome, which activates caspase 3, a key powerful mediator of apoptotic cell death. Loss of mitochondrial integrity also allows the release of the apoptosis-inducing factor (AIF) into the cytosol where it enters the nucleus and causes DNA fragmentation, chromatin condensation, and nucleus shrinkage through a caspase-independent mechanism of apoptosis

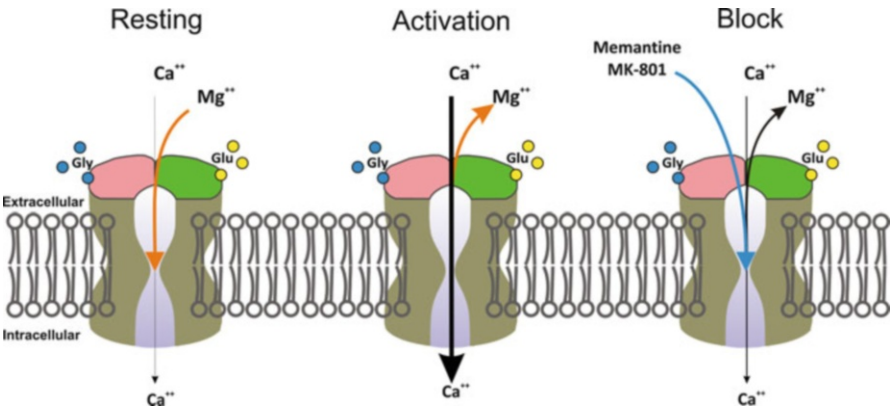

Fig. 3 Preferential block of pathologically active GluRs. Ca^{2+}-permeable NMDARs are an important mediator of GluR excitotoxicity. Under resting conditions, a steep electrochemical gradient favors Ca^{2+} influx through NMDARs while a voltage-dependent block by extracellular Mg^{2+} interferes with Ca^{2+} influx. During NMDAR activation, transient depolarization is required to force Mg^{2+} out of the channel and allow Ca^{2+} influx into the cell. Once the cell returns to its normal resting potential, Mg^{2+} block of Ca^{2+} influx is restored while excess Ca^{2+} is pumped out of the cell. Selective NMDAR antagonists, such as memantine, act as activity-dependent open-channel blockers that, similar to Mg^{2+}, can gain access to the inside the ion channel pore and block the influx of Ca^{2+} through NMDARs during excessive pathological activation. Because its blocking effect requires prior activation of the receptor by glutamate, which exposes binding sites that are otherwise hidden during the resting state, the efficacy of the drug increases as the activity of the receptor increases and vice versa. In addition to this property, preferential block of pathologically active GluRs requires drugs to be highly selective for their specific target, to avoid interference with other neurotransmitter systems and unwanted side effects, and to have low affinity for its binding site (in the case of memantine, produced by a relatively rapid off-rate from the channel) in order to minimize inhibition of the receptor during normal physiological function. In contrast, MK-801 has a very slow off-rate from the channel, blocking physiological activity and producing multiple side effects

Recently, ketamine, an NMDAR open-channel blocker and dissociative anesthetic with anti-excitotoxic properties (Choi et al. 1989; Choi 1990), has been shown to possess strong antidepressant activity in both preclinical and clinical studies of depression (Skolnick et al. 2009).

An alternative anti-excitotoxic strategy that has produced positive clinical results makes use of drugs that boost the function of glutamate transport systems to reduce extracellular levels of glutamate (Fumagalli et al. 2008; Rothstein 1996). An example of such drugs is riluzole (Fumagalli et al. 2008), which is currently approved by the FDA for the treatment of amyotrophic lateral sclerosis (Bensimon et al. 1994; Lacomblez et al. 1996; Miller et al. 2012).

A growing body of evidence suggests GluR-mediated neurotoxicity facilitates growth of gliomas, one of the most aggressive and malignant forms of brain cancers (Takano et al. 2001; Watkins and Sontheimer 2012; Ye and Sontheimer 1999). Glioma cells can cause excitotoxic cell death in the surrounding brain tissue by releasing toxic amounts of glutamate through the cystine-glutamate antiporter

(SXC, SLC7A11) (Buckingham et al. 2011; Watkins and Sontheimer 2012). Clinical trials are currently under way to test the neuroprotective efficacy of the NMDAR antagonist memantine and the SXC inhibitor sulfasalazine in patients suffering from glioma (*ClinicalTrials.gov*: NCT01260467; NCT01430351, NCT01577966).

8 Conclusion

Although the full implications of excitotoxicity are not yet fully understood, a large body of evidence points towards a role for excitotoxic processes in brain disease. Either as a cause or consequence of other pathological process, the role of excitotoxicity in brain disease has become increasingly undeniable and as a result, difficult to ignore when trying to understand a series of neurodegenerative and neuropsychiatric disorders.

References

Abramov, A. Y., & Duchen, M. R. (2008). Mechanisms underlying the loss of mitochondrial membrane potential in glutamate excitotoxicity. *Biochimica et Biophysica Acta, 1777*, 953–964.

Alexander, S. P., Mathie, A., & Peters, J. A. (2011). Guide to Receptors and Channels (GRAC), 5th edition. *British Journal of Pharmacology, 164*(Suppl 1), S1–S324.

Ankarcrona, M., Dypbukt, J. M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S. A., & Nicotera, P. (1995). Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on mitochondrial function. *Neuron, 15*, 961–973.

Arundine, M., & Tymianski, M. (2004). Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. *Cellular and Molecular Life Sciences, 61*, 657–668.

Bano, D., Young, K. W., Guerin, C. J., Lefeuvre, R., Rothwell, N. J., Naldini, L., Rizzuto, R., Carafoli, E., & Nicotera, P. (2005). Cleavage of the plasma membrane Na^+/Ca^{2+} exchanger in excitotoxicity. *Cell, 120*, 275–285.

Beal, M. F. (1998). Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. *Annals of Neurology, 44*, S110–S114.

Bensimon, G., Lacomblez, L., & Meininger, V. (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *The New England Journal of Medicine, 330*, 585–591.

Benveniste, H., Drejer, J., Schousboe, A., & Diemer, N. H. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *Journal of Neurochemistry, 43*, 1369–1374.

Bernardi, P. (1999). Mitochondrial transport of cations: Channels, exchangers, and permeability transition. *Physiological Reviews, 79*, 1127–1155.

Bernardi, P., & Von Stockum, S. (2012). The permeability transition pore as a Ca^{2+} release channel: New answers to an old question. *Cell Calcium, 52*, 22–27.

Besancon, E., Guo, S., Lok, J., Tymianski, M., & Lo, E. H. (2008). Beyond NMDA and AMPA glutamate receptors: Emerging mechanisms for ionic imbalance and cell death in stroke. *Trends in Pharmacological Sciences, 29*, 268–275.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature, 361*, 31–39.

Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P., & Lipton, S. A. (1995). Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 7162–7166.

Boulter, J., Hollmann, M., O'shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C., & Heinemann, S. (1990). Molecular cloning and functional expression of glutamate receptor subunit genes. *Science, 249*, 1033–1037.

Brorson, J. R., Manzolillo, P. A., & Miller, R. J. (1994). Ca^{2+} entry via AMPA/KA receptors and excitotoxicity in cultured cerebellar Purkinje cells. *The Journal of Neuroscience, 14*, 187–197.

Brorson, J. R., Marcuccilli, C. J., & Miller, R. J. (1995). Delayed antagonism of calpain reduces excitotoxicity in cultured neurons. *Stroke, 26*, 1259–1266.

Brouns, R., & De Deyn, P. P. (2009). The complexity of neurobiological processes in acute ischemic stroke. *Clinical Neurology and Neurosurgery, 111*, 483–495.

Brown, G. C. (2010). Nitric oxide and neuronal death. *Nitric Oxide, 23*, 153–165.

Buckingham, S. C., Campbell, S. L., Haas, B. R., Montana, V., Robel, S., Ogunrinu, T., & Sontheimer, H. (2011). Glutamate release by primary brain tumors induces epileptic activity. *Nature Medicine, 17*, 1269–1274.

Budd, S. L., & Nicholls, D. G. (1996). Mitochondria, calcium regulation, and acute glutamate excitotoxicity in cultured cerebellar granule cells. *Journal of Neurochemistry, 67*, 2282–2291.

Budd, S. L., Tenneti, L., Lishnak, T., & Lipton, S. A. (2000). Mitochondrial and extramitochondrial apoptotic signaling pathways in cerebrocortical neurons. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 6161–6166.

Bullock, R., Zauner, A., Woodward, J., & Young, H. F. (1995). Massive persistent release of excitatory amino acids following human occlusive stroke. *Stroke, 26*, 2187–2189.

Cao, G., Xing, J., Xiao, X., Liou, A. K., Gao, Y., Yin, X. M., Clark, R. S., Graham, S. H., & Chen, J. (2007). Critical role of calpain I in mitochondrial release of apoptosis-inducing factor in ischemic neuronal injury. *The Journal of Neuroscience, 27*, 9278–9293.

Chen, H. S., & Lipton, S. A. (2006). The chemical biology of clinically tolerated NMDA receptor antagonists. *Journal of Neurochemistry, 97*, 1611–1626.

Chen, H. S., Pellegrini, J. W., Aggarwal, S. K., Lei, S. Z., Warach, S., Jensen, F. E., & Lipton, S. A. (1992). Open-channel block of *N*-methyl-D-aspartate (NMDA) responses by memantine: Therapeutic advantage against NMDA receptor-mediated neurotoxicity. *The Journal of Neuroscience, 12*, 4427–4436.

Chinopoulos, C., & Adam-Vizi, V. (2006). Calcium, mitochondria and oxidative stress in neuronal pathology. Novel aspects of an enduring theme. *The FEBS Journal, 273*, 433–450.

Cho, D. H., Nakamura, T., & Lipton, S. A. (2010). Mitochondrial dynamics in cell death and neurodegeneration. *Cellular and Molecular Life Sciences, 67*, 3435–3447.

Choi, D. W. (1985). Glutamate neurotoxicity in cortical cell culture is calcium dependent. *Neuroscience Letters, 58*, 293–297.

Choi, D. W. (1987). Ionic dependence of glutamate neurotoxicity. *The Journal of Neuroscience, 7*, 369–379.

Choi, D. W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron, 1*, 623–634.

Choi, D. W. (1990). Ketamine reduces NMDA receptor mediated neurotoxicity in cortical cultures. In E. F. Domino (Ed.), *Status of ketamine in anesthesiology* (pp. 549–555). Ann Arbor, MI: NPP Books.

Choi, D. W., Viseskul, V., Amirthanayagam, M., & Monyer, H. (1989). Aspartate neurotoxicity on cultured cortical neurons. *Journal of Neuroscience Research, 23*, 116–121.

Cook, D. J., Teves, L., & Tymianski, M. (2012a). Treatment of stroke with a PSD-95 inhibitor in the gyrencephalic primate brain. *Nature, 483*, 213–217.

Cook, D. J., Teves, L., & Tymianski, M. (2012b). A translational paradigm for the preclinical evaluation of the stroke neuroprotectant Tat-NR2B9c in gyrencephalic nonhuman primates. *Science Translational Medicine, 4*, 154ra133.

Cooke, S. F., & Bliss, T. V. (2006). Plasticity in the human central nervous system. *Brain, 129*, 1659–1673.

Curtis, D. R., Phillis, J. W., & Watkins, J. C. (1959). Chemical excitation of spinal neurones. *Nature, 183*, 611–612.

Curtis, D. R., Phillis, J. W., & Watkins, J. C. (1960). The chemical excitation of spinal neurones by certain acidic amino acids. *The Journal of Physiology, 150*, 656–682.

D'orsi, B., Bonner, H., Tuffy, L. P., Dussmann, H., Woods, I., Courtney, M. J., Ward, M. W., & Prehn, J. H. (2012). Calpains are downstream effectors of bax-dependent excitotoxic apoptosis. *The Journal of Neuroscience, 32*, 1847–1858.

David, G., Talbot, J., & Barrett, E. F. (2003). Quantitative estimate of mitochondrial [Ca^{2+}] in stimulated motor nerve terminals. *Cell Calcium, 33*, 197–206.

Dawson, V. L., Dawson, T. M., London, E. D., Bredt, D. S., & Snyder, S. H. (1991). Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proceedings of the National Academy of Sciences of the United States of America, 88*, 6368–6371.

Dawson, V. L., Kizushi, V. M., Huang, P. L., Snyder, S. H., & Dawson, T. M. (1996). Resistance to neurotoxicity in cortical cultures from neuronal nitric oxide synthase-deficient mice. *The Journal of Neuroscience, 16*, 2479–2487.

Doyle, K. P., Simon, R. P., & Stenzel-Poore, M. P. (2008). Mechanisms of ischemic brain damage. *Neuropharmacology, 55*, 310–318.

Duchen, M. R. (2000a). Mitochondria and Ca^{2+} in cell physiology and pathophysiology. *Cell Calcium, 28*, 339–348.

Duchen, M. R. (2000b). Mitochondria and calcium: From cell signalling to cell death. *The Journal of Physiology, 529*(Pt 1), 57–68.

Duchen, M. R. (2012). Mitochondria, calcium-dependent neuronal death and neurodegenerative disease. *Pflugers Archiv: European Journal of Physiology, 464*, 111–121.

Dugan, L. L., Sensi, S. L., Canzoniero, L. M., Handran, S. D., Rothman, S. M., Lin, T. S., Goldberg, M. P., & Choi, D. W. (1995). Mitochondrial production of reactive oxygen species in cortical neurons following exposure to *N*-methyl-D-aspartate. *The Journal of Neuroscience, 15*, 6377–6388.

Dumuis, A., Sebben, M., Haynes, L., Pin, J. P., & Bockaert, J. (1988). NMDA receptors activate the arachidonic acid cascade system in striatal neurons. *Nature, 336*, 68–70.

Dykens, J. A. (1994). Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated Ca^{2+} and Na^{+}: Implications for neurodegeneration. *Journal of Neurochemistry, 63*, 584–591.

Dykens, J. A., Stern, A., & Trenkner, E. (1987). Mechanism of kainate toxicity to cerebellar neurons in vitro is analogous to reperfusion tissue injury. *Journal of Neurochemistry, 49*, 1222–1228.

Farooqui, A. A., Ong, W. Y., & Horrocks, L. A. (2008). *Neurochemical aspects of excitotoxicity*. New York, NY: Springer.

Fleischhacker, W. W., Buchgeher, A., & Schubert, H. (1986). Memantine in the treatment of senile dementia of the Alzheimer type. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 10*, 87–93.

Fumagalli, E., Funicello, M., Rauen, T., Gobbi, M., & Mennini, T. (2008). Riluzole enhances the activity of glutamate transporters GLAST, GLT1 and EAAC1. *European Journal of Pharmacology, 578*, 171–176.

Garthwaite, J., Charles, S. L., & Chess-Williams, R. (1988). Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature, 336*, 385–388.

Gereau, R. W., & Swanson, G. (2008). *The glutamate receptors*. Totowa, NJ: Humana Press.

Gillessen, T., Budd, S. L., & Lipton, S. A. (2002). Excitatory amino acid neurotoxicity. *Advances in Experimental Medicine and Biology, 513*, 3–40.

Greenamyre, J. T. (1986). The role of glutamate in neurotransmission and in neurologic disease. *Archives of Neurology, 43*, 1058–1063.

Gunter, K. K., Zuscik, M. J., & Gunter, T. E. (1991). The Na^+(-independent Ca^{2+} efflux mechanism of liver mitochondria is not a passive $Ca^{2+}/2H^+$ exchanger. *The Journal of Biological Chemistry, 266*, 21640–21648.

Hagberg, H., Lehmann, A., Sandberg, M., Nystrom, B., Jacobson, I., & Hamberger, A. (1985). Ischemia-induced shift of inhibitory and excitatory amino acids from intra- to extracellular compartments. *Journal of Cerebral Blood Flow and Metabolism, 5*, 413–419.

Hara, M. R., & Snyder, S. H. (2007). Cell signaling and neuronal death. *Annual Review of Pharmacology and Toxicology, 47*, 117–141.

Hara, M. R., Agrawal, N., Kim, S. F., Cascio, M. B., Fujimuro, M., Ozeki, Y., Takahashi, M., Cheah, J. H., Tankou, S. K., Hester, L. D., Ferris, C. D., Hayward, S. D., Snyder, S. H., & Sawa, A. (2005). S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nature Cell Biology, 7*, 665–674.

Hayashi, T. (1952). A physiological study of epileptic seizures following cortical stimulation in animals and its application to human clinics. *The Japanese Journal of Physiology, 3*, 46–64.

Hayashi, T. (1954). Effects of sodium glutamate on the nervous system. *The Keio Journal of Medicine, 3*, 183–192.

Hollmann, M., & Heinemann, S. (1994). Cloned glutamate receptors. *Annual Review of Neuroscience, 17*, 31–108.

Hollmann, M., O'shea-Greenfield, A., Rogers, S. W., & Heinemann, S. (1989). Cloning by functional expression of a member of the glutamate receptor family. *Nature, 342*, 643–648.

Hu, N. W., Ondrejcak, T., & Rowan, M. J. (2012). Glutamate receptors in preclinical research on Alzheimer's disease: Update on recent advances. *Pharmacology, Biochemistry, and Behavior, 100*, 855–862.

Ichas, F., Jouaville, L. S., & Mazat, J. P. (1997). Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals. *Cell, 89*, 1145–1153.

Jeffery, B., Barlow, T., Moizer, K., Paul, S., & Boyle, C. (2004). Amnesic shellfish poison. *Food and Chemical Toxicology, 42*, 545–557.

Jensen, F. E. (2005). Role of glutamate receptors in periventricular leukomalacia. *Journal of Child Neurology, 20*, 950–959.

Kaul, M., Garden, G. A., & Lipton, S. A. (2001). Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature, 410*, 988–994.

Keinanen, K., Wisden, W., Sommer, B., Werner, P., Herb, A., Verdoorn, T. A., Sakmann, B., & Seeburg, P. H. (1990). A family of AMPA-selective glutamate receptors. *Science, 249*, 556–560.

Kim, M. J., Jo, D. G., Hong, G. S., Kim, B. J., Lai, M., Cho, D. H., Kim, K. W., Bandyopadhyay, A., Hong, Y. M., Kim, D. H., Cho, C., Liu, J. O., Snyder, S. H., & Jung, Y. K. (2002). Calpain-dependent cleavage of cain/cabin1 activates calcineurin to mediate calcium-triggered cell death. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 9870–9875.

Kirichok, Y., Krapivinsky, G., & Clapham, D. E. (2004). The mitochondrial calcium uniporter is a highly selective ion channel. *Nature, 427*, 360–364.

Koumura, A., Nonaka, Y., Hyakkoku, K., Oka, T., Shimazawa, M., Hozumi, I., Inuzuka, T., & Hara, H. (2008). A novel calpain inhibitor, ((1S)-1((((1S)-1-benzyl-3-cyclopropylamino-2,3-di-oxopropyl)amino)carbonyl)-3-methylbutyl) carbamic acid 5-methoxy-3-oxapentyl ester, protects neuronal cells from cerebral ischemia-induced damage in mice. *Neuroscience, 157*, 309–318.

Kroemer, G., Galluzzi, L., & Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. *Physiological Reviews, 87*, 99–163.

Kumar, J., & Mayer, M. L. (2012). Functional insights from glutamate receptor ion channel structures. *Annual Review of Physiology, 75*, 313–337.

Kupina, N. C., Nath, R., Bernath, E. E., Inoue, J., Mitsuyoshi, A., Yuen, P. W., Wang, K. K., & Hall, E. D. (2001). The novel calpain inhibitor SJA6017 improves functional outcome after delayed administration in a mouse model of diffuse brain injury. *Journal of Neurotrauma, 18*, 1229–1240.

Lacomblez, L., Bensimon, G., Leigh, P. N., Guillet, P., & Meininger, V. (1996). Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet, 347*, 1425–1431.

Lafon-Cazal, M., Pietri, S., Culcasi, M., & Bockaert, J. (1993). NMDA-dependent superoxide production and neurotoxicity. *Nature, 364*, 535–537.

Lai, M. M., Burnett, P. E., Wolosker, H., Blackshaw, S., & Snyder, S. H. (1998). Cain, a novel physiologic protein inhibitor of calcineurin. *The Journal of Biological Chemistry, 273*, 18325–18331.

Landshamer, S., Hoehn, M., Barth, N., Duvezin-Caubet, S., Schwake, G., Tobaben, S., Kazhdan, I., Becattini, B., Zahler, S., Vollmar, A., Pellecchia, M., Reichert, A., Plesnila, N., Wagner, E., & Culmsee, C. (2008). Bid-induced release of AIF from mitochondria causes immediate neuronal cell death. *Cell Death and Differentiation, 15*, 1553–1563.

Latremoliere, A., & Woolf, C. J. (2009). Central sensitization: A generator of pain hypersensitivity by central neural plasticity. *The Journal of Pain, 10*, 895–926.

Lau, A., & Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Archiv: European Journal of Physiology, 460*, 525–542.

Lazarewicz, J. W., Wroblewski, J. T., Palmer, M. E., & Costa, E. (1988). Activation of *N*-methyl-D-aspartate-sensitive glutamate receptors stimulates arachidonic acid release in primary cultures of cerebellar granule cells. *Neuropharmacology, 27*, 765–769.

Lefebvre, K. A., & Robertson, A. (2010). Domoic acid and human exposure risks: A review. *Toxicon, 56*, 218–230.

Lipton, S. A. (2004). Turning down, but not off. *Nature, 428*, 473.

Lipton, S. A. (2007). Pathologically activated therapeutics for neuroprotection. *Nature Reviews Neuroscience, 8*, 803–808.

Lipton, S. A., & Rosenberg, P. A. (1994). Excitatory amino acids as a final common pathway for neurologic disorders. *The New England Journal of Medicine, 330*, 613–622.

Lipton, S. A., Choi, Y. B., Pan, Z. H., Lei, S. Z., Chen, H. S., Sucher, N. J., Loscalzo, J., Singel, D. J., & Stamler, J. S. (1993). A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature, 364*, 626–632.

Liu, X., Kim, C. N., Yang, J., Jemmerson, R., & Wang, X. (1996). Induction of apoptotic program in cell-free extracts: Requirement for dATP and cytochrome c. *Cell, 86*, 147–157.

Liu, J., Liu, M. C., & Wang, K. K. (2008). Calpain in the CNS: From synaptic function to neurotoxicity. *Science Signaling, 1*, re1.

Lodge, D. (2009). The history of the pharmacology and cloning of ionotropic glutamate receptors and the development of idiosyncratic nomenclature. *Neuropharmacology, 56*, 6–21.

Lu, Y. M., Yin, H. Z., Chiang, J., & Weiss, J. H. (1996). Ca^{2+}-permeable AMPA/kainate and NMDA channels: High rate of Ca^{2+} influx underlies potent induction of injury. *The Journal of Neuroscience, 16*, 5457–5465.

Lubisch, W., Beckenbach, E., Bopp, S., Hofmann, H. P., Kartal, A., Kastel, C., Lindner, T., Metz-Garrecht, M., Reeb, J., Regner, F., Vierling, M., & Moller, A. (2003). Benzoylalanine-derived ketoamides carrying vinylbenzyl amino residues: Discovery of potent water-soluble calpain inhibitors with oral bioavailability. *Journal of Medicinal Chemistry, 46*, 2404–2412.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *Archives of Ophthalmology, 58*, 193–201.

Majewska, M. D., & Bell, J. A. (1990). Ascorbic acid protects neurons from injury induced by glutamate and NMDA. *Neuroreport, 1*, 194–196.

Martinez-Ruiz, A., Cadenas, S., & Lamas, S. (2011). Nitric oxide signaling: Classical, less classical, and nonclassical mechanisms. *Free Radical Biology & Medicine, 51*, 17–29.

Mattson, M. P. (2003). Excitotoxic and excitoprotective mechanisms: Abundant targets for the prevention and treatment of neurodegenerative disorders. *Neuromolecular Medicine, 3*, 65–94.

Mattson, M. P., Haughey, N. J., & Nath, A. (2005). Cell death in HIV dementia. *Cell Death and Differentiation, 12*(Suppl. 1), 893–904.

Mcshane, R., Areosa Sastre, A., & Minakaran, N. (2006). Memantine for dementia. *Cochrane Database of Systematic Reviews, 2*, CD003154.

Mehta, A., Prabhakar, M., Kumar, P., Deshmukh, R., & Sharma, P. L. (2013). Excitotoxicity: Bridge to various triggers in neurodegenerative disorders. *European Journal of Pharmacology, 698*, 6–18.

Meldrum, B. S. (1993). Excitotoxicity and selective neuronal loss in epilepsy. *Brain Pathology, 3*, 405–412.

Miller, R. G., Mitchell, J. D., & Moore, D. H. (2012). Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database of Systematic Reviews, 3*, CD001447.

Mucke, L., & Selkoe, D. J. (2012). Neurotoxicity of amyloid beta-protein: Synaptic and network dysfunction. *Cold Spring Harbor Perspectives in Medicine, 2*, a006338.

Nakamura, T., & Lipton, S. A. (2010). Preventing Ca^{2+}-mediated nitrosative stress in neurodegenerative diseases: Possible pharmacological strategies. *Cell Calcium, 47*, 190–197.

Nakamura, T., & Lipton, S. A. (2011). Redox modulation by *S*-nitrosylation contributes to protein misfolding, mitochondrial dynamics, and neuronal synaptic damage in neurodegenerative diseases. *Cell Death and Differentiation, 18*, 1478–1486.

Nakanishi, S. (1992). Molecular diversity of glutamate receptors and implications for brain function. *Science, 258*, 597–603.

Nicholls, J. G., Martin, A. R., Fuchs, P. A., Brown, D. A., Diamond, M. E., & Weisblat, D. (2012). *From neuron to brain* (5th ed.). Sunderland, MA: Sinauer Associates.

Nicoletti, F., Bockaert, J., Collingridge, G. L., Conn, P. J., Ferraguti, F., Schoepp, D. D., Wroblewski, J. T., & Pin, J. P. (2011). Metabotropic glutamate receptors: From the workbench to the bedside. *Neuropharmacology, 60*, 1017–1041.

Nimmrich, V., Reymann, K. G., Strassburger, M., Schoder, U. H., Gross, G., Hahn, A., Schoemaker, H., Wicke, K., & Moller, A. (2010). Inhibition of calpain prevents NMDA-induced cell death and beta-amyloid-induced synaptic dysfunction in hippocampal slice cultures. *British Journal of Pharmacology, 159*, 1523–1531.

Niswender, C. M., & Conn, P. J. (2010). Metabotropic glutamate receptors: Physiology, pharmacology, and disease. *Annual Review of Pharmacology and Toxicology, 50*, 295–322.

O'collins, V. E., Macleod, M. R., Donnan, G. A., Horky, L. L., Van Der Worp, B. H., & Howells, D. W. (2006). 1,026 experimental treatments in acute stroke. *Annals of Neurology, 59*, 467–477.

Okamoto, S. (1951). Epileptogenic action of glutamate directly applied into the brains of animals and inhibitory effects of protein and tissue emulsions on its action. *Journal of the Physiological Society, Japan, 13*, 555–562.

Olney, J. W. (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science, 164*, 719–721.

Olney, J. W. (1978). Neurotoxicity of excitatory amino acids. In E. G. McGeer, J. W. Olney, & P. L. McGeer (Eds.), *Kainic acid as a tool in neurobiology* (pp. 95–121). New York, NY: Raven Press.

Olney, J. W. (2003). Excitotoxicity, apoptosis and neuropsychiatric disorders. *Current Opinion in Pharmacology, 3*, 101–109.

Olney, J. W., & Ho, O. L. (1970). Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature, 227*, 609–611.

Olney, J. W., & Sharpe, L. G. (1969). Brain lesions in an infant rhesus monkey treated with monosodium glutamate. *Science, 166*, 386–388.

Olney, J. W., Price, M. T., Labruyere, J., Salles, K. S., Frierdich, G., Mueller, M., & Silverman, E. (1987). Anti-parkinsonian agents are phencyclidine agonists and *N*-methyl-D-aspartate antagonists. *European Journal of Pharmacology, 142*, 319–320.

Orrenius, S., Zhivotovsky, B., & Nicotera, P. (2003). Regulation of cell death: The calcium-apoptosis link. *Nature Reviews. Molecular Cell Biology, 4*, 552–565.

Ow, Y. P., Green, D. R., Hao, Z., & Mak, T. W. (2008). Cytochrome *c*: Functions beyond respiration. *Nature Reviews. Molecular Cell Biology, 9*, 532–542.

Perez-Pinzon, M. A., Stetler, R. A., & Fiskum, G. (2012). Novel mitochondrial targets for neuroprotection. *Journal of Cerebral Blood Flow and Metabolism, 32*, 1362–1376.

Perl, T. M., Bedard, L., Kosatsky, T., Hockin, J. C., Todd, E. C., & Remis, R. S. (1990). An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *The New England Journal of Medicine, 322*, 1775–1780.

Pivovarova, N. B., Hongpaisan, J., Andrews, S. B., & Friel, D. D. (1999). Depolarization-induced mitochondrial Ca accumulation in sympathetic neurons: Spatial and temporal characteristics. *The Journal of Neuroscience, 19*, 6372–6384.

Polster, B. M., Basanez, G., Etxebarria, A., Hardwick, J. M., & Nicholls, D. G. (2005). Calpain I induces cleavage and release of apoptosis-inducing factor from isolated mitochondria. *The Journal of Biological Chemistry, 280*, 6447–6454.

Puttfarcken, P. S., Lyons, W. E., & Coyle, J. T. (1992). Dissociation of nitric oxide generation and kainate-mediated neuronal degeneration in primary cultures of rat cerebellar granule cells. *Neuropharmacology, 31*, 565–575.

Rabey, J. M., Nissipeanu, P., & Korczyn, A. D. (1992). Efficacy of memantine, an NMDA receptor antagonist, in the treatment of Parkinson's disease. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 4*, 277–282.

Randall, R. D., & Thayer, S. A. (1992). Glutamate-induced calcium transient triggers delayed calcium overload and neurotoxicity in rat hippocampal neurons. *The Journal of Neuroscience, 12*, 1882–1895.

Raymond, L. A., Andre, V. M., Cepeda, C., Gladding, C. M., Milnerwood, A. J., & Levine, M. S. (2011). Pathophysiology of Huntington's disease: Time-dependent alterations in synaptic and receptor function. *Neuroscience, 198*, 252–273.

Rego, A. C., Santos, M. S., & Oliveira, C. R. (2000). Glutamate-mediated inhibition of oxidative phosphorylation in cultured retinal cells. *Neurochemistry International, 36*, 159–166.

Reisberg, B., Doody, R., Stoffler, A., Schmitt, F., Ferris, S., & Mobius, H. J. (2003). Memantine in moderate-to-severe Alzheimer's disease. *The New England Journal of Medicine, 348*, 1333–1341.

Reynolds, I. J., & Hastings, T. G. (1995). Glutamate induces the production of reactive oxygen species in cultured forebrain neurons following NMDA receptor activation. *The Journal of Neuroscience, 15*, 3318–3327.

Rigoulet, M., Yoboue, E. D., & Devin, A. (2011). Mitochondrial ROS generation and its regulation: Mechanisms involved in H_2O_2 signaling. *Antioxidants & Redox Signaling, 14*, 459–468.

Rizzuto, R., De Stefani, D., Raffaello, A., & Mammucari, C. (2012). Mitochondria as sensors and regulators of calcium signalling. *Nature Reviews. Molecular Cell Biology, 13*, 566–578.

Rothman, S. (1984). Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. *The Journal of Neuroscience, 4*, 1884–1891.

Rothman, S. M., & Olney, J. W. (1986). Glutamate and the pathophysiology of hypoxic – ischemic brain damage. *Annals of Neurology, 19*, 105–111.

Rothstein, J. D. (1996). Therapeutic horizons for amyotrophic lateral sclerosis. *Current Opinion in Neurobiology, 6*, 679–687.

Sabbagh, M. N., Hake, A. M., Ahmed, S., & Farlow, M. R. (2005). The use of memantine in dementia with Lewy bodies. *Journal of Alzheimer's Disease, 7*, 285–289.

Sanz-Blasco, S., Valero, R. A., Rodriguez-Crespo, I., Villalobos, C., & Nunez, L. (2008). Mitochondrial Ca^{2+} overload underlies Aβ oligomers neurotoxicity providing an unexpected mechanism of neuroprotection by NSAIDs. *PLoS One, 3*, e2718.

Sattler, R., Xiong, Z., Lu, W. Y., Hafner, M., Macdonald, J. F., & Tymianski, M. (1999). Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science, 284*, 1845–1848.

Savitz, S. I., & Fisher, M. (2007). Future of neuroprotection for acute stroke: In the aftermath of the SAINT trials. *Annals of Neurology, 61*, 396–402.

Siman, R., Noszek, J. C., & Kegerise, C. (1989). Calpain I activation is specifically related to excitatory amino acid induction of hippocampal damage. *The Journal of Neuroscience, 9*, 1579–1590.

Simon, R. P., Swan, J. H., Griffiths, T., & Meldrum, B. S. (1984). Blockade of *N*-methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science, 226*, 850–852.

Skolnick, P., Popik, P., & Trullas, R. (2009). Glutamate-based antidepressants: 20 years on. *Trends in Pharmacological Sciences, 30*, 563–569.

Sladeczek, F., Pin, J. P., Recasens, M., Bockaert, J., & Weiss, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurones. *Nature, 317*, 717–719.

Sugiyama, H., Ito, I., & Hirono, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature, 325*, 531–533.

Surmeier, D. J., Guzman, J. N., Sanchez-Padilla, J., & Goldberg, J. A. (2011). The origins of oxidant stress in Parkinson's disease and therapeutic strategies. *Antioxidants & Redox Signaling, 14*, 1289–1301.

Susin, S. A., Zamzami, N., Castedo, M., Hirsch, T., Marchetti, P., Macho, A., Daugas, E., Geuskens, M., & Kroemer, G. (1996). Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. *The Journal of Experimental Medicine, 184*, 1331–1341.

Sutherland, B. A., Minnerup, J., Balami, J. S., Arba, F., Buchan, A. M., & Kleinschnitz, C. (2012). Neuroprotection for ischaemic stroke: Translation from the bench to the bedside. *International Journal of Stroke, 7*, 407–418.

Syntichaki, P., & Tavernarakis, N. (2003). The biochemistry of neuronal necrosis: Rogue biology? *Nature Reviews Neuroscience, 4*, 672–684.

Takano, T., Lin, J. H., Arcuino, G., Gao, Q., Yang, J., & Nedergaard, M. (2001). Glutamate release promotes growth of malignant gliomas. *Nature Medicine, 7*, 1010–1015.

Teitelbaum, J. S., Zatorre, R. J., Carpenter, S., Gendron, D., Evans, A. C., Gjedde, A., & Cashman, N. R. (1990). Neurologic sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. *The New England Journal of Medicine, 322*, 1781–1787.

Tenneti, L., & Lipton, S. A. (2000). Involvement of activated caspase-3-like proteases in *N*-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. *Journal of Neurochemistry, 74*, 134–142.

Tenneti, L., D'emilia, D. M., Troy, C. M., & Lipton, S. A. (1998). Role of caspases in *N*-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. *Journal of Neurochemistry, 71*, 946–959.

Thayer, S. A., & Wang, G. J. (1995). Glutamate-induced calcium loads: Effects on energy metabolism and neuronal viability. *Clinical and Experimental Pharmacology & Physiology, 22*, 303–304.

Trapp, B. D., & Stys, P. K. (2009). Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. *Lancet Neurology, 8*, 280–291.

Traynelis, S. F., Wollmuth, L. P., Mcbain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., & Dingledine, R. (2010). Glutamate receptor ion channels: Structure, regulation, and function. *Pharmacological Reviews, 62*, 405–496.

Troy, C. M., Akpan, N., & Jean, Y. Y. (2011). Regulation of caspases in the nervous system implications for functions in health and disease. *Progress in Molecular Biology and Translational Science, 99*, 265–305.

Tymianski, M. (2010). Can molecular and cellular neuroprotection be translated into therapies for patients?: Yes, but not the way we tried it before. *Stroke, 41*, S87–S90.

Van Houten, B., Woshner, V., & Santos, J. H. (2006). Role of mitochondrial DNA in toxic responses to oxidative stress. *DNA Repair, 5*, 145–152.

Vandenabeele, P., Orrenius, S., & Zhivotovsky, B. (2005). Serine proteases and calpains fulfill important supporting roles in the apoptotic tragedy of the cellular opera. *Cell Death and Differentiation, 12*, 1219–1224.

Vandongen, A. M. (2009). *Biology of the NMDA receptor*. Boca Raton, FL: CRC Press.

Volterra, A., Trotti, D., Cassutti, P., Tromba, C., Salvaggio, A., Melcangi, R. C., & Racagni, G. (1992). High sensitivity of glutamate uptake to extracellular free arachidonic acid levels in rat cortical synaptosomes and astrocytes. *Journal of Neurochemistry, 59*, 600–606.

Vosler, P. S., Brennan, C. S., & Chen, J. (2008). Calpain-mediated signaling mechanisms in neuronal injury and neurodegeneration. *Molecular Neurobiology, 38*, 78–100.

Wang, K. K., Nath, R., Posner, A., Raser, K. J., Buroker-Kilgore, M., Hajimohammadreza, I., Probert, A. W., Jr., Marcoux, F. W., Ye, Q., Takano, E., Hatanaka, M., Maki, M., Caner, H., Collins, J. L., Fergus, A., Lee, K. S., Lunney, E. A., Hays, S. J., & Yuen, P. (1996). An alpha-mercaptoacrylic acid derivative is a selective nonpeptide cell-permeable calpain inhibitor and is neuroprotective. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 6687–6692.

Wang, H. G., Pathan, N., Ethell, I. M., Krajewski, S., Yamaguchi, Y., Shibasaki, F., Mckeon, F., Bobo, T., Franke, T. F., & Reed, J. C. (1999). Ca^{2+}-induced apoptosis through calcineurin dephosphorylation of BAD. *Science, 284*, 339–343.

Ward, M. W., Rego, A. C., Frenguelli, B. G., & Nicholls, D. G. (2000). Mitochondrial membrane potential and glutamate excitotoxicity in cultured cerebellar granule cells. *The Journal of Neuroscience, 20*, 7208–7219.

Watkins, J. C. (1962). The synthesis of some acidic amino acids possessing neuropharmacological activity. *Journal of Medicinal and Pharmaceutical Chemistry, 91*, 1187–1199.

Watkins, J. C. (2000). L-Glutamate as a central neurotransmitter: Looking back. *Biochemical Society Transactions, 28*, 297–309.

Watkins, J. C., & Evans, R. H. (1981). Excitatory amino acid transmitters. *Annual Review of Pharmacology and Toxicology, 21*, 165–204.

Watkins, S., & Sontheimer, H. (2012). Unique biology of gliomas: Challenges and opportunities. *Trends in Neurosciences, 35*, 546–556.

Weil-Malherbe, H. (1950). Significance of glutamic acid for the metabolism of nervous tissue. *Physiological Reviews, 30*, 549–568.

Wu, H. Y., Tomizawa, K., & Matsui, H. (2007). Calpain-calcineurin signaling in the pathogenesis of calcium-dependent disorder. *Acta Medica Okayama, 61*, 123–137.

Yang, J., Liu, X., Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T. I., Jones, D. P., & Wang, X. (1997). Prevention of apoptosis by Bcl-2: Release of cytochrome *c* from mitochondria blocked. *Science, 275*, 1129–1132.

Ye, Z. C., & Sontheimer, H. (1999). Glioma cells release excitotoxic concentrations of glutamate. *Cancer Research, 59*, 4383–4391.

Yi, J. H., & Hazell, A. S. (2006). Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. *Neurochemistry International, 48*, 394–403.

Yu, S. W., Wang, H., Poitras, M. F., Coombs, C., Bowers, W. J., Federoff, H. J., Poirier, G. G., Dawson, T. M., & Dawson, V. L. (2002). Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. *Science, 297*, 259–263.

Zoratti, M., & Szabo, I. (1995). The mitochondrial permeability transition. *Biochimica et Biophysica Acta, 1241*, 139–176.

Role of Ionotropic Glutamate Receptors in Neurodegenerative and Other Disorders

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Abstract

Disorders of the central nervous system (CNS) are amongst the most complex disease states and lead to some of the most devastating conditions found in medicine today. It is well established that both genetic and epigenetic factors influence the pathogenesis of neurodegenerative disorders. More recently a growing body of evidence suggests that changes in neurotransmitter systems may underlie the pathogenic mechanisms of neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease. Excitatory amino acids (EAA) are known to play key roles in neurotransmission, neuromodulation, and neurotoxicity. EAA receptors are classified into two major subdivisions, ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). L-Glu-gated ion channels, more commonly known as (iGluRs), are the primary mediators of fast excitatory synaptic transmission between neurons in the mammalian CNS. Evidence suggests that the iGluR receptor systems are involved in a number of pathophysiology conditions. This chapter will detail the current state of knowledge regarding the involvement of iGluRs in neurodegenerative and other disorders.

Keywords

Alzheimer's disease • Excitotoxicity • Ionotropic glutamate receptor • Neurodegeneration • Parkinson's disease

List of Abbreviations

AD	Alzheimer's disease
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate
AMPAR	AMPA receptor
CNS	Central nervous system
EAA	Excitatory amino acid
iGluR	Ionotropic glutamate receptor
Kainate	(2S,3S,4S)-3-(carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylic acid
L-Glu	L-glutamate
NMDA	(2R)-2-(methylamino)-butanedioic acid
NMDAR	NMDA receptor

1 History of Central Nervous System Disorders

Disorders of the central nervous system (CNS) have been a part of recorded history since ancient times. The Greek Athenian philosopher Socrates (469–399 BC) is

believed to have experienced neuropsychological disturbances in the form of "demonic orders" or what are now medically referred to as auditory command hallucinations. Similarly, the Greek historian Aristotle (384–322 BC) who was said to have suffered from epilepsy was the first to propose that all mental illness was caused by physical problems of the body (see Traicté de l'Epilepsie by Jean Taxol, 1602). Texts also allude to various other notable historical figures being afflicted with neurological disorders including Julius Caesar (epilepsy), Mozart (manic depression), and Joan of Arc (schizophrenia). Neurological disorders have not only been part of the human condition since the beginning of civilization but also played pivotal roles in human history.

2 Multifactorial Etiology of CNS Disorders

Disorders of the CNS are complex disease states with more than 600 disorders currently recognized. Of all CNS disorders, neurodegenerative diseases are the most prevalent. Etymologically, neurodegeneration is made up from prefix "neuro-," which refers to cells of neuronal origin, and "degeneration," which in the context of an organ or tissue system refers to the process of losing function or structure. Clinically, neurodegenerative diseases comprise a diverse assemblage of disorders that present with heterogeneous pathological and clinical expression patterns. These diseases are typically characterized by nervous system dysfunction resulting from the progressive loss of neuron structure or function often with accompanying neuronal cell death. Cell death primarily affects subsets of neurons in specific functional systems and may involve either the entire neuron (neuronopathy) or be restricted to the axon (axonopathy). While the etiology of neurodegenerative diseases is often unknown, a variety of factors have been identified that determine an individual's susceptibility to disease, including an individual's age, race, gender, and genetic makeup. Environmental factors such as exposure to chemicals and infectious agents have also been shown to increase the risk of neurodegenerative diseases. The most prevalent neurodegenerative diseases include Alzheimer's disease and other dementias, Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease).

3 Prevalence of Neurodegenerative Disorders

Dementia is the term typically used when referring to a decline in intellectual functioning, including problems with episodic memory, critical reasoning, and objective thinking. Within the general population, the most common disorders that cause neurological impairments include Alzheimer's disease, dementia with Lewy bodies, frontotemporal dementia, and microvascular brain injury. The primary risk factor for dementia is age, with incidence rates doubling for every 5 years after the age of 65. Gender has also been found to affect the incidence of dementia, with woman having a higher risk of developing the condition. Cohort longitudinal

studies estimate that the prevalence rate for all dementias is between 10 and 15 per 1,000 person-years. According to estimates from the World Health Organization, 0.441 % of people worldwide will have dementia by 2015 and that the prevalence will increase to 0.556 % by 2030 (World Health Organization 2012). As of 2010, there were an estimated 35.6 million people worldwide affected by dementia. According to the World Alzheimer Report, released by Alzheimer's Disease International, the prevalence of dementia will double every 20 years to 65.7 million in 2030 and to 115.4 million in 2050 (ASD International 2012). Currently, 58 % of people afflicted with dementia live in developing countries. It is estimated that by 2050 this will rise to 71 %. Alzheimer's disease is the most common form of dementia, accounting for between 50 % and 70 % of all dementia cases.

4 Societal Cost of Neurodegenerative Disorders

With a general trend for increasing life expectancies worldwide, neurodegenerative disorders are becoming one of the most costly diseases for societies. In 2009, it was estimated that the approximate cost of dementia worldwide was approximately US$422 billion yearly. The costs associated with neurodegenerative disorders can be categorized into direct medical costs and indirect costs. Direct medical costs include those associated with long-term care by health-care professionals in addition to the cost of nursing home care. It is estimated that over two-thirds of nursing home residents suffer from some sort of dementia. It is often the case that the primary caregiver for a person suffering from a neurodegenerative disorder is a relative or close friend. This can result in significant indirect costs that include caregiving time and the loss of economic productivity for both the patient and caregiver. It has been estimated that the total costs for an Alzheimer's patient are between $18,000 and $77,500 per year in the United States. Dementia caregivers themselves often suffer psychosocial and physical problem which can be directly related to the duties of the caregiver toward the sufferer. It has been estimated that a caregiver may give up to 47 h per week nonwork-related time to the care of a sufferer. It therefore remains that neurodegenerative disorders will continue to represent a major challenge for any society.

5 A Brief History of L-Glutamate

In 1866, the German chemist K. Heinrich Ritthausen discovered and identified L-glutamate. However, the role of L-Glu as a nervous system excitant was not fully elucidated until the mid-twentieth century. In 1954, Hayashi observed that direct injection of glutamate into the brain resulted in seizure activities. Similarly, Curtis et al. (Curtis and Eccles 1959; Curtis et al. 1959) showed glutamic acid to be effective for evoking repetitive discharges from interneurons located in the spinal cord of the cat. Subsequent studies by Curtis revealed that application of glutamic acid could induce neuron depolarization with concomitant increases in the firing of

action potentials (Curtis et al. 1959). Concomitant with the presence of high concentrations of glutamate in nervous tissue leads to speculation that L-Glu had an important neurophysiological role. In 1963, a seminal paper by McLennan was published that outlined the criteria required to establish substances as neurotransmitters. In the same year, iontophoretic studies of the effects of L-Glu on neurons in the mammalian cerebral cortex proposed that L-Glu was a putative excitatory neurotransmitter in the CNS (Krnjevic and Phillis 1963). The discovery by Takeuchi and Takeuchi of L-Glu-induced depolarization in muscle at locations corresponding to sites of synaptic innervation added further evidence for the role of L-Glu as a neurotransmitter (Takeuchi and Takeuchi 1963). Since then, numerous experiments have unequivocally determined that L-Glu is the major excitatory neurotransmitter in the vertebrate CNS.

6 Glutamate Receptors

The receptors mediating L-Glu neurotransmission can be classified into two major groups, ionotropic and metabotropic (Fig. 1a). L-Glu-gated ion channels, more commonly known as ionotropic glutamate receptors (iGluRs), are the primary mediators of fast excitatory synaptic transmission between neurons in the mammalian CNS. iGluRs have been typically classified by the synthetic agonists that mimic the effects of L-Glu: (2R)-2-(methylamino)butanedioic acid (*N*-methyl-D-aspartate; NMDA), 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA), and (2S,3S,4S)-3-(carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylic acid (kainic acid; kainate) (Fig. 1b). The first cloning of an L-Glu receptor was by Gunderson (1984) who observed that messenger RNA (mRNA) from human brain injected into *Xenopus* oocytes was able to produce both L-Glu- and kainate-sensitive channels in addition to voltage-operated channels. In 1989, Hollmann screened a rat brain cDNA library for kainate-gated ion channels that were subsequently expressed in *Xenopus* oocytes (Hollmann et al. 1989). The resulting cDNA clone was found to encode a single protein of 99,800 relative molecular mass that formed a functional ion channel that possessed both pharmacological and electrophysiological properties of the kainate subtype of L-Glu receptor. Based on its sensitivity to kainate, the receptor was named GluR-K1. However subsequent binding studies of this channel revealed that the receptor was in fact an AMPA receptor protein based on its non-desensitizing responses to kainate. The GluR-K1 receptor was subsequently renamed to GluR1 to reflect this (Sommer et al. 1990).

Over the next few years, additional AMPA receptor family members were cloned including GluR1 (Keinanen et al. 1990), GluR2 (Nakanishi et al. 1990), GluR3 (Bettler et al. 1990; Boulter et al. 1990), and GluR4 (Gallo et al. 1992). In 1990, Bettler et al. cloned a low-affinity kainate-binding subunit they termed GluR5 (Bettler et al. 1990). This receptor subunit displayed only 40–41 % amino acid identity with the other AMPA/kainate receptor subunits known to exist (GluRs1–4). Expression of this clone in Xenopus oocytes resulted in homomeric channels that were only weakly responsive to L-Glu. Investigations determined that

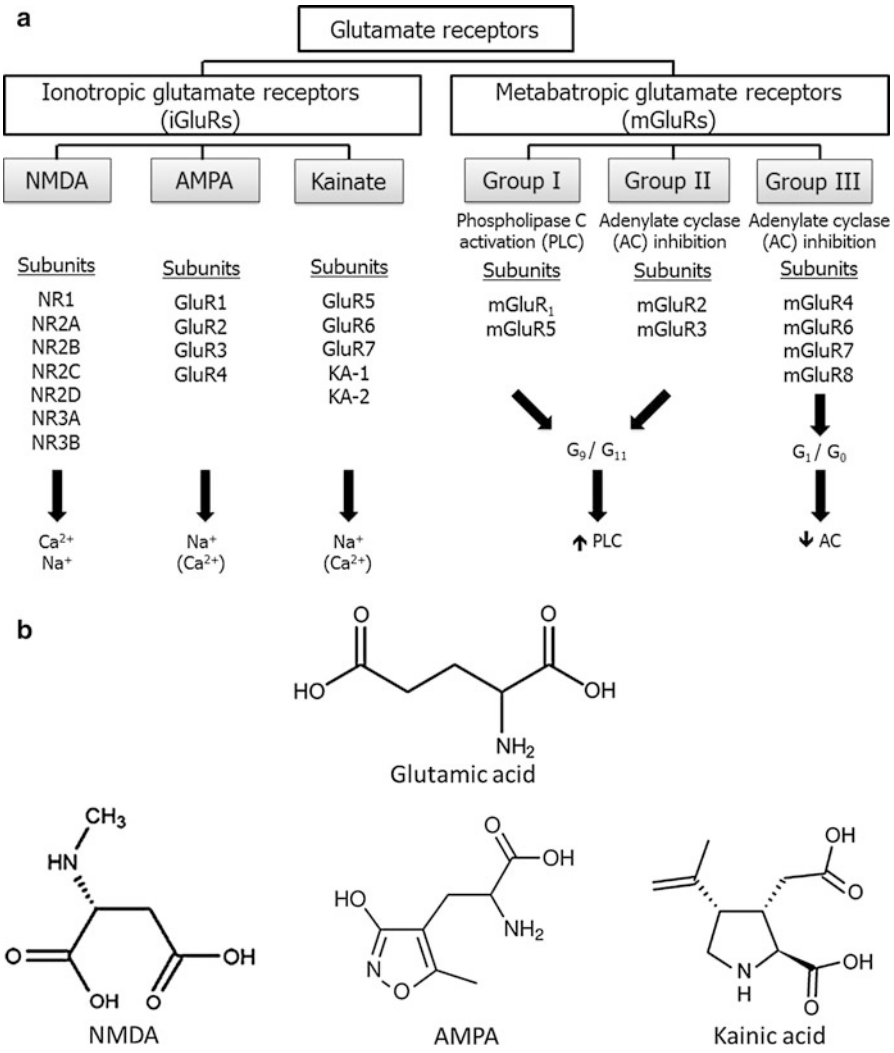

Fig. 1 (**a**) Family of glutamate receptors. (**b**) Structures of L-Glu, NMDA, AMPA and Kainate

the sequence similarity of GluR5 was significantly below the 70 % inter-subunit identity that was characteristic of the other AMPA/kainate receptors identified suggesting that GluR5 was in fact a different subtype of L-Glu receptor. This was confirmed in 1992 by Sommer (Sommer et al. 1992) who reported the desensitization of this subunit to kainate. Other kainate-selective receptors were cloned in the early 1990s including GluR6 (Egebjerg et al. 1991), GluR7 (Bettler et al. 1992; Lomeli et al. 1992), KA1 (Werner et al. 1991), and KA2 (Herb et al. 1992; Kamboj et al. 1992). It should be noted that when KA1 and KA2 subunits are expressed either alone or with each other, it does not result in the formation of functional

channels. However, co-expression of KA1 and KA2 with either Glu5 or Glu6 is able to form functional channels albeit with modified properties (Fisher and Mott 2011). The NMDA receptor subunits were first cloned by Moriyoshi et al. (1991), Yamazaki et al. (1992), and Karp et al. (1993) and were termed NR1 (also referred to as NMDAR1). In 1993, Ishii et al. (1993) cloned an additional four subunits, which they termed NR2A-D. Two NR3 subunits have also been cloned termed NR3A and NR3B (Sucher et al. 1995; Das et al. 1998).

Each functional iGluR subtype is formed by the heteromeric assembly of four subunits from a single class, e.g., NMDA subunits can only assemble with other members of the NMDA subfamily. Of the 16 iGluR subunits identified in human, 7 belong to the NMDA class (NR1, NR2A-D, NR3A-B), with the remaining 9 being members of the non-NMDA class, 4 to the AMPA class (GluR1–4) and 5 to the kainate class (GluR5–7 and KA1–2) (Fig. 1a). Two other subunits, known as δ1 and δ2, are termed orphan receptors because while they share high sequence similarity to other iGluR subunits, there is currently no evidence to indicate that they form functional iGluRs (Schmid et al. 2008). Recently, a new nomenclature for ligand-gated ion channels has been proposed by Collingridge et al. (2009) and is currently being reviewed by the nomenclature committee of International Union of Basic and Clinical Pharmacology (IUPHAR) IUPHAR (NC-IUPHAR).

7 NMDA Receptors

The NMDA subtype of iGluRs is involved in activity-dependent forms of synaptic plasticity, synapse consolidation, and excitatory amino acid (EAA)-mediated neuronal toxicity (Hynd et al. 2004a). NMDA receptors (NMDARs) are found on most CNS neurons (Petralia et al. 1994a, b, c) and mediate Ca^{2+} influx in response to the coincident binding of L-Glu and glycine (Constantine-Paton 1990; Bliss and Collingridge 1993; Seeburg 1993).

Functional NMDARs exist as tetrameric or pentameric complexes comprising proteins from three families of homologous subunits, designated NR1, NR2A-D, and NR3A-B (Monyer et al. 1992; Ishii et al. 1993; Dingledine et al. 1999). The presence of the NR3 subunits serves to have an inhibitory effect on NMDAR activity. The NR1 subunit functions to bind glycine, whereas the NR2 subunits bind L-Glu. For both the NR1 and NR2 mRNA transcripts and subunit proteins, distinct spatial and temporal expression patterns have been found (Bockers et al. 1994). While the NR1 subunit is expressed ubiquitously in the brain, the NR2 subunits show marked regional and developmental variations (Watanabe et al. 1992; Akazawa et al. 1994; Monyer et al. 1994). The function and modulation of NMDAR channels is both spatially and temporally regulated (Bockers et al. 1994; Rigby et al. 1996). The NR1 mRNA undergoes alternate splicing of three exons (4, 20, and 21) to give eight functionally distinct subunit variants (Sugihara et al. 1992; Durand et al. 1993; Hollmann et al. 1993). Exon 4 encodes a 21-amino acid amino-terminal cassette (designated "N1"), while exons 20 and 21 encode two consecutive carboxy-terminal 37- ("C1") and 38-amino acid ("C2") cassettes

(Zimmer et al. 1995). Both the gene sequence and the intron/exon organization of the human NR1 gene show a high degree of homology to the rat gene, with exception of the deletion of rat exon #3 (Hollmann et al. 1993). In vivo, NMDARs are regulated by a variety of agents including glycine, Mg^{2+}, Zn^{2+} and H^{+} ions, and polyamines. Alternative splicing of the N1 cassette has been shown to modulate both the ligand affinity and channel characteristics of NMDARs. Insertion of the N1 cassette results in the formation of receptors with decreased sensitivity to proton inhibition, increased current amplitudes, and altered responses to protein kinase C activation (Durand et al. 1993; Traynelis et al. 1995). Deletion of the cassette potentiates responses to the polyamines spermine, spermidine, and Zn^{2+} (Zhang et al. 1994; Traynelis et al. 1995). In contrast deletion of the N1 cassette has been shown to potentiate the response to polyamines in the presence of saturating concentrations of glycine (Durand et al. 1993) in addition to low-micromolar Zn^{2+} concentrations (Hollmann et al. 1993). In contrast, both N-terminal variants are inhibited by high-micromolar concentrations of Zn^{2+} and at low glycine concentrations. Potentiation of NMDARs occurs dose dependently and involves an increase in both peak channel open probability and peak channel current duration (Araneda et al. 1993).

8 AMPA Receptors

The AMPA type of iGluRs (AMPARs) are believed to be the most common type of neurotransmitter receptor in the CNS. AMPARs are multimeric ligand-gated ion channels that like NMDARs play crucial roles in synaptic transmission and plasticity (Ozawa et al. 1998). AMPARs are heteromeric and are composed from four different subunit types, designated as GluR1, GluR2, GluR3, and GluR4 (previously referred to as GluRA-D, respectively) (Collingridge et al. 2009). Each of the subunits is encoded for by a separate gene. Native AMPARs typically occur in a symmetric "dimer of dimers" configuration, consisting of GluR2 and either GluR1, GluR3, or GluR4. Dimerization of the subunits is initiated in the endoplasmic reticulum and occurs via interaction of a conserved N-terminal domain consisting of the amino acids leucine, isoleucine, and valine-binding protein motif. The association of different GluR subunits gives rise to receptors with different channel properties and ionic conductances (Derkach et al. 2007). Each GluR subunit consists of approximately 900 amino acids with each exhibiting 65–75 % sequence homology when compared to the other subunits (Hollmann and Heinemann 1994).

Similar to other iGluRs, the GluR subunits possess a structure consisting of an extracellular N-terminal domain, an intracellular C-terminal domain, and three membrane-spanning hydrophobic domains termed M1, M3, and M4. In addition, one membrane reentrant loop (M2) is also present (Bennett and Dingledine 1995). Within each subunit, the ligand-binding domain is made up from an N-terminal region designated as S1 and S2. However, AMPAR subunits show the greatest difference in their C-terminal sequence, which determines how each subunit interacts with cellular scaffolding proteins. Each AMPAR has four agonist-binding

sites, each of which is formed by the N-terminal region and the extracellular loops located between TM's III and IV. The binding of an agonist (such as L-Glu) results in a structural rearrangement of these two loops, such that each is moved toward each other, resulting in opening of the channel pore. Ion flow through AMPARs only occurs upon coincident binding of two agonists with increases in current flow occurring following binding of two more agonists at the unoccupied sites. Due to the rapid nature for both AMPAR desensitization and resensitization kinetics, it has been suggested that they are the primary mediators for fast excitatory neurotransmission in the CNS. Long-term potentiation (LTP) via AMPARs occurs by both an increase in the number of AMPARs at the postsynaptic surface and increases in the single channel conductance of the receptor channel (Lee and Kirkwood 2011).

Native AMPAR subunits exist as two splice variants, known as the "flip" and "flop" types. Alternative splicing is based on the insertion/deletion of a 38-amino acid sequence located at the C-terminal end of an extracellular loop located between transmembrane segments III and IV (Seeburg et al. 1998). The resulting AMPARs show altered desensitization kinetics including the speed of receptor desensitization, the speed at which the receptor is subsequently resensitized in addition to the rate at which the AMPAR channel closes (Frandsen and Schousboe 2003). The flip variant has been shown to occur in the prenatal CNS, while the flop variant is the form found in the adult CNS.

AMPARs are permeable to the monovalent cations, Na^+ and K^+. However, in contrast to NMDARs, AMPARs are typically impermeable to the divalent cation, calcium. Ca^{2+} permeability of AMPARs is controlled by the GluR2 subunit. Posttranscriptional editing of the GluR2 mRNA results in a single amino acid change from the uncharged amino acid glutamine (Q) to the positively charged arginine (R). This substitution occurs in transmembrane region II, known as the Q/R editing site. AMPARs containing glutamine, GluR2(Q), are permeable to Ca^{2+}, while receptors containing arginine, GluR2(R), are not due to the unfavorable energetics required for calcium to enter past the positive-charged amino acid. In the adult CNS, the majority of GluR2 subunits exist as the GluR2(R) form, resulting in Ca^{2+}-impermeable AMPARs. This impermeability is believed to protect against neuronal excitotoxicity. The GluR2 subunit has also been shown to modulation of the receptor by polyamines (Stromgaard and Mellor 2004). AMPARs lacking a GluR2 subunit are capable of being blocked in a voltage-dependent manner, preventing the passage of positive ions (K^+, Na^+) through the channel pore.

The phosphorylation of AMPAR subunits has been shown to alter both synaptic transmission and plasticity in the CNS. Phosphorylation of AMPARs at the intracellular carboxy-terminus has been well established. Serine/threonine phosphorylation sites have been identified on all four AMPA subunits and serve to regulate channel localization, open probability, and ionic conductance (Santos et al. 2009). The GluR1 subunit has four phosphorylation sites which are located at serine 818 (S818), S831, and S845 and threonine 840 (T840) (Lee et al. 2007). Phosphorylation of S818 by protein kinase C (PKC) has been shown to play a crucial role in

long-term potentiation (LTP) (Boehm et al. 2006). Similarly, during LTP, S831 is phosphorylated resulting in the trafficking of GluA1-containing AMPARs to the synapse (Lee et al. 2000). The phosphorylation of S845 by protein kinase A has been shown to regulate channel open probability (Castellani et al. 2005). In contrast, the T840 site has been implicated in long-term depression (LTD) (Lee et al. 2007).

9 Kainate Receptors

The kainate types of iGluRs are formed from five distinct subunit types – GluR5 (GluK1), GluR6 (GluK2), GluR7 (GluK3), KA1 (GluK4), and KA2 (GluK5). Similar to both the NMDA and AMPA receptors, kainate receptor subunits can associate in multiple ways to form tetrameric receptors (Contractor et al. 2011). In vivo, the GluR5–7 (GluK1–3) subunits associate to form both homomeric receptors (i.e., receptors that are composed only of one type of GluR subunit) and heteromeric receptors (i.e., receptors whose subunit composition consists of different GluR subunits). In contrast, both the KA1 and KA2 (GluK4 and GluK5) subunits can only form functional receptors when combined with one of the GluR5–7 (GluK1–3) subunits.

Similar to other iGluRs, kainate receptors share a high degree of structural similarity to other subunits of the iGluR family. Receptors consist of an extracellular N-terminal domain that is involved in receptor assembly that, together with a loop between TMIII and TMIV, forms the ligand-binding domain (S1) and a reentrant loop (TMII) that forms the lining of the pore region of the ion channel. Entry of the TMII segment occurs only partially into the plasma membrane before dipping back to the cytoplasm. This segment has been termed the "p loop" and determines the calcium permeability of the receptor. GluRs 5–7 (GluK1–3) have been shown to occur in numerous splice variants and to undergo both RNA editing, giving rise to a variety of receptor types with differing pharmacological and functional properties (Jaskolski et al. 2004). The ion channels formed by kainate receptors are permeable to K^+ and Na^+, but typically impermeable to Ca^{2+}. Similar to AMPARs, the ionic conductance of kainate receptors is approximately 20pS; however, their channel kinetics are significantly shorter in duration when compared to AMPARs. Association of receptors with the protein Neto1 has been shown to slow the activation and deactivation kinetics of synaptic responses (Lerma 2011). By modulating the likelihood of depolarization of the postsynaptic neuron, kainate receptors can directly affect the amount of neurotransmitter that is released into the synaptic cleft (Huettner 2003).

Similar to both NMDA and AMPA receptors, kainate receptors are widely distributed throughout the brain. Based on this distribution it has been suggested that rather than being direct mediators of synaptic transmission, the receptors may instead play a role in modulating synaptic plasticity. The spatial distribution for the GluR5–7 subunits is found in somewhat higher levels in the CA3 region of the hippocampus, the striatum, and inner layers of the cerebral cortex. In contrast, both

the KA1 and KA2 subunits are more spatially restricted, with KA1 present only in the hippocampus (Darstein et al. 2003) while KA2 subunits found in the striatum and the inner/outer layers of the cortex (Gallyas et al. 2003). Kainate receptors are the least studied amongst those in the iGluR family, which is largely due to the lack of selective agonists and antagonists for this subtype. Kainate receptors have been implicated in several disease states from clinical data, autopsy brain studies, and animal models including knockout mice (Swanson 2009; Matute 2011). However, due to the crossover activity of many antagonists with AMPARs, it has been difficult to specifically determine the contribution of kainate receptor block to specific neurodegenerative disorders. NBQX, which has shown improvement of akinesia in rat models of Parkinson's disease, is an antagonist at both AMPA and kainate receptors, but is more potent at AMPA receptors. Thus, for the purpose of this chapter, mainly the effects of NMDA and AMPA receptors will be discussed.

10 L-Glu-Mediated Neuronal Excitotoxicity

The first report of the neurotoxic effects of L-Glu was published by Hayashi in 1954 following the observation that direct application of L-Glu was sufficient to induce seizure activity. Subsequent studies by Lucas and Newhouse (1957) showed that subcutaneous injection of L-Glu into postnatal mice resulted in degeneration of neurons in the retina. In 1969 Olney coined the term "excitotoxicity" when he showed that L-Glu-mediated cell death was not limited to the retina but could also occur in the CNS. Application of L-Glu could result in the selective death of postsynaptic neurons and that glutamate agonists could act as potent neurotoxins. Based on the ability of L-Glu to activate iGluRs and that L-Glu antagonists were able to mediate the resulting neurotoxicity, only the term "excitotoxicity" was coined, a portmanteau of excitatory and neurotoxicity. Excitotoxicity is thus defined as the pathological processes by which neurons are either damaged or killed as a result of excessive stimulation by excitatory amino acids (EAAs).

Neuronal excitotoxicity occurs when iGluRs are repetitively activated by endogenous EEAs. As the primary mediator of excitatory neurotransmission in the CNS, L-Glu is considered to be the major endogenous excitotoxin in the brain. Within the brain, L-Glu is synthesized from L-glutamine and Krebs cycle intermediates. When viewed as a complete organ, the concentration of *intracellular* L-Glu is between 5 and 15 mmol/kg wet weight, which far exceeds the *extracellular* L-Glu concentration, which is normally in the range of 0.2–5 mmol/L (Erecinska and Silver 1990). Within presynaptic neurons, L-Glu is stored in vesicles whose contents are subsequently released into the synaptic cleft via a Ca^{2+}-dependent mechanism. Once in the synaptic cleft, L-Glu binds to – and activates – postsynaptic iGluRs. Under normal physiological conditions, L-Glu is present in the synaptic cleft at a concentration of 0.6 μM (Bouvier et al. 1992). During synaptic transmission, the concentration of L-Glu in the synaptic cleft is increased up to 1 mM, which is then rapidly returned to normal over a millisecond time course

(Clements et al. 1992). The excitatory action of L-Glu is terminated by its removal from the synaptic cleft. To date, two primary mechanisms for synaptic L-Glu removal have been identified. The first involves the reuptake of L-Glu into the presynaptic neuron, while the second occurs via a glial cell-based high-affinity reuptake system (Castagna et al. 1997; Kanai et al. 1994). As a result of these sodium-dependent transport systems, a 5,000–10,000-fold concentration gradient of L-Glu exists between the intracellular and extracellular neuronal environments (Ferrarese et al. 1993).

The overactivation of iGluRs by L-Glu results in excessive amounts of Ca^{2+} entering the postsynaptic neuron. This influx of calcium can result in the sequential activation of a number of degradative enzymatic, nucleolytic, and proteolytic pathways (Zundorf and Reiser 2011). As a result, cellular DNA, RNA, and proteins begin to be degraded along with dismantling of the cytoskeleton and cell membrane. With continued L-Glu stimulation and concomitant calcium ion influx, the neuron undergoes cellular degeneration. Excitotoxic theories suggest that the cellular mechanisms normally involved in excitatory neurotransmission can be transformed into mechanisms of neuronal cell destruction (Olney et al. 1986; Whetsell and Shapira 1993). EAA neurotoxicity in animal models has been found to be cellularly selective, with distinctive degenerative patterns produced by different EAAs (Coyle et al. 1981).

11 Excitotoxicity and Neurodegenerative Disorders

Excitotoxicity is thought to be a major contributing factor to a variety of degenerative disorders, including spinal cord injury and hearing loss (ototoxicity), and in neurodegenerative diseases of the central nervous system (CNS). Excessive activation of iGluRs has been implicated in a variety of neurodegenerative diseases including traumatic brain injury (Luo et al. 2011), amyotrophic lateral sclerosis (ALS) (Grosskreutz et al. 2010), multiple sclerosis (Frigo et al. 2012), epilepsy (Czuczwar 2000), hypoxia-ischemia, and chronic neurodegenerative disorders such as Alzheimer's (Woods and Padmanabhan 2012), Parkinson's (Surmeier et al. 2010), and Huntington's diseases (Raymond et al. 2011).

12 Involvement of iGluRs in Alzheimer's Disease

Alzheimer's disease (AD) is the most frequent cause of cognitive deficit in the aged population with the prevalence of probable AD is 3 % in subjects aged 65–74, 19 % in those aged 75–84, and 47 % in those over 85. AD represents the fourth most common cause of death in Western countries (Forsyth and Ritzline 1998). With the continuing increase in the world's aged population, projections indicate that by 2050 over 115 million people will suffer from AD (World Health Organization 2012). While normal cognitive losses do occur as part of the normal aging process, the cognitive impairments observed in dementia patients are both qualitatively and

quantitatively more severe (Roses 1998a, b). Following postmortem histopathological examination, ~75 % of patients that presented with a clinical diagnosis of dementia are confirmed to have AD (Evans 1989).

The impairments in memory and cognition observed in AD can be correlated to the neuropathological features of the disease, including neuronal loss and plaque and tangle formation in both the cortex and hippocampus (Hyman et al. 1986; Albin and Greenamyre 1992; Bobinski et al. 1998). While neuronal loss is generally restricted to the pyramidal cells in layers III and IV, damage to L-Glu-innervated neurons is also observed. In AD, neuronal cell death is generally restricted to neuronal cell bodies and dendrites, with axons, terminal boutons, and glial, endothelial, and ependymal cells relatively spared. There is mounting evidence that the region-selective pattern of neuronal cell destruction may occur as a consequence of EAA dysfunction, resulting in the cognitive symptomatology observed. The neurodegeneration observed in AD may therefore arise from both the regional and cellular distribution of an EAA defect, with the observed pathology resulting from the heterogeneous distribution of EAA receptor subtype(s), including NMDA, AMPA, and the kainate receptor types (Hynd et al. 2004a).

12.1 AMPA Receptors in AD

Data from both experimental and clinical trials have suggested that modulation of AMPA receptors may be therapeutically effective in the treatment of neurodegenerative diseases. Reduced levels of the AMPA receptor subunits GluR1, GluR2, and GluR2/3 have been found in the pathologically susceptible areas of the AD brain, including the CA1 region of the hippocampus (Armstrong et al. 1994; Aronica et al. 1998; Garcia-Ladona et al. 1994; Ikonomovic et al. 1997; Wakabayashi et al. 1994). In AD patients, reductions in AMPAR expression can be correlated to disease progression and disease severity (Carter et al. 2004). Within the subiculum, a reduction in GluR2 and GluR2/3 protein levels was found to correlate with Braak stages I–II and stages III–IV, respectively. While in Braak stages V–VI, both GluR2 and GluR2/3 protein levels were similar to those of Braak stages I–II. In contrast, despite reduced subiculum GluR1 protein levels in patients with severe AD, a direct correlation between Braak staging and protein loss throughout all stages of the disease has not been demonstrated (Armstrong et al. 1994; Schaeffer and Gattaz 2008; Yasuda et al. 1995). Within the pathologically spared regions, hippocampal regions, CA2 and CA3, and the dentate gyrus, AMPAR subunits show relatively little change across all Braak stages.

AD is typically characterized by the presence of amyloid plaques and intracellular neurofibrillary tangles. Amyloid is the general term given for peptides produced by the cleavage of amyloid precursor protein (APP). The amyloid beta (Aβ) peptide is the primary component of amyloid plaques, which are thought to be triggered by the self-aggregation of Aβ into oligomers. It is the near ubiquitous presence of both amyloid plaques and neurofibrillary tangles that is thought to contribute to the degeneration of neurons and subsequently to the progressive

decline in cognitive function observed in AD (Shankar and Walsh 2009). Loss of GluR2/3 immunoreactivity has been shown to precede the formation of neurofibrillary tangles in both the hippocampus and entorhinal cortex in the brains of AD sufferers (Ikonomovic et al. 1997). A reduction in the binding of AMPA to receptors has also been observed in AD cases when analyzed by quantitative autoradiography (Dewar et al. 1991). The trafficking of AMPARs has also been shown to be disrupted by soluble Aβ peptides (Shankar et al. 2007).

Ca^{2+}/calmodulin-dependent protein kinases II (CaMKII) are serine/threonine-specific protein kinases involved in signaling cascades that are important in mediating long-term potentiation (LTP). Aβ has been found to impair AMPAR receptor trafficking and function by reducing CaMKII synaptic distribution (Gu et al. 2009). By competition with the proteolytic maturation of brain-derived neurotrophic factor (BDNF), Aβ can also interfere with synaptic potentiation by reducing AMPAR trafficking (Zheng et al. 2010). Similarly, overexpression of APP in cultured hippocampal neurons has been shown to result in reductions in the recycling of presynaptic vesicles. PSD-95 is a neuronal protein that associates with receptors and cytoskeletal elements at synapses. In PSD-95 knockout mice, reduced AMPAR receptor activity is observed. The loss of PSD-95 may contribute to AMPAR-dependent LTP deficits observed in AD. Significantly decreased levels of PSD-95 have been found in the AD brain (Sultana et al. 2010). Altered AMPA receptor currents that correlate with the reduced GluR2/3 immunoreactivity precede NFT deposition and synapse loss (Ikonomovic et al. 1997).

12.2 NMDA Receptors in AD

Many neurodegenerative disorders, including AD, involve abnormalities in synaptic structure and function. NMDARs show altered expression in a variety of neurodegenerative diseases, including AD and Parkinson's disease (PD). Immunohistochemical analysis of the distribution of NMDAR subunits in AD has to date yielded conflicting results. In a study of the AD patients with mild/modest pathology, staining for the NMDAR in the hippocampus was found to be indistinguishable from that of controls. However, in cases with severe AD changes, a more complex staining pattern was observed (Ikonomovic et al. 1999). Furthermore, quantitative autoradiography has revealed substantially higher binding to NMDARs in the striatum and nucleus accumbens in AD patients (Ulas et al. 1994). A follow-up study however showed significantly lower NR1 mRNA levels in layer III of the entorhinal cortex in AD brains using in situ hybridization. Similar reductions were also observed in layers II and IV–VI of the entorhinal cortex and in granule cells of the dentate gyrus. However, no differences in mRNA expression were observed in the CA1 region of the hippocampus or the subiculum (Ulas and Cotman, 1997). Locally lower NMDAR levels have been reported in the AD hippocampus, although no reductions were noted in either the occipital cortex or caudate (Sze et al. 2001). The basis of these discrepancies may be a result of alternative splicing of the NMDA NR1 mRNA that yields distinct protein subunit variants.

In the AD brain, regional variations in the expression of NMDA NR1 splice variants have been shown to occur between areas that are pathologically susceptible and those that remain relatively spared (Hynd et al. 2004b, 2001). The N1 (exon 4) cassette of the NR1 mRNA is particularly important in the modulation of the mature receptor. Exon 4 encodes a 21-amino acid segment in the N-terminal domain (Durand et al. 1993). Insertion/deletion of the N1 cassette yields NMDAR splice variants (designated the $NR1_{1XX}$ and $NR1_{0XX}$ variants, respectively) that are subject to distinct modulation by endogenous molecules, including the polyamines spermidine, spermine, and putrescine. Polyamines are a group of organic polycations that are protonated at physiological pH. Polyamines are present in all nucleated cells and occur in the mammalian brain in micromolar concentrations (Sarhan and Seiler 1989). They modulate various cellular functions, including cellular growth and differentiation (Tabor and Tabor 1984), regulation of gene expression (Celano et al. 1989), protein synthesis and phosphorylation (Lenzen et al. 1986), and programmed cell death (Ha et al. 1997). The action of polyamines on the NMDAR complex consists of both stimulatory and inhibitory components (Reynolds and Miller 1989; Williams et al. 1989; Rock and Macdonald 1995). While polyamines do not directly activate the NMDAR, they act to potentiate or inhibit L-Glu-mediated responses in the presence of glycine. Both $NR1_{0XX}$ and $NR1_{1XX}$ variants are inhibited by high-micromolar Zn^{2+} concentrations and potentiated by polyamines at low glycine concentrations (Zhang et al. 1994). However, only $NR1_{0XX}$ variants are potentiated by polyamines at saturating glycine concentrations (Durand et al. 1993). The concentration of polyamines in the brain shows marked changes in response to pathological state (Paschen 1992). In particular, levels of brain polyamines are altered in AD (Morrison et al. 1995; Morrison and Kish 1995). In AD cases, mean levels of spermidine, the immediate biosynthetic precursor to spermine, are markedly increased compared to controls in the temporal cortex of patients. Conversely, putrescine levels are decreased in the same region in AD cases. In one study, the ratio of NR1 1XX:0XX was found to be significantly reduced in regions of the AD brain most susceptible to pathological damage (Hynd et al. 2001), while levels of the $NR1_{1XX}$ mRNA were found to be lower in cingulate gyrus, the hippocampal formation, and superior temporal gyrus in AD cases. Conversely, $NR1_{1XX}$ levels were unchanged in both the motor and occipital cortices in AD cases and controls. These results suggest that, at least in the pathologically susceptible temporal cortex of AD patients, stimulation of the NMDAR (as a consequence of elevated spermidine levels) may be increased and its antagonism (through lowered putrescine levels) would be decreased. The selective vulnerability of neurons in AD may be attributed, in part to local variations in the composition and hence function of the NMDA NR1 receptor.

The co-expression of NR1 variants with an NR2(A–D) subunit results in heteromeric channels with distinct electrophysiological and pharmacological profiles. Significantly lower levels of both the NR2A and NR2B mRNAs were found in susceptible brain regions, including the hippocampus, temporal, and cingulate cortices (Hynd et al. 2004b).

13 Involvement of iGluRs in Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world after Alzheimer's disease, affecting about 1 % of adults over 60 years of age. PD is a disease which is more common in the elderly and prevalence rises from 1 % in those over 60 years of age to 4 % of the population over 80 (de Lau and Breteler 2006). Although the mean age of onset is around 60 years, 5–10 % of cases are diagnosed as young onset PD between the ages of 20 and 50 (Samii et al. 2004).

The hallmark of PD pathology is loss of dopaminergic neurons in the substantia nigra pars compacta of the midbrain, which manifests itself with symptoms such as bradykinesia, muscle rigidity, and tremor. Bradykinesia involves delayed voluntary movements, difficulty in undertaking rapid motor tasks and multitasking, and also decreased facial movements. Rigidity affects the patient's posture because of an increase in tone throughout all movements. The tremor or shaking primarily affects the hands, arms, legs, jaw, and face. As these symptoms become more pronounced, patients may have difficulty walking, talking, or completing other simple tasks. The first symptoms appear after a large proportion of the dopaminergic neurons are lost. The severity of how PD affects different people varies greatly and there is no way to predict the symptoms. Although some people become severely disabled, others experience only minor motor dysfunctions.

Current established therapies focus primarily on alleviating the motor symptoms using dopamine replacement therapy with levodopa (L-DOPA) (Calabresi et al. 2010; Wu and Frucht 2005), which is a dopamine precursor. However, long-term complications after continuous use of levodopa involve disabling fluctuations and dyskinesias (Calabresi et al. 2010).

However, the chronic and progressive neurodegeneration in PD also affects non-dopaminergic pathways, which causes nonmotor symptoms, such as autonomic dysfunction, cognitive and psychiatric changes, sensory symptoms, and sleep disturbances (Samii et al. 2004). The striatum is the principal input structure of the basal ganglia, where excitatory glutamatergic input and dopaminergic input convey and interact. The depletion of nigrostriatal dopaminergic neurons leads to overactivity of the glutamatergic pathways within the striatum and output nuclei. The involvement of the glutamatergic system in the pathogenesis and symptomatology of PD is well established (Blandini et al. 1996; Chase and Oh 2000; Lange et al. 1997; Starr 1995), and the iGluRs are of interest as potential targets for anti-PD treatment therapies.

13.1 NMDA Receptors in PD

NMDARs are widely expressed in the CNS, including all of the structures of the basal ganglia, but with different subunit compositions. The abundance of NMDA receptors in the postsynaptic density of glutamatergic synapses suggests an important role in rapid response to neurotransmitter release and modulation of glutamate excitation in both physiological and pathological conditions, including PD.

Due to the presumed involvement of NMDARs in PD, NMDA receptor antagonists have therefore been of major interest for the treatment of PD in recent years, but results from both experimental studies and human trials have given mixed results. In one study by Calon (Calon et al. 2003), tissue obtained at autopsy showed increased binding of 3H-Ro 25–6981 (an NR1/NR2B-selective antagonist) in the lateral putamen when compared to controls, while binding of 3H-CGP39653 (an NR1/NR2A-selective antagonist) was unchanged in PD patients with levodopa-induced motor complications. Another study reported a decrease in the abundance of NR1/NR2B subunits in the striatum of a parkinsonian model of the rat. In contrast, no changes in the NR2A subunit were found (Dunah et al. 2000). An in vivo study used positron emission tomography to compare NMDA receptor activation in PD patients with and without levodopa-induced dyskinesias showed an increased activity in patients with dyskinesias compared to those without dyskinesias (Ahmed et al. 2011).

The noncompetitive low-affinity NMDA receptor antagonist amantadine in combination with levodopa or as monotherapy has showed limited antiparkinsonian effects and reduction in dyskinesia in PD patients (Crosby et al. 2003). Remacemide, another low-affinity NMDA receptor antagonist, showed promising antiparkinsonian effects in rodent and primate models of PD (Greenamyre et al. 1994), but when tested in 279 PD patients with motor symptoms and treated with levodopa, it showed only modest improvements although not significant (Shoulson et al. 2001). Therapeutic levels of MK-801, a noncompetitive NMDA receptor antagonist, suppress dyskinesia but also worsen parkinsonism such as sensorimotor deficits in the 6-hydroxydopamine (6-OHDA) rat model (Paquette et al. 2010). Memantine, an NMDA receptor antagonist, showed promise in treating PD patients with motor symptoms taking levodopa (Rabey et al. 1992), but in a larger study with PD patients with dementia, there was no effect on motor symptoms (Aarsland et al. 2009). However, there was an overall improvement in cognitive functions. More recent studies have focused on subtype-selective NMDA receptor antagonists, primarily those selective for the NR2B subunit, with expectation to achieve antiparkinsonian effects without the adverse effects seen from nonspecific antagonists. However the effects of NR2B-selective NMDA receptor antagonists as PD therapeutics have been mixed. The NR1/NR2B-specific NMDA receptor antagonist CP-101,606 showed limited effect on parkinsonian symptoms when administered as monotherapy but showed only a modest potentiation of antiparkinsonian actions of levodopa treatment in PD patients (Nutt et al. 2008) and in a marmoset model of PD (Nash et al. 2004). Another NR2B subtype-selective antagonist, CI-1041, successfully prevented levodopa induced dyskinesias when coadministered, in parkinsonian monkeys (Hadj Tahar et al. 2004). Ro 25–6981 is subtype-specific for active NR2B-containing NMDA receptors and has showed antiparkinsonian effect in both rat and marmoset models of PD, in addition to potentiating levodopa treatment and potentiating dopamine receptor agonists apomorphine, A68930, and quinpirole in rats (Loschmann et al. 2004). Single-dose monotherapy of MK-0657 did not improve motor functions in PD patients, and although the drug was well tolerated, it had adverse effects such as an increase in

blood pressure (Addy et al. 2009). NR2B subtype-selective NMDA receptor antagonists may have potential as therapeutic agents to treat PD, but maybe the mode of action needs to be further investigated so that a drug with fine-tuned properties for best antiparkinsonian effects can be developed.

13.2 AMPA Receptors in PD

AMPARs mediate the vast majority of fast excitatory neurotransmission in the brain and are therefore of interest in regards to the glutamatergic involvement in PD. Increased binding of AMPA in the lateral putamen in PD patients experiencing motor complications, suggests an involvement of AMPA receptors in levodopa-induced motor complications (Calon et al. 2003). Several AMPA receptor antagonists have been tested for their antiparkinsonian effect and for their ability to relieve levodopa-induced dyskinesia in rodents (Bibbiani et al. 2005; Megyeri et al. 2007; Stauch Slusher et al. 1995) and monkey models of PD (Bibbiani et al. 2005; Konitsiotis et al. 2000). However, the results are not totally conclusive. The AMPA antagonist GYKI-47261 given in combination with levodopa produced an improvement in dyskinesias; however, significance was not reached (Bibbiani et al. 2005). In contrast, another study showed that the AMPA antagonist LY300164, in combination with various concentrations of levodopa, increased motor activity by up to 86 % and decreased levodopa-induced dyskinesia by up to 40 % (Konitsiotis et al. 2000). GYKI-52466 and GYKI-53405, two noncompetitive AMPA receptor antagonists, exerted only limited effect on dopamine turnover in striatum tissue of rats with experimental Parkinson's when administered alone, but potentiated the effect of levodopa when given in combination (Megyeri et al. 2007). Significant improvements for akinesia were shown in parkinsonian rats after administration of the AMPAR antagonists NBQX, GYKI-52466, and YM90K (Stauch Slusher et al. 1995). Perampanel, another noncompetitive AMPAR antagonist, has been in clinical trials for treatment of PD after showing encouraging results in relieving motor symptoms in animal models. Perampanel was found to be effective in the treatment of refractory partial-onset seizures in three pivotal (Phase 3) clinical trials, although no evidence for improved cognitive functions was found (French et al. 2012).

Although excitotoxicity of the glutamatergic pathways is suggested to play role in PD, stimulatory modulation of AMPA receptors may also have therapeutic potential for the disease. Potentiators of the AMPA receptors, which stimulate synaptic transmission, synaptic plasticity, and cognition, also increase the production of BDNF (Hyman et al. 1991; Lauterborn et al. 2000). BDNF is associated with reduced neurotoxicity and improved neurological signs in cultured dopaminergic neurons (Hyman et al. 1991) and in animal models of Parkinson's disease as shown after LY503430 treatment (Murray et al. 2003). In contrast, the positive AMPA receptor modulator CX516 was reported to cause dyskinesia in a primate model of PD when administered alone or in combination with levodopa (Konitsiotis et al. 2000). The variable results of AMPA potentiators as therapeutics for PD may

therefore depend on which class of potentiators they belong to, since they have been suggested to show distinct mode of actions, potencies, and selectivities at different AMPA receptors (O'Neill and Witkin 2007). Further investigations will be required in order to fully determine the extent of AMPAR modulation in PD.

14 Traumatic Brain Injury

Traumatic brain injury (TBI), also known as intracranial injury, is a form of acquired primary brain injury that occurs when an external force causes damage to the brain. TBIs can be classified based on the mechanism (i.e., closed or penetrating head injury) and severity of the injury, in addition to the physical location of the injury (e.g., localized or global) (Bruns and Hauser 2003). In addition to the initial primary injury, TBI is often associated with secondary injuries that occur in the hours and days following the primary injury. These injuries result in a cascade of events that serve to alter cerebral blood flow and further contribute to the damage resulting from the primary traumatic insult (Chang et al. 2009). Trauma to the brain can result in vascular damage that results in reduced blood perfusion and may lead to transient ischemia. Ischemia is defined as a restriction in blood supply, with concomitant damage or dysfunction to both cells and tissue. The resulting cerebral hypoxia in which blood flow is reduced to inadequate levels leads to the death of brain tissue or cerebral infarction/ischemic stroke (Bjornsson et al. 2006). TBI typically causes impairment in both cognitive performance and fine motor functions that often persist for months after the initial injury occurs.

14.1 NMDA Receptors in TBI

TBI has been shown to cause a marked elevation in the concentrations of extracellular glutamate and aspartate proximal to the trauma site. This results in the excessive activation of NMDARs and neuronal cell death (Bullock 1995; Hovda et al. 1995). Microdialysis studies in humans reveal that there is sustained increases in the concentration of L-Glu lasting between 6 h and several days following TBI (Faden et al. 1989; Choi et al. 1990). Along with this increase, the density of activated NMDARs increases in the hippocampus but decreases in the cortex at the impact site in mice. Interestingly, a study by Kumar (Kumar et al. 2002) using RT-PCR analysis did not show any significant change in the mRNA levels of NR1, NR2A, and NR2B following injury in comparison with controls, suggesting nontranscriptional changes (i.e., protein) occur in the levels of these subunits. Interestingly, protein levels of NR1, NR2A, and NR2B were found to transiently decrease from the time of TBI up until 12 h post-injury before returning to pre-injury levels at 24 h. In contrast, a study by Schumann showed that the expression of the NR1, NR2A, and NR2B subunits were increased in the hippocampus, but decreased in the cortex at the site of the TBI (Schumann et al. 2008). This study revealed that the increase in NMDAR subunit expression in the

hippocampus was associated with an increase in NR2B tyrosine phosphorylation. Furthermore, that inhibition of NR2B phosphorylation PP2, a tyrosine kinase inhibitor, was able to restore NMDAR subunit expression to pre-TBI levels. Importantly, inhibition of NR2B phosphorylation was also found to result in significant improvements in long-term recovery of motor functions following TBI.

Administration of NMDA receptor antagonists has been shown to reduce injury severity after TBI (Faden et al. 1989). Pretreatment with either the noncompetitive NMDA antagonist dextrorphan or the competitive NMDA antagonist CGS 19755 was found to limit the release of extracellular L-glutamate following TBI (Panter and Faden 1992). In mice subject to mild TBI, it was observed that cellular viability was reduced in the hippocampus of the mice subjected to trauma while animals preconditioned with 75 mg/kg NMDA no reductions in cellular viability were observed. Similarly, in rats subjected to percussion trauma of the cortex, pretreatment with the NMDAR antagonist 3-((+/−)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP) was successful in mitigating cortical damage (Bernert and Turski 1996). Based on their role in activity-dependent forms of synaptic plasticity (Constantine-Paton 1990; Constantine-Paton et al. 1990) and long-term potentiation (Bliss and Collingridge 1993), NMDARs are believed to play a role in memory and learning following TBI. Mice preconditioned with NMDA and subject to object recognition tasks following TBI were found to be protected against impairments in both long- and short-term memory. In contrast, in mice treated with NMDA, long-term impairments in memory were found when assessed in the step-down inhibitory avoidance test at 7 days following the initial training session (Moojen et al. 2012). Long-term behavioral deficits are often observed in patients following TBI. A study by Hayes (Hayes et al. 1988) showed that pretreatment with phencyclidine, an NMDA antagonist, was able to attenuate the long-term behavioral deficits in the rat produced by TBI. Preconditioning of mice with low-dose concentrations of NMDA has also been used to protect against locomotor deficits observed after TBI. Mice preconditioned with NMDA (75 mg/kg) 24 h prior to TBI induction were found to be protected against all motor deficits revealed by footprint tests. However, this protective effect was not seen when mice were tested in rotarod tasks (Costa et al. 2010). These data suggest that excitotoxicity is mediated by NMDARs with the degree of activation contributing to the pathophysiology of brain injury and ultimately the severity of locomotor dysfunction.

Cerebral hypoperfusion following TBI often occurs as a result of loss of responsiveness to cerebrovascular dilator stimuli via NMDAR-mediated impairment of cerebral hemodynamics. Tissue plasminogen activator is a known activator of NMDARs and is upregulated following TBI. Treatment of mice with a catalytically inactive variant of tissue plasminogen activator was able to prevent impairment of cerebral hemodynamics and decrease excitotoxic neuronal death post-TBI (Armstead et al. 2012). Similarly, the polyamine-site NMDAR antagonist ifenprodil was able to protect against both brain edema and breakdown of the blood–brain barrier after TBI (Dempsey et al. 2000). MK-801, a noncompetitive blocker of the NMDAR, has also been used to treat the neuropathological insults following fluid-percussion brain injury in the rat. In a study by McIntosh, MK-801

treatment significantly attenuated the development of focal brain edema at the site of injury 48 h after TBI (McIntosh et al. 1990). At the molecular level, influx of Ca^{2+} may not be the only factor contributing to neuronal cell death following elevated concentrations of L-Glu. Recent studies have shown that activation of extrasynaptic NMDARs triggered by L-Glu or hypoxic/ischemic following TBI activates a CREB (cAMP response element-binding protein) pathway. This pathway results in the loss of mitochondrial membrane potential and neuronal apoptosis. These results suggest that pharmacological inhibition of EAA neurotransmission including the use of NMDA antagonists may be used to improve neurological outcome after TBI.

14.2 AMPA Receptors in TBI

AMPARs are critical for synaptic plasticity, and a number of recent studies have focused on the role of AMPARs in the physiological and pathophysiological processes following TBI. In vivo, AMPARs are subject to alterations based on subunit composition and receptor trafficking to and from the plasma membrane. A number of recent reports have shown that both the composition and function of synaptic AMPARs change after mechanical injury (Arundine and Tymianski 2003). As a result of these changes, the normally Ca^{2+}-impermeable AMPARs can become Ca^{2+} permeable, thereby affecting calcium signaling and the pathophysiology following TBI. In 2010, Beattie (Beattie et al. 2010) showed that the pro-inflammatory cytokine tumor necrosis factor (TNF)α, a potent regulator of AMPAR trafficking, can rapidly increase the surface expression of Ca^{2+}-permeable AMPARs following TBI. This increase in combination with increased levels of extracellular L-Glu after TBI may play an important role in enhancing excitotoxic cell death after CNS injury. Using an in vitro model of TBI, Spaethling (Spaethling et al. 2008) showed that Ca^{2+}-permeable AMPARs appear 4 h following injury to cortical neurons. Interestingly, mechanical injury has been shown to modulate AMPAR kinetics via an NMDA receptor-dependent pathway (Goforth et al. 2004). Activation of the NMDAR can lead to CaMKII activation to produce a sustained reduction of AMPA receptor desensitization. Expression of the GluR1 subunit of the AMPAR has also been shown to be significantly decreased in the rat hippocampus following TBI (Kharlamov et al. 2011).

15 Involvement of iGluRs in Epilepsy

Epilepsy is a common name for a variety of chronic neurological disorders characterized by seizures resulting from excessive neuronal activity in the brain. The highest incidence occurs in young children and the elderly. These seizures can involve specific systems of the brain (partial seizures) or develop in a restricted area of the brain but then spread to involve multiple cortical and subcortical circuits. In the early 1940s, it was suggested that epileptic seizures occur when the "quantity of the

glutamic acid in human brain surpasses the critical level" after observing that injection of glutamate into the grey matter of the motor cortex of dogs and humans caused epileptic seizures. As the major excitotoxic neurotransmitter in the brain, L-Glu plays a major role in the initiation and spread of seizure activity by acting on iGluRs.

16 AMPA Receptors in Epilepsy

Due to their role in excitatory neurotransmission in the brain, AMPARs have recently become the subject of interest in the pathogenesis of epilepsy. AMPAR antagonists have proven therapeutic potential as antiepileptic drugs in clinical trials. Both talampanel and perampanel, two noncompetitive AMPA receptor antagonists, have proven to reduce seizure frequency in patients with refractory partial seizures (Chappell et al. 2002; Stephen and Brodie 2011). Adverse effects such as dizziness and ataxia were the most common.

17 NMDA Receptors in Epilepsy

At present, the NMDAR is probably the iGluR which has been studied the most as a therapeutic target for treatment of epilepsy. However, despite a large number of studies demonstrating up- or downregulation of particular NMDA receptor subunits' mRNA expression in various animal models of epilepsy, and several NMDA receptor antagonists showing anticonvulsant effect in in vivo animal studies, there is debated evidence for NMDA receptor antagonists as useful treatment for epilepsy in humans (Ghasemi and Schachter 2011). Felbamate, a promiscuous NMDA receptor inhibitor, was approved by the FDA in 1993 but is only used for patients with intractable partial seizures, infantile spasms, or Lennox-Gastaut syndrome, because the potential benefit outweighs the side effects. Remacemide, a low-affinity NMDA receptor blocker, showed improvement as adjunctive therapy in several clinical trials in patients with drug-resistant seizures (Ghasemi and Schachter 2011). Ketamine, an open-channel blocker, has shown both positive and adverse effects in clinical trials. Amantadine, an NMDA receptor blocker, has shown improvements in patients with refractory absence seizures as both mono- and add-on treatment. The results from memantine, an open-channel NMDA receptor blocker, in patients with seizures have been inconclusive despite promising animal studies. In summary, lower doses of NMDA receptor antagonists, such as memantine and remacemide, have shown antiepileptic effects with less severe side effects as adjunct therapy compared with monotherapy (Ghasemi and Schachter 2011).

18 Kainate Receptors in Epilepsy

Kainate receptors may play a more modulatory role in fine-tuning excitatory and inhibitory neurotransmission to prevent over excitation, which may lead to epilepsy.

Systemic or intracerebral injection of kainate, an analog of L-Glu, is well known to cause epileptic seizures, originating from the CA3 region of the hippocampus (Vincent and Mulle 2009). Thus, modulating the kainate receptors may have clinical implications in the treatment of epilepsy. Using knockout mice, two independent studies have shown the involvement of the kainate receptor subunit GluK2 in kainate-induced seizures, which are a model for temporal lobe epilepsy (Fisahn et al. 2004; Mulle et al. 1998). Therefore, interfering with GluK2 activity may have potential as antiepileptic drugs. In addition, GluK1 has shown increased expression in the hippocampus of patients suffering from temporal lobe epilepsy (Li et al. 2010), which supports that GluK1 antagonists LY37770 and LY382884 can block seizures in vivo and epileptiform activity in hippocampal slices induced by pilocarpine or electrical stimulation (Barton et al. 2003; Smolders et al. 2002). Topiramate, a GluK1 antagonist, was approved by the Food and Drug Administration (FDA) in 1996 for the treatment of epilepsy both as a mono- and adjunctive therapy.

19 Conclusion

Since its discovery in the 1950s, our understanding of the role that L-Glu plays in both normal synaptic transmission and neurodegenerative disorders has increased dramatically. Numerous experiments have revealed how the molecular mechanism of the human brain can be transformed into implements of neuronal cell destruction. With the advent of gene cloning and transgenic animal models, our understanding of iGluRs and the role they play in neurodegenerative disorders is increasing at an exponential rate. Despite the relative lack of new therapeutics for the treatment of neurodegenerative disorders, the next decade will undoubtedly be one of significant advances for the diagnosis of neurodegenerative diseases and in the development of new and improved treatment regimens for sufferers.

References

Aarsland, D., et al. (2009). Memantine in patients with Parkinson's disease dementia or dementia with Lewy bodies: A double-blind, placebo-controlled, multicentre trial. *Lancet Neurology, 8*(7), 613–618.

Addy, C., et al. (2009). Single-dose administration of MK-0657, an NR2B-selective NMDA antagonist, does not result in clinically meaningful improvement in motor function in patients with moderate Parkinson's disease. *Journal of Clinical Pharmacology, 49*(7), 856–864.

Ahmed, I., et al. (2011). Glutamate NMDA receptor dysregulation in Parkinson's disease with dyskinesias. *Brain, 134*(Pt 4), 979–986.

Akazawa, C., et al. (1994). Differential expression of five *N*-methyl-D-aspartate receptor subunit mRNAs in the cerebellum of developing and adult rats. *The Journal of Comparative Neurology, 347*(1), 150–160.

Albin, R. L., & Greenamyre, J. T. (1992). Alternative excitotoxic hypotheses. *Neurology, 42*(4), 733–738.

Araneda, R. C., Zukin, R. S., & Bennett, M. V. (1993). Effects of polyamines on NMDA-induced currents in rat hippocampal neurons: A whole-cell and single-channel study. *Neuroscience Letters, 152*(1–2), 107–112.

Armstead, W. M., et al. (2012). tPA-S481A prevents neurotoxicity of endogenous tPA in traumatic brain injury. *Journal of Neurotrauma, 29*(9), 1794–1802.

Armstrong, D. M., et al. (1994). AMPA-selective glutamate receptor subtype immunoreactivity in the entorhinal cortex of non-demented elderly and patients with Alzheimer's disease. *Brain Research, 639*(2), 207–216.

Aronica, E., et al. (1998). Non-plaque dystrophic dendrites in Alzheimer hippocampus: A new pathological structure revealed by glutamate receptor immunocytochemistry. *Neuroscience, 82*(4), 979–991.

Arundine, M., & Tymianski, M. (2003). Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. *Cell Calcium, 34*(4–5), 325–337.

ASD International. (2012). World Alzheimer Report – Overcoming the stigma of dementia, World Alzheimer Foundation, http://www.alz.co.uk/research/world-report-2012

Barton, M. E., Peters, S. C., & Shannon, H. E. (2003). Comparison of the effect of glutamate receptor modulators in the 6 Hz and maximal electroshock seizure models. *Epilepsy Research, 56*(1), 17–26.

Beattie, M. S., Ferguson, A. R., & Bresnahan, J. C. (2010). AMPA-receptor trafficking and injury-induced cell death. *The European Journal of Neuroscience, 32*(2), 290–297.

Bennett, J. A., & Dingledine, R. (1995). Topology profile for a glutamate receptor: Three transmembrane domains and a channel-lining reentrant membrane loop. *Neuron, 14*(2), 373–384.

Bernert, H., & Turski, L. (1996). Traumatic brain damage prevented by the non-*N*-methyl-D-aspartate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f] quinoxaline. *Proceedings of the National Academy of Sciences of the United States of America, 93*(11), 5235–5240.

Bettler, B., et al. (1990). Cloning of a novel glutamate receptor subunit, GluR5: Expression in the nervous system during development. *Neuron, 5*(5), 583–595.

Bettler, B., et al. (1992). Cloning of a putative glutamate receptor: A low affinity kainate-binding subunit. *Neuron, 8*(2), 257–265.

Bibbiani, F., et al. (2005). Combined blockade of AMPA and NMDA glutamate receptors reduces levodopa-induced motor complications in animal models of PD. *Experimental Neurology, 196*(2), 422–429.

Bjornsson, C. S., et al. (2006). Effects of insertion conditions on tissue strain and vascular damage during neuroprosthetic device insertion. *Journal of Neural Engineering, 3*(3), 196–207.

Blandini, F., Porter, R. H., & Greenamyre, J. T. (1996). Glutamate and Parkinson's disease. *Molecular Neurobiology, 12*(1), 73–94.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature, 361*(6407), 31–39.

Bobinski, M., et al. (1998). Neuronal and volume loss in CA1 of the hippocampal formation uniquely predicts duration and severity of Alzheimer disease. *Brain Research, 805*(1–2), 267–269.

Bockers, T. M., et al. (1994). Expression of the NMDA R1 receptor in selected human brain regions. *Neuroreport, 5*(8), 965–969.

Boehm, J., et al. (2006). Synaptic incorporation of AMPA receptors during LTP is controlled by a PKC phosphorylation site on GluR1. *Neuron, 51*(2), 213–225.

Boulter, J., et al. (1990). Molecular cloning and functional expression of glutamate receptor subunit genes. *Science, 249*(4972), 1033–1037.

Bouvier, M., et al. (1992). The glial cell glutamate uptake carrier counter transports pH-changing anions. *Nature, 360*(6403), 471–474.

Bruns, J., Jr., & Hauser, W. A. (2003). The epidemiology of traumatic brain injury: A review. *Epilepsia, 44*(Suppl 10), 2–10.

Bullock, R. (1995). Strategies for neuroprotection with glutamate antagonists. Extrapolating from evidence taken from the first stroke and head injury studies. *Annals of the New York Academy of Sciences, 765*, 272–278 discussion 298.

Calabresi, P., et al. (2010). Levodopa-induced dyskinesias in patients with Parkinson's disease: Filling the bench-to-bedside gap. *Lancet Neurology, 9*(11), 1106–1117.

Calon, F., et al. (2003). Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. *Neurobiology of Disease, 14*(3), 404–416.

Carter, T. L., et al. (2004). Differential preservation of AMPA receptor subunits in the hippocampi of Alzheimer's disease patients according to Braak stage. *Experimental Neurology, 187*(2), 299–309.

Castagna, M., et al. (1997). Molecular characteristics of mammalian and insect amino acid transporters: Implications for amino acid homeostasis. *The Journal of Experimental Biology, 200*(Pt 2), 269–286.

Castellani, G. C., et al. (2005). A model of bidirectional synaptic plasticity: From signaling network to channel conductance. *Learning & Memory, 12*(4), 423–432.

Celano, P., Baylin, S. B., & Casero, R. A., Jr. (1989). Polyamines differentially modulate the transcription of growth-associated genes in human colon carcinoma cells. *The Journal of Biological Chemistry, 264*(15), 8922–8927.

Chang, J. J., et al. (2009). Physiologic and functional outcome correlates of brain tissue hypoxia in traumatic brain injury. *Critical Care Medicine, 37*(1), 283–290.

Chappell, A. S., et al. (2002). A crossover, add-on trial of talampanel in patients with refractory partial seizures. *Neurology, 58*(11), 1680–1682.

Chase, T. N., & Oh, J. D. (2000). Striatal dopamine- and glutamate-mediated dysregulation in experimental parkinsonism. *Trends in Neurosciences, 23*(Suppl 10), S86–S91.

Choi, D. W., et al. (1990). Acute brain injury, NMDA receptors, and hydrogen ions: Observations in cortical cell cultures. *Advances in Experimental Medicine and Biology, 268*, 501–504.

Clements, J. D., et al. (1992). The time course of glutamate in the synaptic cleft. *Science, 258*(5087), 1498–1501.

Collingridge, G. L., et al. (2009). A nomenclature for ligand-gated ion channels. *Neuropharmacology, 56*(1), 2–5.

Constantine-Paton, M. (1990). NMDA receptor as a mediator of activity-dependent synaptogenesis in the developing brain. *Cold Spring Harbor Symposia on Quantitative Biology, 55*, 431–443.

Constantine-Paton, M., Cline, H. T., & Debski, E. (1990). Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathways. *Annual Review of Neuroscience, 13*, 129–154.

Contractor, A., Mulle, C., & Swanson, G. T. (2011). Kainate receptors coming of age: Milestones of two decades of research. *Trends in Neurosciences, 34*(3), 154–163.

Costa, T., et al. (2010). *N*-methyl-D-aspartate preconditioning improves short-term motor deficits outcome after mild traumatic brain injury in mice. *Journal of Neuroscience Research, 88*(6), 1329–1337.

Coyle, J. T., et al. (1981). Excitatory amino acid neurotoxins: Selectivity, specificity, and mechanisms of action. Based on an NRP one-day conference held June 30, 1980. *Neurosciences Research Program Bulletin, 19*(4), 1–427.

Crosby, N. J., Deane, K., & Clarke, C. E. (2003). Amantadine in Parkinson's disease. *Cochrane Database of Systematic Reviews* (1). Art. No.: CD003468.

Crosby, N. J., Deane, K., & Clarke, C. E. (2003). Amantadine for dyskinesia in Parkinson's disease. *Cochrane Database of Systematic Reviews* (2). Art. No.: CD003467.

Curtis, D. R., & Eccles, J. C. (1959). The time courses of excitatory and inhibitory synaptic actions. *The Journal of Physiology, 145*(3), 529–546.

Curtis, D. R., Phillis, J. W., & Watkins, J. C. (1959). Chemical excitation of spinal neurones. *Nature, 183*(4661), 611–612.

Czuczwar, S. J. (2000). Perspectives for the use of excitatory amino acid ionotropic receptor antagonists as antiepileptic drugs. *Polish Journal of Pharmacology, 52*(1), 67–70.

Darstein, M., et al. (2003). Distribution of kainate receptor subunits at hippocampal mossy fiber synapses. *The Journal of Neuroscience, 23*(22), 8013–8019.

Das, S., et al. (1998). Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature, 393*(6683), 377–381.

de Lau, L. M., & Breteler, M. M. (2006). Epidemiology of Parkinson's disease. *Lancet Neurology, 5*(6), 525–535.

Dempsey, R. J., Baskaya, M. K., & Dogan, A. (2000). Attenuation of brain edema, blood–brain barrier breakdown, and injury volume by ifenprodil, a polyamine-site *N*-methyl-D-aspartate receptor antagonist, after experimental traumatic brain injury in rats. *Neurosurgery, 47*(2), 399–404 discussion, 404–406.

Derkach, V. A., et al. (2007). Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nature Reviews. Neuroscience, 8*(2), 101–113.

Dewar, D., et al. (1991). Glutamate metabotropic and AMPA binding sites are reduced in Alzheimer's disease: An autoradiographic study of the hippocampus. *Brain Research, 553*(1), 58–64.

Dingledine, R., et al. (1999). The glutamate receptor ion channels. *Pharmacological Reviews, 51*(1), 7–61.

Dunah, A. W., et al. (2000). Alterations in subunit expression, composition, and phosphorylation of striatal *N*-methyl-D-aspartate glutamate receptors in a rat 6-hydroxydopamine model of Parkinson's disease. *Molecular Pharmacology, 57*(2), 342–352.

Durand, G. M., Bennett, M. V., & Zukin, R. S. (1993). Splice variants of the *N*-methyl-D-aspartate receptor NR1 identify domains involved in regulation by polyamines and protein kinase C. *Proceedings of the National Academy of Sciences of the United States of America, 90*(14), 6731–6735.

Egebjerg, J., et al. (1991). Cloning of a cDNA for a glutamate receptor subunit activated by kainate but not AMPA. *Nature, 351*(6329), 745–748.

Erecinska, M., & Silver, I. A. (1990). Metabolism and role of glutamate in mammalian brain. *Progress in Neurobiology, 35*(4), 245–296.

Evans, L. K. (1989). Nursing the hospitalized dementia patient. *Journal of Advanced Medical-Surgical Nursing, 1*(2), 18–31.

Faden, A. I., et al. (1989). The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science, 244*(4906), 798–800.

Ferrarese, C., et al. (1993). Assessment of reliability and biological significance of glutamate levels in cerebrospinal fluid. *Annals of Neurology, 33*(3), 316–319.

Fisahn, A., et al. (2004). Distinct roles for the kainate receptor subunits GluR5 and GluR6 in kainate-induced hippocampal gamma oscillations. *The Journal of Neuroscience, 24*(43), 9658–9668.

Fisher, J. L., & Mott, D. D. (2011). Distinct functional roles of subunits within the heteromeric kainate receptor. *The Journal of Neuroscience, 31*(47), 17113–17122.

Forsyth, E., & Ritzline, P. D. (1998). An overview of the etiology, diagnosis, and treatment of Alzheimer disease. *Physical Therapy, 78*(12), 1325–1331.

Frandsen, A., & Schousboe, A. (2003). AMPA receptor-mediated neurotoxicity: Role of Ca^{2+} and desensitization. *Neurochemical Research, 28*(10), 1495–1499.

French, J. A., et al. (2012). Adjunctive perampanel for refractory partial-onset seizures: Randomized phase III study 304. *Neurology, 79*(6), 589–596.

Frigo, M., et al. (2012). Glutamate and multiple sclerosis. *Current Medicinal Chemistry, 19*(9), 1295–1299.

Gallo, V., et al. (1992). Molecular cloning and development analysis of a new glutamate receptor subunit isoform in cerebellum. *The Journal of Neuroscience, 12*(3), 1010–1023.

Gallyas, F., Jr., Ball, S. M., & Molnar, E. (2003). Assembly and cell surface expression of KA-2 subunit-containing kainate receptors. *Journal of Neurochemistry, 86*(6), 1414–1427.

Garcia-Ladona, F. J., et al. (1994). Excitatory amino acid AMPA receptor mRNA localization in several regions of normal and neurological disease affected human brain. An in situ hybridization histochemistry study. *Brain Research. Molecular Brain Research, 21*(1–2), 75–84.

Ghasemi, M., & Schachter, S. C. (2011). The NMDA receptor complex as a therapeutic target in epilepsy: A review. *Epilepsy & Behavior, 22*(4), 617–640.

Goforth, P. B., Ellis, E. F., & Satin, L. S. (2004). Mechanical injury modulates AMPA receptor kinetics via an NMDA receptor-dependent pathway. *Journal of Neurotrauma, 21*(6), 719–732.

Greenamyre, J. T., et al. (1994). Antiparkinsonian effects of remacemide hydrochloride, a glutamate antagonist, in rodent and primate models of Parkinson's disease. *Annals of Neurology, 35*(6), 655–661.

Grosskreutz, J., Van Den Bosch, L., & Keller, B. U. (2010). Calcium dysregulation in amyotrophic lateral sclerosis. *Cell Calcium, 47*(2), 165–174.

Gu, Z., Liu, W., & Yan, Z. (2009). {beta}-Amyloid impairs AMPA receptor trafficking and function by reducing Ca^{2+}/calmodulin-dependent protein kinase II synaptic distribution. *The Journal of Biological Chemistry, 284*(16), 10639–10649.

Gunderson, C. B., Miledi, R., & Parker. I. (1984). mRNA from human brain induces drug- and voltage-operated channels in Xenopus oocytes. *Nature, 308*, 421–424.

Ha, H. C., et al. (1997). The role of polyamine catabolism in polyamine analogue-induced programmed cell death. *Proceedings of the National Academy of Sciences of the United States of America, 94*(21), 11557–11562.

Hadj Tahar, A., et al. (2004). Effect of a selective glutamate antagonist on L-dopa-induced dyskinesias in drug-naive parkinsonian monkeys. *Neurobiology of Disease, 15*(2), 171–176.

Hayes, R. L., et al. (1988). Pretreatment with phencyclidine, an *N*-methyl-D-aspartate antagonist, attenuates long-term behavioral deficits in the rat produced by traumatic brain injury. *Journal of Neurotrauma, 5*(4), 259–274.

Herb, A., et al. (1992). The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. *Neuron, 8*(4), 775–785.

Hollmann, M., & Heinemann, S. (1994). Cloned glutamate receptors. *Annual Review of Neuroscience, 17*, 31–108.

Hollmann, M., et al. (1989). Cloning by functional expression of a member of the glutamate receptor family. *Nature, 342*(6250), 643–648.

Hollmann, M., et al. (1993). Zinc potentiates agonist-induced currents at certain splice variants of the NMDA receptor. *Neuron, 10*(5), 943–954.

Hovda, D. A., et al. (1995). The neurochemical and metabolic cascade following brain injury: Moving from animal models to man. *Journal of Neurotrauma, 12*(5), 903–906.

Huettner, J. E. (2003). Kainate receptors and synaptic transmission. *Progress in Neurobiology, 70*(5), 387–407.

Hyman, B. T., et al. (1986). Perforant pathway changes and the memory impairment of Alzheimer's disease. *Annals of Neurology, 20*(4), 472–481.

Hyman, C., et al. (1991). BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature, 350*(6315), 230–232.

Hynd, M. R., Scott, H. L., & Dodd, P. R. (2001). Glutamate(NMDA) receptor NR1 subunit mRNA expression in Alzheimer's disease. *Journal of Neurochemistry, 78*(1), 175–182.

Hynd, M. R., Scott, H. L., & Dodd, P. R. (2004a). Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochemistry International, 45*(5), 583–595.

Hynd, M. R., Scott, H. L., & Dodd, P. R. (2004b). Selective loss of NMDA receptor NR1 subunit isoforms in Alzheimer's disease. *Journal of Neurochemistry, 89*(1), 240–247.

Ikonomovic, M. D., et al. (1997). The loss of GluR2(3) immunoreactivity precedes neurofibrillary tangle formation in the entorhinal cortex and hippocampus of Alzheimer brains. *Journal of Neuropathology and Experimental Neurology, 56*(9), 1018–1027.

Ikonomovic, M. D., et al. (1999). Distribution of glutamate receptor subunit NMDAR1 in the hippocampus of normal elderly and patients with Alzheimer's disease. *Experimental Neurology, 160*(1), 194–204.

Ishii, T., et al. (1993). Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. *The Journal of Biological Chemistry, 268*(4), 2836–2843.

Jaskolski, F., et al. (2004). Subunit composition and alternative splicing regulate membrane delivery of kainate receptors. *The Journal of Neuroscience, 24*(10), 2506–2515.

Jean Taxil, TRAICTE DE L'EPILEPSIE, http://asklepios.chez.com/taxil/Livre1_Chap15.htm
Kamboj, R. K., et al. (1992). Molecular structure and pharmacological characterization of humEAA2, a novel human kainate receptor subunit. *Molecular Pharmacology, 42*(1), 10–15.
Kanai, Y., et al. (1994). The neuronal and epithelial human high affinity glutamate transporter. Insights into structure and mechanism of transport. *The Journal of Biological Chemistry, 269*(32), 20599–20606.
Karp, S. J., et al. (1993). Molecular cloning and chromosomal localization of the key subunit of the human *N*-methyl-D-aspartate receptor. *The Journal of Biological Chemistry, 268*(5), 3728–3733.
Keinanen, K., et al. (1990). A family of AMPA-selective glutamate receptors. *Science, 249*(4968), 556–560.
Kharlamov, E. A., et al. (2011). Alterations of GABA(A) and glutamate receptor subunits and heat shock protein in rat hippocampus following traumatic brain injury and in posttraumatic epilepsy. *Epilepsy Research, 95*(1–2), 20–34.
Konitsiotis, S., et al. (2000). AMPA receptor blockade improves levodopa-induced dyskinesia in MPTP monkeys. *Neurology, 54*(8), 1589–1595.
Krnjevic, K., & Phillis, J. W. (1963). Iontophoretic studies of neurones in the mammalian cerebral cortex. *The Journal of Physiology, 165*, 274–304.
Kumar, A., et al. (2002). *N*-methyl-D-aspartate receptors: Transient loss of NR1/NR2A/NR2B subunits after traumatic brain injury in a rodent model. *Journal of Neuroscience Research, 67*(6), 781–786.
Lange, K. W., Kornhuber, J., & Riederer, P. (1997). Dopamine/glutamate interactions in Parkinson's disease. *Neuroscience and Biobehavioral Reviews, 21*(4), 393–400.
Lauterborn, J. C., et al. (2000). Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons. *The Journal of Neuroscience, 20*(1), 8–21.
Lee, H. K., & Kirkwood, A. (2011). AMPA receptor regulation during synaptic plasticity in hippocampus and neocortex. *Seminars in Cell & Developmental Biology, 22*(5), 514–520.
Lee, H. K., et al. (2000). Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature, 405*(6789), 955–959.
Lee, H. K., et al. (2007). Identification and characterization of a novel phosphorylation site on the GluR1 subunit of AMPA receptors. *Molecular and Cellular Neurosciences, 36*(1), 86–94.
Lenzen, S., Hickethier, R., & Panten, U. (1986). Interactions between spermine and Mg^{2+} on mitochondrial Ca^{2+} transport. *The Journal of Biological Chemistry, 261*(35), 16478–16483.
Lerma, J. (2011). Net(o) excitement for kainate receptors. *Nature Neuroscience, 14*(7), 808–810.
Li, J. M., et al. (2010). Aberrant glutamate receptor 5 expression in temporal lobe epilepsy lesions. *Brain Research, 1311*, 166–174.
Lomeli, H., et al. (1992). High-affinity kainate and domoate receptors in rat brain. *FEBS Letters, 307*(2), 139–143.
Loschmann, P. A., et al. (2004). Antiparkinsonian activity of Ro 25–6981, a NR2B subunit specific NMDA receptor antagonist, in animal models of Parkinson's disease. *Experimental Neurology, 187*(1), 86–93.
Lucas, D. R., & Newhouse, J. P. (1957). *AMA Arch Ophthalmol, 58*(2), 193–201.
Luo, P., et al. (2011). The role of glutamate receptors in traumatic brain injury: Implications for postsynaptic density in pathophysiology. *Brain Research Bulletin, 85*(6), 313–320.
Matute, C. (2011). Therapeutic potential of kainate receptors. *CNS Neuroscience & Therapeutics, 17*(6), 661–669.
McIntosh, T. K., et al. (1990). Effect of noncompetitive blockade of *N*-methyl-D-aspartate receptors on the neurochemical sequelae of experimental brain injury. *Journal of Neurochemistry, 55*(4), 1170–1179.
Megyeri, K., et al. (2007). Effects of 2,3-benzodiazepine AMPA receptor antagonists on dopamine turnover in the striatum of rats with experimental parkinsonism. *Brain Research Bulletin, 71*(5), 501–507.

Monyer, H., et al. (1992). Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science, 256*(5060), 1217–1221.

Monyer, H., et al. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron, 12*(3), 529–540.

Moojen, V. K., et al. (2012). NMDA preconditioning prevents object recognition memory impairment and increases brain viability in mice exposed to traumatic brain injury. *Brain Research, 1466*, 82–90.

Moriyoshi, K., et al. (1991). Molecular cloning and characterization of the rat NMDA receptor. *Nature, 354*(6348), 31–37.

Morrison, L. D., & Kish, S. J. (1995). Brain polyamine levels are altered in Alzheimer's disease. *Neuroscience Letters, 197*(1), 5–8.

Morrison, L. D., et al. (1995). Polyamines in human brain: Regional distribution and influence of aging. *Journal of Neurochemistry, 65*(2), 636–642.

Mulle, C., et al. (1998). Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. *Nature, 392*(6676), 601–605.

Murray, T. K., et al. (2003). LY503430, a novel alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor potentiator with functional, neuroprotective and neurotrophic effects in rodent models of Parkinson's disease. *The Journal of Pharmacology and Experimental Therapeutics, 306*(2), 752–762.

Nakanishi, N., Shneider, N. A., & Axel, R. (1990). A family of glutamate receptor genes: Evidence for the formation of heteromultimeric receptors with distinct channel properties. *Neuron, 5*(5), 569–581.

Nash, J. E., et al. (2004). The NR2B-selective NMDA receptor antagonist CP-101,606 exacerbates L-DOPA-induced dyskinesia and provides mild potentiation of anti-parkinsonian effects of L-DOPA in the MPTP-lesioned marmoset model of Parkinson's disease. *Experimental Neurology, 188*(2), 471–479.

Nutt, J. G., et al. (2008). Effects of a NR2B selective NMDA glutamate antagonist, CP-101,606, on dyskinesia and Parkinsonism. *Movement Disorders, 23*(13), 1860–1866.

O'Neill, M. J., & Witkin, J. M. (2007). AMPA receptor potentiators: Application for depression and Parkinson's disease. *Current Drug Targets, 8*(5), 603–620.

Olney, J. W., et al. (1986). The role of specific ions in glutamate neurotoxicity. *Neuroscience Letters, 65*(1), 65–71.

Ozawa, S., Kamiya, H., & Tsuzuki, K. (1998). Glutamate receptors in the mammalian central nervous system. *Progress in Neurobiology, 54*(5), 581–618.

Panter, S. S., & Faden, A. I. (1992). Pretreatment with NMDA antagonists limits release of excitatory amino acids following traumatic brain injury. *Neuroscience Letters, 136*(2), 165–168.

Paquette, M. A., et al. (2010). MK-801 inhibits L-DOPA-induced abnormal involuntary movements only at doses that worsen parkinsonism. *Neuropharmacology, 58*(7), 1002–1008.

Paschen, W. (1992). Polyamine metabolism in different pathological states of the brain. *Molecular and Chemical Neuropathology, 16*(3), 241–271.

Petralia, R. S., Wang, Y. X., & Wenthold, R. J. (1994a). Histological and ultrastructural localization of the kainate receptor subunits, KA2 and GluR6/7, in the rat nervous system using selective antipeptide antibodies. *The Journal of Comparative Neurology, 349*(1), 85–110.

Petralia, R. S., Wang, Y. X., & Wenthold, R. J. (1994b). The NMDA receptor subunits NR2A and NR2B show histological and ultrastructural localization patterns similar to those of NR1. *The Journal of Neuroscience, 14*(10), 6102–6120.

Petralia, R. S., Yokotani, N., & Wenthold, R. J. (1994c). Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *The Journal of Neuroscience, 14*(2), 667–696.

Rabey, J. M., Nissipeanu, P., & Korczyn, A. D. (1992). Efficacy of memantine, an NMDA receptor antagonist, in the treatment of Parkinson's disease. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 4*, 277–282.

Raymond, L. A., et al. (2011). Pathophysiology of Huntington's disease: Time-dependent alterations in synaptic and receptor function. *Neuroscience, 198*, 252–273.

Reynolds, I. J., & Miller, R. J. (1989). Ifenprodil is a novel type of *N*-methyl-D-aspartate receptor antagonist: Interaction with polyamines. *Molecular Pharmacology, 36*(5), 758–765.

Rigby, M., et al. (1996). The messenger RNAs for the *N*-methyl-D-aspartate receptor subunits show region-specific expression of different subunit composition in the human brain. *Neuroscience, 73*(2), 429–447.

Rock, D. M., & Macdonald, R. L. (1995). Polyamine regulation of *N*-methyl-D-aspartate receptor channels. *Annual Review of Pharmacology and Toxicology, 35*, 463–482.

Roses, A. D. (1998a). Genetic testing and Alzheimer disease: The promise. *Alzheimer Disease and Associated Disorders, 12*(Suppl 3), S3–S9.

Roses, A. D. (1998b). Alzheimer diseases: A model of gene mutations and susceptibility polymorphisms for complex psychiatric diseases. *American Journal of Medical Genetics, 81*(1), 49–57.

Samii, A., Nutt, J. G., & Ransom, B. R. (2004). Parkinson's disease. *Lancet, 363*(9423), 1783–1793.

Santos, S. D., et al. (2009). Regulation of AMPA receptors and synaptic plasticity. *Neuroscience, 158*(1), 105–125.

Sarhan, S., & Seiler, N. (1989). On the subcellular localization of the polyamines. *Biological Chemistry Hoppe-Seyler, 370*(12), 1279–1284.

Schaeffer, E. L., & Gattaz, W. F. (2008). Cholinergic and glutamatergic alterations beginning at the early stages of Alzheimer disease: Participation of the phospholipase A2 enzyme. *Psychopharmacology, 198*(1), 1–27.

Schmid, A., et al. (2008). Activity-dependent site-specific changes of glutamate receptor composition in vivo. *Nature Neuroscience, 11*(6), 659–666.

Schumann, J., et al. (2008). Inhibition of NR2B phosphorylation restores alterations in NMDA receptor expression and improves functional recovery following traumatic brain injury in mice. *Journal of Neurotrauma, 25*(8), 945–957.

Seeburg, P. H. (1993). The TINS/TiPS lecture. The molecular biology of mammalian glutamate receptor channels. *Trends in Neurosciences, 16*(9), 359–365.

Seeburg, P. H., Higuchi, M., & Sprengel, R. (1998). RNA editing of brain glutamate receptor channels: Mechanism and physiology. *Brain Research. Brain Research Reviews, 26*(2–3), 217–229.

Shankar, G. M., & Walsh, D. M. (2009). Alzheimer's disease: Synaptic dysfunction and Abeta. *Molecular Neurodegeneration, 4*, 48.

Shankar, G. M., et al. (2007). Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *The Journal of Neuroscience, 27*(11), 2866–2875.

Shoulson, I., et al. (2001). A randomized, controlled trial of remacemide for motor fluctuations in Parkinson's disease. *Neurology, 56*(4), 455–462.

Smolders, I., et al. (2002). Antagonists of GLU(K5)-containing kainate receptors prevent pilocarpine-induced limbic seizures. *Nature Neuroscience, 5*(8), 796–804.

Sommer, B., et al. (1990). Flip and flop: A cell-specific functional switch in glutamate-operated channels of the CNS. *Science, 249*(4976), 1580–1585.

Sommer, B., et al. (1992). A glutamate receptor channel with high affinity for domoate and kainate. *The EMBO Journal, 11*(4), 1651–1656.

Spaethling, J. M., et al. (2008). Calcium-permeable AMPA receptors appear in cortical neurons after traumatic mechanical injury and contribute to neuronal fate. *Journal of Neurotrauma, 25*(10), 1207–1216.

Starr, M. S. (1995). Antiparkinsonian actions of glutamate antagonists–alone and with L-DOPA: A review of evidence and suggestions for possible mechanisms. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 10*(2–3), 141–185.

Stauch Slusher, B., et al. (1995). Centrally-administered AMPA antagonists increase locomotion in parkinsonian rats. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 9*(2–3), 145–149.

Stephen, L. J., & Brodie, M. J. (2011). Pharmacotherapy of epilepsy: Newly approved and developmental agents. *CNS Drugs, 25*(2), 89–107.

Stromgaard, K., & Mellor, I. (2004). AMPA receptor ligands: Synthetic and pharmacological studies of polyamines and polyamine toxins. *Medicinal Research Reviews, 24*(5), 589–620.

Sucher, N. J., et al. (1995). Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. *The Journal of Neuroscience, 15*(10), 6509–6520.

Sugihara, H., et al. (1992). Structures and properties of seven isoforms of the NMDA receptor generated by alternative splicing. *Biochemical and Biophysical Research Communications, 185*(3), 826–832.

Sultana, R., Banks, W. A., & Butterfield, D. A. (2010). Decreased levels of PSD95 and two associated proteins and increased levels of BCl2 and caspase 3 in hippocampus from subjects with amnestic mild cognitive impairment: Insights into their potential roles for loss of synapses and memory, accumulation of Abeta, and neurodegeneration in a prodromal stage of Alzheimer's disease. *Journal of Neuroscience Research, 88*(3), 469–477.

Surmeier, D. J., et al. (2010). What causes the death of dopaminergic neurons in Parkinson's disease? *Progress in Brain Research, 183*, 59–77.

Swanson, G. T. (2009). Targeting AMPA and kainate receptors in neurological disease: Therapies on the horizon? *Neuropsychopharmacology, 34*(1), 249–250.

Sze, C., et al. (2001). *N*-Methyl-D-aspartate receptor subunit proteins and their phosphorylation status are altered selectively in Alzheimer's disease. *Journal of the Neurological Sciences, 182*(2), 151–159.

Tabor, C. W., & Tabor, H. (1984). Polyamines. *Annual Review of Biochemistry, 53*, 749–790.

Takeuchi, A., & Takeuchi, N. (1963). Glutamate-induced depolarization in crustacean muscle. *Nature, 198*, 490–491.

Traynelis, S. F., Hartley, M., & Heinemann, S. F. (1995). Control of proton sensitivity of the NMDA receptor by RNA splicing and polyamines. *Science, 268*(5212), 873–876.

Ulas, J., & Cotman, C. W. (1997). Decreased expression of *N*-methyl-D-aspartate receptor 1 messenger RNA in select regions of Alzheimer brain. *Neuroscience, 79*(4), 973–82.

Ulas, J., et al. (1994). Selective increase of NMDA-sensitive glutamate binding in the striatum of Parkinson's disease, Alzheimer's disease, and mixed Parkinson's disease/Alzheimer's disease patients: An autoradiographic study. *The Journal of Neuroscience, 14*(11 Pt 1), 6317–6324.

Vincent, P., & Mulle, C. (2009). Kainate receptors in epilepsy and excitotoxicity. *Neuroscience, 158*(1), 309–323.

Wakabayashi, K., Honer, W. G., & Masliah, E. (1994). Synapse alterations in the hippocampal-entorhinal formation in Alzheimer's disease with and without Lewy body disease. *Brain Research, 667*(1), 24–32.

Watanabe, M., et al. (1992). Developmental changes in distribution of NMDA receptor channel subunit mRNAs. *Neuroreport, 3*(12), 1138–1140.

Werner, P., et al. (1991). Cloning of a putative high-affinity kainate receptor expressed predominantly in hippocampal CA3 cells. *Nature, 351*(6329), 742–744.

Whetsell, W. O., Jr., & Shapira, N. A. (1993). Neuroexcitation, excitotoxicity and human neurological disease. *Laboratory Investigation, 68*(4), 372–387.

Williams, K., Romano, C., & Molinoff, P. B. (1989). Effects of polyamines on the binding of [^{3}H] MK-801 to the *N*-methyl-D-aspartate receptor: Pharmacological evidence for the existence of a polyamine recognition site. *Molecular Pharmacology, 36*(4), 575–581.

Woods, N. K., & Padmanabhan, J. (2012). Neuronal calcium signaling and Alzheimer's disease. *Advances in Experimental Medicine and Biology, 740*, 1193–1217.

World Health Organization. (2012). *Dementia: A public health priority*. Geneva: World Health Organization.

Wu, S. S., & Frucht, S. J. (2005). Treatment of Parkinson's disease: What's on the horizon? *CNS Drugs, 19*(9), 723–743.

Yamazaki, M., et al. (1992). Cloning, expression and modulation of a mouse NMDA receptor subunit. *FEBS Letters, 300*(1), 39–45.

Yasuda, R. P., et al. (1995). Reduction of AMPA-selective glutamate receptor subunits in the entorhinal cortex of patients with Alzheimer's disease pathology: A biochemical study. *Brain Research, 678*(1–2), 161–167.

Zhang, L., et al. (1994). Spermine potentiation of recombinant *N*-methyl-D-aspartate receptors is affected by subunit composition. *Proceedings of the National Academy of Sciences of the United States of America, 91*(23), 10883–10887.

Zheng, Z., Sabirzhanov, B., & Keifer, J. (2010). Oligomeric amyloid-{beta} inhibits the proteolytic conversion of brain-derived neurotrophic factor (BDNF), AMPA receptor trafficking, and classical conditioning. *The Journal of Biological Chemistry, 285*(45), 34708–34717.

Zimmer, M., et al. (1995). Cloning and structure of the gene encoding the human *N*-methyl-D-aspartate receptor (NMDAR1). *Gene, 159*(2), 219–223.

Zundorf, G., & Reiser, G. (2011). Calcium dysregulation and homeostasis of neural calcium in the molecular mechanisms of neurodegenerative diseases provide multiple targets for neuroprotection. *Antioxidants & Redox Signaling, 14*(7), 1275–1288.

Ionotropic Receptors in the Central Nervous System and Neurodegenerative Disease

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Abstract

Glutamate was identified as the main excitatory neurotransmitter in the mammalian central nervous system (CNS) following the observation in the early 1950s that glutamate can induce seizure activity and excite neurons in the mammalian brain. Over the last two decades, selective ligands, including competitive agonists and antagonists and allosteric modulators, have been developed to further investigate the functional role of glutaminergic receptors. Glutamate released from synapses can activate ligand-gated cation channels at postsynaptic cells to mediate fast postsynaptic potentials. These ion channel-forming ionotropic glutamate receptors (iGluRs) are divided into *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxyl-5-methyl-isoxazole-4-prorionate (AMPA), and kainate (KA) receptors. While only 20–30 % of the amino acid sequence is shared among these receptor subtypes, they share similar structural features and their activity is based on specific pharmacological preference. In this chapter, we will describe the structure and composition of iGluRs and infer their pharmacology, with a particular focus on their role in the CNS and their relevance to the pathogenesis of neurodegenerative diseases.

1 Introduction

Human neurodegenerative diseases are characterized by extensive loss of neurons, resulting in a number of neurological deficits (Hague, Klaffe et al. 2005). Excitatory amino acids serve as major neurotransmitters in the cerebral cortex and hippocampus. Under normal physiological conditions, excitatory amino acids are essential for the maintenance of normal psychological functions including learning and memory. Dysregulation of the excitatory amino acid system in various CNS diseases is associated with the development of several cognitive pathologies including schizophrenia, delirium, and dementia (Javitt and Zukin 1990). Overactivation of the excitatory amino acid system can also lead to cell death, giving rise to the concept of excitotoxicity (Olney 1978). Excitotoxicity refers to the process of neuronal cell death arising from prolonged exposure to excitatory amino acids with different permeabilities, leading to elevated Ca^{2+} influx (Wong, Cai et al. 2002; Berliocchi, Bano et al. 2005). The subsequent Ca^{2+} overload can stimulate the activation of a number of different enzymes which can degrade proteins, lipids, and nucleic acid (Berliocchi, Bano et al. 2005) (Fig. 1). Apart from glutamate, which is the principal excitatory neurotransmitter in the mammalian CNS, other constitutive amino acids,

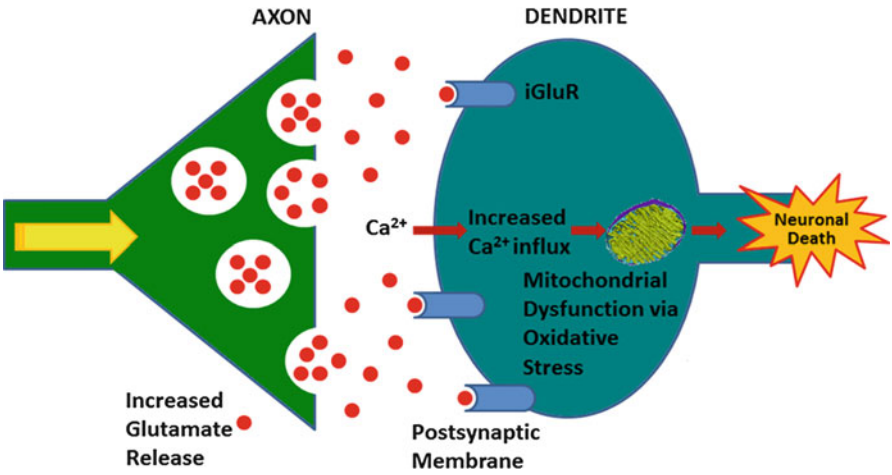

Fig. 1 Schematic representation of glutamate-induced excitotoxicity in neurons. Excitotoxicity due to increased glutamate release from hyperactive neuronal circuits in the brain can activate ionotropic glutamate receptors. Overactivation of these receptors leads to high intracellular calcium influx, disrupts mitochondrial function, and culminates in cell death

such as aspartic acid, and exogenous compounds, such as quinolinic acid and kainate, or of synthetic origin such as NMDA, can induce excitotoxicity (Foster, Collins et al. 1983; Hertz, Dringen et al. 1999).

Neurodegenerative disorders have distinct clinical symptoms and genetic etiologies. While some neurodegenerative conditions are considered "sporadic," due to lack of well-defined genetic associations common to all patients, others do have specific genetic mutations. For instance, the majority of cases of Alzheimer's disease (AD) are sporadic, only around 5 % of these cases are familial forms of autosomal-dominant (not sex-linked) inheritance, that usually occurs before the age of 65 (Blennow, de Leon et al. 2006). While these genetic mutations can affect a variety of proteins, sufficient evidence suggests that excitotoxicity represents a convergence point in neuronal degeneration (Dodd 2002; Kawamata and Shimohama 2002). Overactivation of ionotropic glutamate receptors iGluR (Fig. 2) can participate in fast glutaminergic synaptic transmission, leading to impaired calcium homeostasis and increased free radical generation, culminating in programmed cell death (Rae, Moussa et al. 2006).

2 Structural Features of Ionotropic Glutamate Receptors

The first transmembrane domain of the iGluR family (Fig. 3) contains a pore-forming membrane-residing domain that forms a re-entrant loop entering from and exiting to the cytoplasm (Dingledine and McBain 1994; Dingledine, Borges et al. 1999). A large extracellular loop provides a link between the second and third transmembrane domains, followed by an extracellular carboxy-terminus.

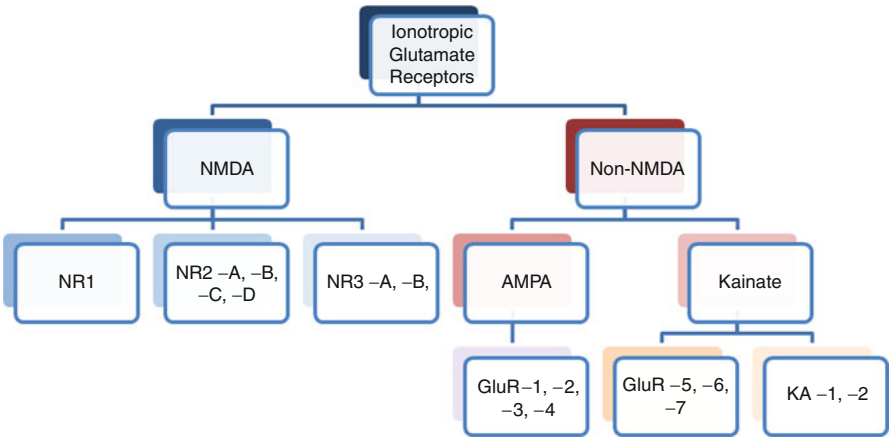

Fig. 2 Family of ionotropic glutamate receptors based on structure and function

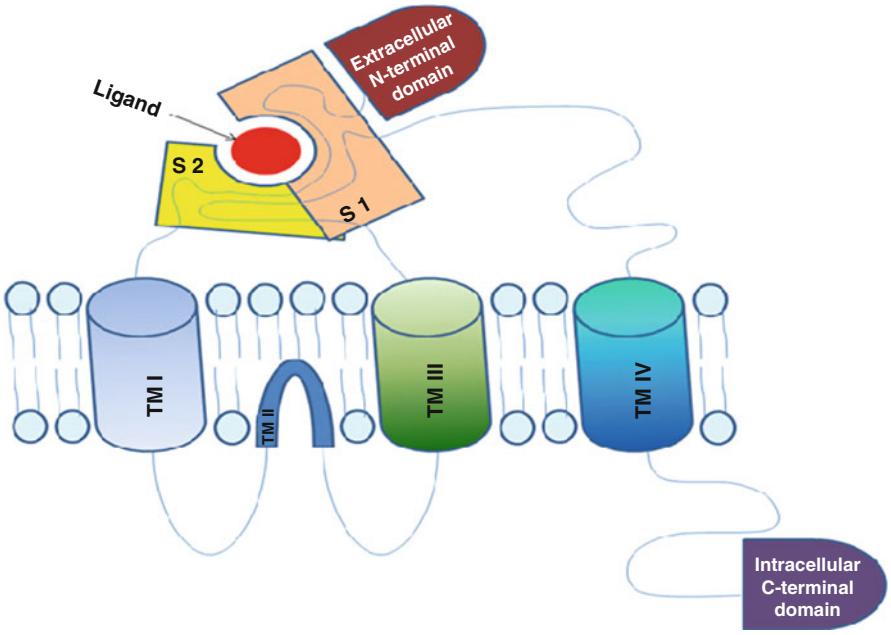

Fig. 3 Schematic diagram of iGluR subunit topology. The extracellular amino-terminal domain is followed by the first transmembrane domain (TM I). The membrane-residing domain (TM II) forms a re-entrant loop. The third and fourth transmembrane domains (TM III and TM IV) are linked by an extracellular loop. The agonist-binding domain is located in a pocket between polypeptide segments S1 and S2

The crystal structure of iGluR ligand binding domains consists of unique polypeptides attached to both the extracellular amino terminus and the extracellular loop between transmembrane domain 3 and 4 (Armstrong, Sun et al. 1998; Mayer and Armstrong 2004).

2.1 NMDA Receptors

NMDA receptors are ligand-gated ion channels that exist as heterotetrameric complexes at the membrane surface. Molecular cloning has identified seven subunits (NR1, NR2A-D, and NR3A and B), each of which represent products of different genes. These amino acid sequences are 18 % (NR1 and NR2), 55 % (NR2A and NR2C), 70 % (NR2A and NR2B), and 25 % (NR3 and NR1, or NR3 and NR2) identical (McBain and Mayer 1994).

Electrophysiological studies have shown that activation of the NMDA receptor requires occupation of two independent glutamate and glycine sites (Clements and Westbrook 1991). Therefore, functional NMDA receptors are composed of the constitutive NR1 subunit and at least one NR2 subunit, or NR1 and both NR2 and NR3 subunits (Mayer and Armstrong 2004). While the exact role of NR3 remains unknown, site-directed mutagenesis has shown that the NR2 subunit contains the glutamate-binding site while the binding site for the co-agonist glycine is carried on the NR1 subunit (Seeburg, Burnashev et al. 1995; Nishi, Hinds et al. 2001; Chatterton, Awobuluyi et al. 2002; Loftis and Janowsky 2003; Matsuda, Fletcher et al. 2003). This glycine-binding domain resembles the clamshell-like architecture reminiscent of the AMPA and kainite subunit-binding pockets (Kew, Koester et al. 2000; Kew and Kemp 2005).

The NR2 and NR3 subunits are differentially expressed in a distinct spatiotemporal pattern (Monyer, Sprengel et al. 1992). In the rodent and human brain, the NR2A and NR2B subunits are predominantly expressed in the forebrain, while the NR2C is expressed in cerebellar granule cells and several nuclei (Monyer, Sprengel et al. 1992; Monyer, Burnashev et al. 1994). However, the expression of the NR2D subunits is confined to the diencephalon and midbrain where it is upregulated significantly during development (Monyer, Sprengel et al. 1992). The NR3 subunit also shares similar spatiotemporal distribution, with the NR3A receptor also highly expressed during development, although expression of the NR3A receptor persists in some neuronal populations during adulthood (Nishi, Hinds et al. 2001). Unlike the NR3A subunit, NR3B is localized in somatic motor neurons in the brainstem and spinal cord. When expressed with NR1 and NR2, the NR3 subunit suppresses NMDA channel unitary conductance and Ca^{2+} permeability relative to NR1/NR2-containing receptors (Nishi, Hinds et al. 2001; Chatterton, Awobuluyi et al. 2002; Loftis and Janowsky 2003; Matsuda, Fletcher et al. 2003). Moreover, NR3A knockout mice exhibit increased NMDA-mediated currents and increased spine density in cortical neurons (Das, Sasaki et al. 1998). Lee et al. (2011) recently showed that adult and fetal human primary astrocytes express all known NMDA receptor subunits (Lee, Ting et al. 2011). This is in accordance to previous

immunohistochemical studies showing that NR1 and NR2A/NR2B are present in rodent astrocytes (Conti, DeBiasi et al. 1996; Conti, Barbaresi et al. 1999). Native NMDA receptors are therefore composed of NR1 in combination with one or more NR2 subunits (e.g., NR1/NR2A, NR1/NR2B, or NR1/NR2A/NR2B), or alternatively, NR1 may exist in combination with both NR2 and NR3 subunits (e.g., NR1/NR2B/NR3A) (Kew and Kemp 2005).

2.2 AMPA Receptors

Cloning studies have shown that AMPA receptors are composed of four closely related subunits (GluR1, GluR2, GluR3, and GluR4) (Borges and Dingledine 1998). These four subunits appear to be products of separate genes. The polypeptide sequence of each subunit is about 900 amino acids in length and sharing 70 % (GluR1 and 2) and 73 % (GluR2 and 3) homology (Rosenmund, Stern-Bach et al. 1998). Like NMDA receptors, native AMPA receptors are heteromeric in composition and expressed differentially in the brain. GluR2 plays a predictive role in determining the permeability of AMPA receptors to Ca^{2+} (Bowie and Mayer 1995). AMPA receptors lacking the GluR2 subunit are Ca^{2+} permeable and exhibit evidence of inward channel rectification due to voltage-dependent block of the ion channel by intracellular polyamines (Bowie and Mayer 1995). The molecular property determining the Ca^{2+} impermeability of the GluR2 subunit can be attributed to the presence of an arginine (R) positioned at a critical site in the pore loop, M2 domain which is replaced by glutamine (Q) in other AMPA subunits (Seeburg and Hartner 2003).

The diversity of AMPA receptor subunits is due to two genetic processes: RNA editing and alternative splicing. Editing is a posttranscriptional change in at least one base in the pre-mRNA where the codon encoded by the gene differs to the mRNA. In AMPA receptors, editing is restricted to the GluR2 subunit. In the primary transcript of GluR2, glutamine at position 586 in the M2 putative membrane is replaced with an arginine (Dingledine, Borges et al. 1999). The arginine codon is absent in the GluR2 gene and is introduced in the mRNA by dsRNA adenosine deaminase which converts adenosine to inosine which is subsequently read as guanosine. Editing is regulated during development, such that 99 % of the GluR2 subunit is in the arginine form in the postnatal stage (Dingledine, Borges et al. 1999).

Alternative splicing affects the 38 amino acid sequence in the extracellular S2 domain preceding the C-terminal transmembrane domain M4 of each of the four subunits (Dingledine, Borges et al. 1999). The small segment exists in two alternatively spliced forms referred to as Flip and Flop which are encoded by adjacent exons of the receptor genes. The Flip form is expressed prenatally while the Flop variants are expressed postnatally. These variants can influence the rate and effect of desensitization of heteromeric AMPA receptors and also their sensitivity to specific allosteric modulators (Dingledine, Borges et al. 1999).

AMPA receptors lacking edited GluR2 receptors are expressed in the hippocampus and amygdala. These receptors demonstrate high Ca^{2+} permeability and

predominate in inhibitory interneurons, while non-edited, Ca^{2+} impermeable GluR2 receptors are found in pyramidal cells (Geiger, Melcher et al. 1995). Extensive evidence in cerebellar stellate cells has shown that activity-induced Ca^{2+} influx through GluR2-lacking AMPA receptors regulates the targeting of GluR2-containing AMPA receptors, suggesting the existence of a self-regulatory mechanism influencing Ca^{2+} permeability at the synapses (Liu and Cull-Candy 2002).

2.3 Kainate Receptors

Five subunits of the kainate family of receptors have been cloned: GluR5, GluR6, GluR7, KA1, and KA2 (Bleakman, Gates et al. 2002). These receptors have been subdivided into two families based not only on sequence homology but also agonist-binding properties. GluR5-7 are approximately 70 % identical with each other and 40 % homologous with AMPA receptors (Bleakman, Gates et al. 2002). KA1 and KA2 also share 70 % amino acid sequence homology with each other but only 40 % with GluR5-7. GluR5-7 have a lower affinity kainate-binding site (K_D of 50–100nM), whereas KA1 and KA2 c have a higher affinity kainate-binding site (K_D of 5–15nM) (Bleakman, Gates et al. 2002).

Kainate receptors are tetrameric with GluR5-7 forming homomeric and heteromeric combinations. However, KA1 and KA2 cannot form functional homomeric receptors, but combine to form heteromeric assemblies with GluR5-7 (Alt, Weiss et al. 2004). Interestingly, KA2 is not expressed on the cellular surface in the absence of GluR5-7, but is translocated to the endoplasmic reticulum, suggesting the existence of an endoplasmic reticulum signal that is shielded in the presence of other kainate receptors (Gallyas, Ball et al. 2003).

Similar to AMPA receptors, GluR5-7 are susceptible to RNA editing and alternative splicing to introduce further heterogeneity. GluR5-6 but not GluR7 mRNA can be edited at the Q/R site on the M2 segment (Dingledine, Borges et al. 1999). Analogous to AMPA receptors, the addition of arginine can foster lower permeability to Ca^{2+}. However, unlike GluR2, RNA editing appears to be incomplete during aging and a large number of both edited and non-edited GluR5-6 receptors are distributed in the adult brain (Dingledine, Borges et al. 1999).

Kainate receptors are distributed throughout the CNS (Bowie and Mayer 1995; Bleakman, Gates et al. 2002). The KA1 and KA2 subunits are both localized in hippocampal CA3 and dentate granule neurons; however, KA2 is more dominant. GluR6 appears to be the major subunit in both presynaptic and postsynaptic receptors at mossy fiber synapses in the hippocampal CA3 region (Kerchner, Wilding et al. 2002). The GluR5 subunit appears to be predominant in the dorsal root ganglion cells (Contractor, Swanson et al. 2000; Kerchner, Wilding et al. 2002). GluR5 is expressed predominantly in hippocampal interneurons where it plays a critical role in regulating the effects of glutamate released by astrocytes (Liu, Xu et al. 2004). GluR5 is also strongly expressed in the temporal lobe. Functional studies have shown that GluR5-containing receptors are involved in synaptic excitation in the amygdala (Rogawski, Gryder et al. 2003).

3 Pharmacology of Ionotropic Glutamate Receptors

Several iGluR agonists and antagonists have been identified. Information regarding the structure and function of these ligands has helped to increase our body of understanding regarding the pharmacology of these receptors and markedly increased our knowledge of receptor physiology and pathophysiology in the CNS.

3.1 NMDA Receptors

NMDA is the most common agonist at the glutamate-binding site (located on the NR2 subunit) of the NMDA receptor (Fig. 4). While it demonstrates lower potency than glutamate, it is not quite a full agonist and shows little selectivity between the NMDA receptor subtypes (Addae, Evans et al. 2000). Another selective full agonist at NMDA receptors is quinolinic acid (QUIN) (De Carvalho, Bochet et al. 1996). QUIN is an endogenous metabolite of the kynurenine pathway (KP). In the CNS, the KP represents the principal route of L-tryptophan metabolism, leading to the formation of the essential pyridine nucleotide nicotinamide adenine dinucleotide (NAD^+) (Stone 2001). During neuroinflammation, increased activity of the primary KP enzyme indoleamine 2,3-dioxygenase (IDO-1) directs the KP to stimulate

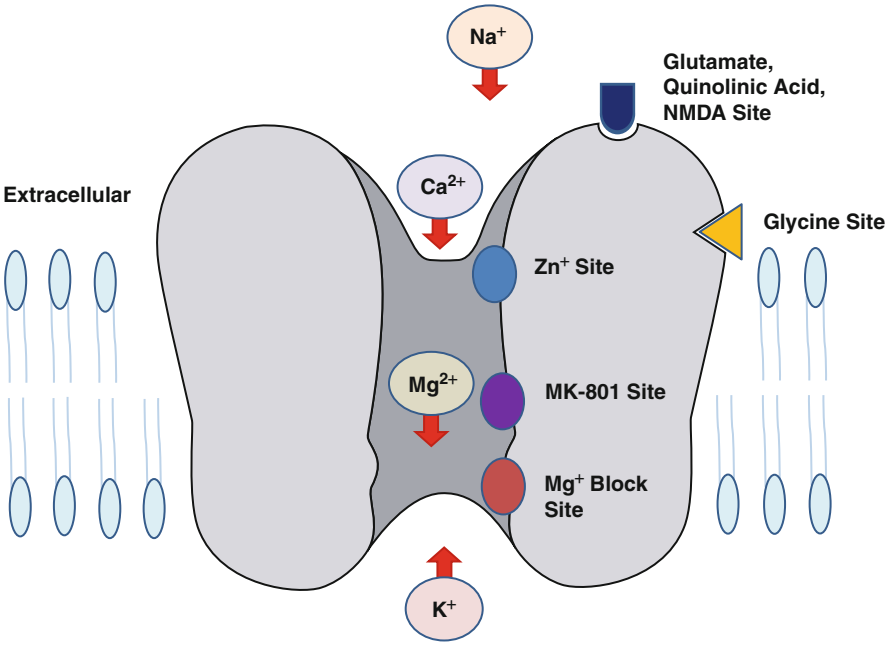

Fig. 4 The NMDA receptor showing binding sites for both glutamate and glycine. The ligand-gated ion channel facilitates movement of calcium, sodium, and potassium across the postsynaptic membrane

QUIN production by activated microglia and invading macrophages, leading to neuronal and glial cell death (Guillemin 2003; Guillemin, Smythe et al. 2004).

Other NMDA receptor agonists include L-aspartate, homocysteate, and homoquinolinate, while *cis*-2,3-piperidinedicarboxylic acid is a low efficacy agonist (Jane, Olverman et al. 1994; Christie, Jane et al. 2000). Additional compounds demonstrate agonist or partial agonist activity at the glycine co-agonist site on the NR1 subunit. Vinyl substituted glycine derivatives such as *S*-hydroxyvinyl glycine can also act as agonists at the glycine site (Kemp, Foster et al. 1988; Priestley, Horne et al. 1990; Priestley, Ochu et al. 1994; Priestley, Laughton et al. 1995). While D-serine is an agonist at low concentrations, it can act as a competitive antagonist at the glutamate site at high concentrations. Other glycine-site partial agonists include L-alanine and D-cycloserine (Kemp and Leeson 1993).

Continuous activation of the NMDA receptor can lead to increased intracellular Ca^{2+} permeability and conductance which can trigger a cascade of events leading to either apoptosis or necrosis. The downstream effectors include free radical production, mitochondrial membrane depolarization, and caspase activation (Chen, Harris et al. 1995; Colwell, Altemus et al. 1996; Catts, Ward et al. 1997; Araque, Sanzgiri et al. 1998; Budd, Tenneti et al. 2000; Comoletti, Muzio et al. 2001). Given the importance of NMDA receptors in normal physiological function and in the etiology of neurodegenerative diseases, several NMDA receptor antagonists have been developed to target the glutamate and glycine recognition sites and the NMDA ion channel pore. An example of a high-affinity competitive antagonist of the glutamate site is (*R*)-2-amino-5-phosphonopentanoate (D-AP5) (Jane, Olverman et al. 1994). While this compound shows high in vivo affinity due to the appropriately placed ω-phosphonic acid group, it cannot penetrate the blood-brain barrier (BBB) due to its highly charged nature (Kew and Kemp 2005). Recently, adenosine triphosphate (ATP) has been shown to be a dual modulator of the NMDA receptor. While ATP can antagonize the NMDA receptor at the glutamate site at low glutamate concentrations, it can act simultaneously to enhance NMDA function at high glutamate concentrations (Kloda, Clements et al. 2004).

Similarly, numerous inhibitors have been identified at the glycine recognition site (Bergeron, Meyer et al. 1998). Kynurenic acid (KA), a metabolite of the KP, was the first glycine site antagonist described and displayed weak inhibition of the NMDA receptor against QUIN toxicity. In one study, the required concentration of KA to display neuroprotection was tenfold greater than the agonist, QUIN (Jhamandas, Boegman et al. 2000). Many synthetic antagonists of the glycine site were later developed using KA as the pharmacophore (Hartley, Monyer et al. 1990; Foster, Kemp et al. 1992; Kemp and Leeson 1993; Dannhardt and Kohl 1998; Honer, Benke et al. 1998). These compounds include synthetic high-affinity derivatives such as quinolines, quinolones, and indoles. However, compounds of this class can bind tightly to plasma protein which compromises their ability to cross the BBB and produce potent in vivo activity (Bristow, Hutson et al. 1996).

The most potent NMDA antagonists used to treat neurodegenerative diseases are the NMDA ion channel pore blockers. Following the identification of ketamine and phencyclidine as selective NMDA receptor antagonists, the highly potent in vivo

active antagonist dizocilpine (MK-801) was developed (Foster, Gill et al. 1987, 1988; Farlow 2004). However, long-term NMDA receptor inhibition by MK-801 has previously been shown to be toxic to cultures of rat cortical neurons (Hwang, Kim et al. 1999). Subsequently, lower affinity compounds such as 1,3-dimethyl-5-aminoadamantane (Memantine) have been developed and approved for clinical use (Farlow 2004). These compounds act as open channel blockers and are irreversibly trapped within the closed pore. Memantine has quicker reversibility, making it efficacious and better tolerated than MK-801 (Kew and Kemp 2005).

A more recent development in NMDA receptor antagonists has been the identification of allosteric modulators that can inhibit the receptor by an interaction with the extracellular N-terminal domain of the NR2 subunit (Kew and Kemp 2005). Compounds such as Ifenprodil and Benzimidazole have high NR2 affinity compared with other NMDA subtypes but produce additional off-target effects by modulating adrenergic receptors and human ether-a-go-go (hERG) ion channels (Finlayson, Witchel et al. 2004). Recently, a phase III clinical trial for Troxoprodil used for the treatment of traumatic brain disease, failed due to poor efficacy (Kew and Kemp 2005).

3.2 AMPA Receptors

AMPA receptors have also been implicated in excitotoxicity due to their ability to increase intracellular Ca^{2+} overload (Borges and Dingledine 1998). Apart from AMPA itself, several agonists have been developed including ibotenic acid, quisqualic acid, and willardine (Stensbol, Madsen et al. 2002). While the distal acidic group varies between agonists, the amino acid moiety is highly conserved. Unlike glutamate and AMPA which are full agonists and induce rapidly desensitizing responses, kainate is a partial agonist with demonstrated low desensitization. The degree of desensitization has been linked to the extent of dimerization between pairs of subunits within the AMPA receptor (Stensbol, Madsen et al. 2002).

The first competitive AMPA receptor antagonists were 6-cyano-7-nitroquinoxaline-2,3-dione (CQNX) and 6,7-dinitro-quinoxaline-2,3-dione (DNQX). However, these compound showed low selectivity and could also antagonize the glycine-binding site of the NMDA receptor. More selective AMPA receptor antagonists include 2,3-dihydroxy-6-nitro-7sulfamoyl-benzo(F)quinoxaline (NBQX) (Birch, Grossman et al. 1988; Turski, Huth et al. 1998). However, these compounds show poor water solubility and represent failed clinical trials due to adverse side effects including nephrotoxicity. Several noncompetitive AMPA receptor antagonists have been developed. These include several 2,3-benzodiazepine compounds and quinazoline derivatives. These compounds are selective for AMPA over NMDA and kainate receptors and have recently entered clinical trials (Lazzaro, Paternain et al. 2002; Solyom and Tarnawa 2002).

Additionally, several AMPA-receptor-positive allosteric modulators have been developed. These new class of compounds can modulate receptor

desensitization/deactivation without activating the receptor when supplied alone (Danysz 2002). These compounds include cognitive enhancing "nootropic agents" such as 1-(4-methoxybenzoyl)-2-pyrrolidinone (aniracetam), benzamides, and benzothiadiazides. These compounds demonstrate distinct mechanistic profiles and are selective for individual AMPA receptor subunits (Danysz 2002).

3.3 Kainate Receptors

Kainate receptors have also been associated with increased Ca^{2+} influx, leading to excitotoxic neuronal cell death. Apart from kainate, several kainate-receptor agonists have been identified. These include 5-tert-butyl-4-isoxazolepropionic acid (ATPA), a substituted analogue of AMPA, and (*S*)-5-iodowillardine (Lerma, Paternain et al. 2001; Bleakman, Gates et al. 2002). These compounds show lower affinity to AMPA receptors. The quinoxalinedione compounds such as CNQX and NBQX are competitive antagonists at kainate receptors but show little discrimination between kainate and AMPA receptors (Lerma, Paternain et al. 2001; Bleakman, Gates et al. 2002). Derivatives of willardine also exhibit antagonist effects at both AMPA and kainate receptors. Kainate receptors also demonstrate rapid desensitization following agonist binding (Bleakman, Gates et al. 2002; Alt, Weiss et al. 2004). The lectin concanavalin A can rapidly inhibit receptor desensitization by interacting with *N*-glycosylated residues and increase receptor agonist affinity. It also exhibits activity at NMDA and AMPA receptors (Lerma, Paternain et al. 2001; Bleakman, Gates et al. 2002).

4 Molecular Mechanisms in Neurodegenerative Diseases

There is an attractive body of evidence incriminating glutaminergic dysfunction in the process of slow excitotoxicity associated with neuronal loss in Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and Amyotrophic Lateral Sclerosis (ALS).

4.1 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with the progressive loss of cholinergic neurons parallel to impairments in cognition and memory (Mattson 2004). The pathological hallmarks of AD include extracellular deposition of the β-amyloid peptide (Aβ) in senile plaques and formation of intracellular neurofibrillary tangles (NFTs) (Hartmann 1999). Accordingly, the Aβ hypothesis suggests that AD occurs as a response to increased production and extracellular deposition of Aβ and intracellular deposition of NFTs. These abnormal pathologies can trigger numerous neurotoxic deleterious processes including excitotoxicity, oxidative stress and energy depletion,

neuroinflammation, and cell death (Bamberger and Landreth 2001). Interestingly, the glutaminergic system is severely compromised by Aβ-mediated regulation of glutamate receptors in various brain regions, correlating with significant cognitive impairment (Parameshwaran, Dhanasekaran et al. 2008).

The neuronal degeneration in AD has been linked to overactivation of the NMDA receptor (Butterfield 1997). Recent studies have shown that Aβ can stimulate free radical formation through a mechanism involving NMDA receptors. QUIN can increase mRNA and protein expression of the inducible (iNOS) and neuronal (nNOS) forms of nitric oxide following overactivation of the NMDA receptor, leading to subsequent induction of nitric oxide synthase in human primary fetal astrocytes and neurons, triggering cell death (Braidy, Grant et al. 2009). Dysregulation of NMDA receptor activation and free radical formation may serve as dual deleterious roles in AD.

We have previously shown that QUIN is significantly increased in postmortem AD brain (Guillemin, Brew et al. 2007). Recently, our group has shown that the KP is activated in the AD brain with QUIN immunoreactivity reported in cortical microglia, astrocytes, and neurons, and in the perimeter of Aβ plaques and NFTs. We recently reported an accumulation of QUIN in dystrophic neurons in cortical sections from AD patients (Guillemin, Brew et al. 2005). Additionally, QUIN can increase tau phosphorylation and stimulates dysregulation of microtubule formation in vitro. We also found that QUIN can induce at least ten important neuronal genes strongly associated with AD pathology (Rahman, Ting et al. 2009). Therefore, excitotoxicity due to increased activation of NMDA receptors can enhance the vulnerability of local neurons consistent with AD pathology.

Restoration of homeostasis in the glutamatergic system using NMDA receptor antagonists has been shown to ameliorate the neurotoxic effects by NMDA activation both in vitro and in vivo. Memantine, a noncompetitive NMDA receptor channel blocker, has received marketing approval from the EMEA (European Medicines Agency), FDA (Food and Drug Administration), and TGA (Therapeutic Goods Administration) for the treatment of mild-to-severe AD in Europe, USA, and Australia (Doggrell 2003). It has strong voltage-dependency and fast kinetics to help provide neuroprotection and reverse deficits in memory and learning (Ferris 2003; Gallarda and Loo 2004; Kato 2004; Gardoni and Di Luca 2006).

4.2 Parkinson's Disease

Parkinson's disease (PD) is a neurological disease caused by degeneration of dopaminergic neurons in the substantia nigra, leading to a significant reduction in dopamine levels in the striatum (Mosley, Benner et al. 2006). Although the exact etiology of PD remains unknown, excessive glutaminergic drive has been proposed as a major source of excitotoxicity in the nigra (Barnum and Tansey 2010). Studies have shown that parkin (hereditary Parkinson disease PARK2 gene product) can modulate the stability and function of excitatory glutaminergic synapses (Yu, Sun et al. 2011). Postsynaptic expression of parkin can inhibit excitatory synaptic

transmission. Knockdown of parkin or PD-linked parkin mutants can promote synaptic function and facilitate the proliferation of glutamatergic synapses. Activation of the NMDA receptor can lead to increased Ca^{2+} influx which has been linked to cell death in PD (Yu, Sun et al. 2011). This is further supported by the observation that dopaminergic neurons expressing the calcium-binding protein calmodulin are selectively preserved in the PD brain (Pierson, Norris et al. 2004).

Several studies have shown that excitotoxicity plays a key role in toxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toward dopaminergic neurons (Araki, Kumagai et al. 2000). MPTP is converted to the 1-methyl-4-phenylpyridinium ion (MPP^+) in glial cells by monoamine oxidase-B. The active metabolite is taken up by the dopamine transporter into dopaminergic neurons where it has been shown to inhibit mitochondrial complex I (Beal 2003). This not only compromises neuronal energy production, but also induces a slow excitotoxicity, leading to the loss of nigral cells. Inhibition of the glutamatergic corticostriatal tract has been shown to reduce the size of MPP^+-mediated lesions in the rat striatum. Furthermore, the glutamate-release inhibitor riluzole has been shown to inhibit glutamate release in the corticostriatal tract to attenuate intrastriatal administration of MPP^+ on dopaminergic neurons (Araki, Kumagai et al. 2000). MK-801 (dizocilipine), a noncompetitive NMDA receptor antagonist, has been shown to protect against the toxicity of MPTP in a rat model for PD (Hsieh, Gu et al. 2012). NBQX, an AMPA/kainate-receptor antagonist, has been shown to prevent the symptoms of PD, but not the neurodegeneration in MPTP-treated monkeys (Luqquin, Obeso et al. 1993). The finding that MK-801 cannot protect against MPP^+ toxicity in primary cultures of dissociated mesencephalic neurons suggests that alternative mechanisms of action for MPTP may exist in vivo.

4.3 Huntington's Disease

Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by cognitive and psychiatric impairment, and dyskinesia (Beal, Kowall et al. 1986). One of the major hypotheses developed to explain the vulnerability of medium-size spiny neurons (MSNs) in the neostriatum is the excitotoxicity hypothesis. Studies performed as early as the 1970s in rats, mice and other rodents demonstrated that striatal kainate injections can destroy MSNs while sparing neighboring axons and the effect is similar to HD pathology observed in humans (Ferrante, Kowall et al. 1993; Figueredo-Cardenas, Anderson et al. 1994; Estrada Sanchez, Mejia-Toiber et al. 2008). Similarly, striatal injections of QUIN in rats are able to reproduce the lesions reported in HD (Ellison, Beal et al. 1987; Bruyn and Stoof 1990; Ceresoli-Borroni, Guidetti et al. 1999). QUIN-induced lesions in experimental rodent models can be attenuated using NMDA receptor antagonists (Ellison, Beal et al. 1987; Bruyn and Stoof 1990; Ceresoli-Borroni, Guidetti et al. 1999). The QUIN model has been extended to primates where similar pathological hallmarks have been observed together with clinical symptoms of chorea (Bruyn and Stoof 1990).

Intrastriatal infusion of kainate has also been shown to induce excitotoxic lesions in the striatum and induces neuronal apoptosis via activation of NF-κB and induction of p53 and other pro-apoptotic proteins (Nakai, Qin et al. 2000). However, AMPA/kainate-receptor antagonists have been shown to be inactive in these models (Levine, Klapstein et al. 1999).

The HD gene and its protein product huntingtin (htt) have been proposed to play a key role in neuronal cell death. It has been demonstrated that mutant expression of htt can stimulate NMDA receptor activity to disrupt intracellular calcium homeostasis (Zhang, Li et al. 2008). Recent studies have shown that the postsynaptic density protein PSD-95 represents a link between htt and NMDA receptor function. One study has shown that PSD-95 can bind to the C-terminal of the NR2 subunit to stabilize the NMDA receptor (Roche, Standley et al. 2001). Co-immunoprecipitation studies have also revealed interactions between PSD-95 and NMDA receptor subunits NR1, NR2A, and NR2B, thus providing further evidence for the association of NMDA receptors with PSD-95 (Sun, Savanenin et al. 2001).

4.4 Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is characterized by progressive muscle weakness, leading to paralysis and death, generally due to respiratory failure. Histologically, ALS is characterized by the selective loss of both upper (corticospinal) and lower (spinomuscular) motor neurons (Boillee, Vande Velde et al. 2006; Boillee, Yamanaka et al. 2006). There are several lines of evidence to support the role of excitotoxicity in the loss of motor neurons. Intrathecal injections of kainate have been shown to induce neuronal damage in the spinal cord (Hugon, Ludolph et al. 1988; Hugon and Vallet 1990). These lesions are characterized by axonal swelling and impaired cytoskeletal architecture similar to those seen in ALS (Hugon and Vallet 1990). In chicken motor neurons, glutamate-initiated neurotoxicity is predominantly due to AMPA/kainate receptors. However, in rats, excitotoxicity is mediated by both NMDA and AMPA/kainate receptors (Estevez, Stutzmann et al. 1995; Carriedo, Yin et al. 1996). The observed toxicity in these cell types can be attenuated using AMPA/kainate-receptor antagonists but not NMDA receptor antagonists (Rothstein 1995). However, in humans, we have shown that QUIN can induce apoptosis of fetal motor neurons in a dose-dependent manner and the effect is ameliorated by blocking the NMDA receptor (Chen, Brew et al. 2011).

If excitotoxicity is indeed a major pathogenic mechanism in ALS, it is necessary to explain why only certain motor neurons are affected. A major factor determining the vulnerability of certain motor neurons to excitotoxicity is associated with the subunit composition of the iGluR that they may express. In situ hybridization studies have shown that the NR2B NMDA receptor subunit is absent in rat motor neurons while the GluR2 AMPA/kainate receptor is not present in human spinal motor neurons (Tolle, Berthele et al. 1993). In the absence of the GluR2 subunit,

AMPA/kainate receptors are more permeable to Ca^{2+} and this may explain the excitotoxicity observed in rats (Williams, Day et al. 1997). Nonetheless, direct electrophysiological studies investigating intracellular calcium influx into motor neurons resulting from AMPA/kainate-receptor-mediated excitotoxicity have not been investigated and no comparison has been made between iGluR in rats and humans.

Although no effective cures have been developed, anti-excitotoxic drugs have provided the first treatment for this debilitating disorder. Early trials conducted using the noncompetitive NMDA receptor antagonist dextromethorphan have shown negative results (Hollander, Pradas et al. 1992). Given that dextromethorphan is a weak NMDA receptor antagonist at the doses used (<150 mg/day), it may be ineffective in achieving complete receptor blockade (Beaton, Stemsrud et al. 1992). Moreover, pharmacokinetic studies have shown that side effects of the drug occur at doses five times higher than those used in the efficacy studies (Hollander, Pradas et al. 1994). Plasma levels of dextromethorphan necessary for significant NMDA receptor blockade were unlikely to be reached in the clinical trials.

Promising results have been obtained with the glutamate-release inhibitor and sodium channel blocker, riluzole. A modest increase in survival was observed following 1 year of treatment which was associated with a significant reduction in the rate of deterioration in motor function (Zoccolella, Beghi et al. 2007). Another trial confirmed the beneficial effects of riluzole on survival but not on motor function (Gurney, Fleck et al. 1998). Accordingly, riluzole has been approved and marketed for the treatment of ALS in various countries (Miller, Mitchell et al. 2007). While this is encouraging to those who argue that blockade of excitotoxicity is an important therapeutic strategy to treat neurodegenerative diseases, it remains to be demonstrated whether riluzole is neuroprotective in ALS.

5 Conclusion

The concept of excitotoxicity is implicated in the pathogenesis of a variety of neurodegenerative diseases. Neurodegenerative diseases may develop as a result of abnormally high concentrations of excitotoxic amino acids, or by a more insidious process whereby energetically weakened neurons become vulnerable to ambient concentrations of glutamate or an exogenous congener. Currently available anti-excitotoxic drugs have played vital roles in examining the factors which facilitate neuronal death in several experimental paradigms. Where suitable animal models are available, such as for AD and PD, iGluR inhibitors have paved the way for defining the etiology and treatment of neurodegenerative diseases in the clinic. The next decade will lead to the development of newer and more specific compounds to block excitatory neurotransmission, and may include NMDA and AMPA/kainate-receptor antagonists. The current century may witness a revolution in the treatment of neurological diseases using anti-excitotoxic drugs.

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References

Addae, J. I., Evans, S. M., et al. (2000). NMDA-induced changes in a cortical network in vivo are prevented by AMPA. *Brain Research, 869*(1–2), 211–215.

Alt, A., Weiss, B., et al. (2004). Pharmacological characterisation of glutamatergic agonists and antagonists at recombinant human homomeric and heteromeric kainate receptors in vitro. *Neuropharmacology, 46*, 793–806.

Araki, T., Kumagai, T., et al. (2000). Protective effect of riluzole on MPTP-induced depletion of dopamine and its metabolite content in mice. *Metabolic Brain Disease, 15*(3), 193–201.

Araque, A., Sanzgiri, R. P., et al. (1998). Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. *The Journal of Neuroscience, 18*(17), 6822–6829.

Armstrong, N., Sun, Y., et al. (1998). Structure of a glutamate-receptor ligand binding core in complex with kainate. *Nature, 395*, 913–917.

Bamberger, M. E., & Landreth, G. E. (2001). Microglial interaction with beta-amyloid: Implications for the pathogenesis of Alzheimer's disease. *Microscopy Research and Technique, 54*(2), 59–70.

Barnum, C., & Tansey, M. (2010). Modeling neuroinflammatory pathogenesis of Parkinson's disease. *Progress in Brain Research, 184*, 113–132.

Beal, M. F. (2003). Mitochondria, oxidative damage, and inflammation in Parkinson's disease. *Annals of the New York Academy of Sciences, 991*, 120–131.

Beal, M. F., Kowall, N. W., et al. (1986). Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature, 321*(6066), 168–171.

Beaton, J. A., Stemsrud, K., et al. (1992). Identification of a novel *N*-methyl-D-aspartate receptor population in the rat medial thalamus. *Journal of Neurochemistry, 59*(2), 754–757.

Bergeron, R., Meyer, T. M., et al. (1998). Modulation of *N*-methyl-D-aspartate receptor function by glycine transport. *Proceedings of the National Academy of Sciences of the United States of America, 95*(26), 15730–15734.

Berliocchi, L., Bano, D., et al. (2005). Ca^{2+} signals and death programmes in neurons. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 360*, 2255–2258.

Birch, P., Grossman, C., et al. (1988). 6,7-Dintro-quinoxaline-2,3-dion and 6-nitro,7-cyano-quinoxaline-2,3-dion antagonise responses to NMDA in the rat spinal cord via an action at the strychine-insensitive glycine receptor. *European Journal of Pharmacology, 156*, 177–180.

Bleakman, D., Gates, M., et al. (2002). Kainate receptor agonists, antagonists and allosteric modulators. *Current Pharmaceutical Design, 8*, 873–885.

Blennow, K., de Leon, M. J., et al. (2006). Alzheimer's disease. *Lancet, 368*(9533), 387–403.

Boillee, S., Vande Velde, C., et al. (2006). ALS: A disease of motor neurons and their nonneuronal neighbors. *Neuron, 52*(1), 39–59.

Boillee, S., Yamanaka, K., et al. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science, 312*(5778), 1389–1392.

Borges, K., & Dingledine, R. (1998). AMPA receptors: Molecular and functional diversity. *Progress in Brain Research, 116*, 153–170.

Bowie, D., & Mayer, M. (1995). Inward rectification of both AMPA and kainate subtype glutamate receptors generated by polyamine-mediated ion channel block. *Neuron, 15*, 453–462.

Braidy, N., Grant, R., et al. (2009). Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons. *Neurotoxicity Research, 16*, 77–86.

Bristow, L., Hutson, P., et al. (1996). Anticonvulsant and behavioural profile of L-701,324, a potent, orally active antagonist at the glycine modulatory site on the *N*-methyl-D-aspartate receptor complex. *The Journal of Pharmacology and Experimental Therapeutics, 279*, 492–501.

Bruyn, R. P., & Stoof, J. C. (1990). The quinolinic acid hypothesis in Huntington's chorea. *Journal of the Neurological Sciences, 95*(1), 29–38.

Budd, S. L., Tenneti, L., et al. (2000). Mitochondrial and extramitochondrial apoptotic signaling pathways in cerebrocortical neurons. *Proceedings of the National Academy of Sciences of the United States of America, 97*(11), 6161–6166.

Butterfield, D. A. (1997). β-Amyloid associated free radical oxidative stress and neurotoxicity: Implications for Alzheimer's disease. *Chemical Research in Toxicology, 10*, 495–506.

Carriedo, S., Yin, H., et al. (1996). Motor neurons are selectively vulnerable to AMPA/kainate receptor-mediated injury in vitro. *The Journal of Neuroscience, 16*, 4069–4079.

Catts, S. V., Ward, P. B., et al. (1997). Molecular biological investigations into the role of the NMDA receptor in the pathophysiology of schizophrenia. *The Australian and New Zealand Journal of Psychiatry, 31*(1), 17–26.

Ceresoli-Borroni, G., Guidetti, P., et al. (1999). Acute and chronic changes in kynurenate formation following an intrastriatal quinolinate injection in rats. *Journal of Neural Transmission, 106* (3–4), 229–242.

Chatterton, J. E., Awobuluyi, M., et al. (2002). Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature, 415*(6873), 793–798.

Chen, Q., Harris, C., et al. (1995). Glutamate-mediated excitotoxic death of cultured striated neurons is mediated by non-NMDA receptors. *Experimental Neurology, 136*, 212–224.

Chen, Y., Brew, B. J., et al. (2011). Characterisation of the kynurenine pathway in NSC-34 cell line: Implications for amyotropic lateral sclerosis. *Journal of Neurochemistry, 118*, 816–825.

Christie, J. M., Jane, D. E., et al. (2000). Native *N*-methyl-D-aspartate receptors containing NR2A and NR2B subunits have pharmacologically distinct competitive antagonist binding sites. *The Journal of Pharmacology and Experimental Therapeutics, 292*(3), 1169–1174.

Clements, J., & Westbrook, G. L. (1991). Activation kinetics reveal the number of glutamate and glycine binding sites on the *N*-methyl-D-aspartate receptor. *Neuron, 7*, 606–613.

Colwell, C. S., Altemus, K. L., et al. (1996). Regulation of *N*-methyl-D-aspartate-induced toxicity in the neostriatum: A role for metabotropic glutamate receptors? *Proceedings of the National Academy of Sciences of the United States of America, 93*(3), 1200–1204.

Comoletti, D., Muzio, V., et al. (2001). Nitric oxide produced by non-motoneuron cells enhances rat embryonic motoneuron sensitivity to excitotoxins: Comparison in mixed neuron/glia or purified cultures. *Journal of the Neurological Sciences, 192*(1–2), 61–69.

Conti, F., DeBiasi, S., et al. (1996). Expression of NR1 and NR2A/B subunits of the NMDA receptor in cortical astrocytes. *Glia, 17*(3), 254–258.

Conti, F., Barbaresi, P., et al. (1999). Neuronal and glial localization of NR1 and NR2A/B subunits of the NMDA receptor in the human cerebral cortex. *Cerebral Cortex, 9*(2), 110–120.

Contractor, A., Swanson, G., et al. (2000). Identification of the kainate receptor subunits underlying modulation of excitatory synaptic transmission in the CA3 region of the hippocampus. *The Journal of Neuroscience, 20*, 8269–8278.

Dannhardt, G., & Kohl, B. K. (1998). The glycine site on the NMDA receptor: Structure-activity relationships and possible therapeutic applications. *Current Medicinal Chemistry, 5*(4), 253–263.

Danysz, W. (2002). CX-516 cortex pharmaceuticals. *Current Opinion in Investigational Drugs, 3*, 1081–1088.

Das, S., Sasaki, Y., et al. (1998). Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature, 393*, 377–381.

De Carvalho, L. P., Bochet, P., et al. (1996). The endogenous agonist quinolinic acid and the non endogenous homoquinolinic acid discriminate between NMDAR2 receptor subunits. *Neurochemistry International, 28*(4), 445–452.

Dingledine, R., & McBain, C. J. (1994). Excitatory amino acid transmitters. In G. J. Siegel, B. W. Agranoff, & R. W. Albers (Eds.), *Basic neurochemistry: Molecular, cellular and medical aspects* (pp. 367–387). New York: Raven Press.

Dingledine, R., Borges, K., et al. (1999). The glutamate receptor ion channels. *Pharmacological Reviews, 51*(1), 7–61.

Dodd, P. R. (2002). Excited to death: Different ways to lose your neurones. *Biogerontology, 3* (1–2), 51–56.

Doggrell, S. (2003). Is memantine a breakthrough in the treatment of moderate-to-severe Alzheimer's disease? *Expert Opinion on Pharmacotherapy, 4*(10), 1857–1860.

Ellison, D. W., Beal, M. F., et al. (1987). Amino acid neurotransmitter abnormalities in Huntington's disease and the quinolinic acid animal model of Huntington's disease. *Brain, 110*(Pt 6), 1657–1673.

Estevez, A., Stutzmann, J., et al. (1995). Protective effect of riluzole on excitatory amino-acid mediated neurotoxicity in motoneurons-enriched cultures. *European Journal of Pharmacology, 280*, 47–53.

Estrada Sanchez, A., Mejia-Toiber, J., et al. (2008). Excitotoxic neuronal death and the pathogenesis of Huntington's disease. *Archives of Medical Research, 39*, 265–276.

Farlow, M. R. (2004). NMDA receptor antagonists. A new therapeutic approach for Alzheimer's disease. *Geriatrics, 59*(6), 22–27.

Ferrante, R. J., Kowall, N. W., et al. (1993). Excitotoxin lesions in primates as a model for Huntington's disease: Histopathologic and neurochemical characterization. *Experimental Neurology, 119*(1), 46–71.

Ferris, S. H. (2003). Evaluation of memantine for the treatment of Alzheimer's disease. *Expert Opinion on Pharmacotherapy, 4*(12), 2305–2313.

Figueredo-Cardenas, G., Anderson, K. D., et al. (1994). Relative survival of striatal projection neurons and interneurons after intrastriatal injection of quinolinic acid in rats. *Experimental Neurology, 129*(1), 37–56.

Finlayson, K., Witchel, H., et al. (2004). Acquired QT interval prolongation and HERG: Implications for drug discovery and development. *European Journal of Pharmacology, 500*, 129–142.

Foster, A. C., Collins, J. F., et al. (1983). On the excitotoxic properties of quinolinic acid, 2,3-piperidine dicarboxylic acids and structurally related compounds. *Neuropharmacology, 22* (12A), 1331–1342.

Foster, A. C., Gill, R., et al. (1987). Systemic administration of MK-801 prevents *N*-methyl-D-aspartate-induced neuronal degeneration in rat brain. *Neuroscience Letters, 76*(3), 307–311.

Foster, A. C., Gill, R., et al. (1988). Neuroprotective effects of MK-801 in vivo: Selectivity and evidence for delayed degeneration mediated by NMDA receptor activation. *The Journal of Neuroscience, 8*(12), 4745–4754.

Foster, A. C., Kemp, J. A., et al. (1992). Kynurenic acid analogues with improved affinity and selectivity for the glycine site on the *N*-methyl-D-aspartate receptor from rat brain. *Molecular Pharmacology, 41*(5), 914–922.

Gallarda, T., & Loo, H. (2004). Memantine (Ebixa): A new therapeutic strategy for the treatment of moderate to severe forms of Alzheimer's disease. *Encephale, 30*(1), 69–79.

Gallyas, F., Ball, S., et al. (2003). Assembly and cell surface expression of KA-2 subunit-containing kainate receptors. *Journal of Neurochemistry, 86*, 1414–1427.

Gardoni, F., & Di Luca, M. (2006). New targets for pharmacological intervention in the glutamatergic synapse. *European Journal of Pharmacology, 545*(1), 2–10.

Geiger, J., Melcher, T., et al. (1995). Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principals neurons and interneurons in rat CNS. *Neuron, 15*, 193–204.

Guillemin, G. J. (2003). Microglial activation. In ANPP (Ed.), *Functional neuroanatomy. Microglia* (Vol. 7, p. 4.1). Paris: ANPP.

Guillemin, G., Smythe, G., et al. (2004). Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes and neurons. *Glia, 49*, 15–23.

Guillemin, G. J., Brew, B. J., et al. (2005). Indoleamine 2,3 dioxygenase and quinolinic acid immunoreactivity in Alzheimer's disease hippocampus. *Neuropathology and Applied Neurobiology, 31*(4), 395–404.

Guillemin, G. J., Brew, B. J. et al. (2007). Mass spectrometric detection of quinolinic acid in microdissected Alzheimer's disease plaques. *International Congress Series*, K. Takai. *1304*, 404–408.

Gurney, M. E., Fleck, T. J., et al. (1998). Riluzole preserves motor function in a transgenic model of familial amyotrophic lateral sclerosis. *Neurology, 50*(1), 62–66.

Hague, S., Klaffe, S., et al. (2005). Neurodegenerative disorders: Parkinson's disease and Huntington's disease. *Journal of Neurology, Neurosurgery, and Psychiatry, 76*, 1058–1063.

Hartley, D. M., Monyer, H., et al. (1990). 7-Chlorokynurenate blocks NMDA receptor-mediated neurotoxicity in murine cortical culture. *The European Journal of Neuroscience, 2*(4), 291–295.

Hartmann, T. (1999). Intracellular biology of Alzheimer's disease amyloid beta peptide. *European Archives of Psychiatry and Clinical Neuroscience, 249*(6), 291–298.

Hertz, L., Dringen, R., et al. (1999). Astrocytes: Glutamate producers for neurons. *Journal of Neuroscience Research, 57*(4), 417–428.

Hollander, D., Pradas, J., et al. (1992). Long-term high dose dextromethorphan in amyotropic lateral sclerosis. *Annals of Neurology, 32*, 280.

Hollander, D., Pradas, J., et al. (1994). High-dose dextromethorphan in amyotropic lateral sclerosis: Phase I safety and pharmacokinetic studies. *Annual Review of Neuroscience, 36*, 920–924.

Honer, M., Benke, D., et al. (1998). Differentiation of glycine antagonist sites of *N*-methyl-D-aspartate receptor subtypes. Preferential interaction of CGP 61594 with NR1/2B receptors. *The Journal of Biological Chemistry, 273*(18), 11158–11163.

Hsieh, M., Gu, S., et al. (2012). Effects of MK-801 on recognition and neurodegeneration in an MPTP-induced Parkinson's disease rat model. *Behavioural Brain Research, 229*, 41–47.

Hugon, J., & Vallet, J. (1990). Abnormal distribution of phosphorylated neurofilaments in neuronal degeneration induced by kainic acid. *Neuroscience Letters, 119*, 45–48.

Hugon, J., Ludolph, A., et al. (1988). Studies on the aetiology and pathogenesis of motor neurons diseases. II. Clincial and electrophysiologica features of pyramidal dysfunction in macaques fed *Lathyrus sativus* and IDPN. *Neurology, 38*, 435–442.

Hwang, J. Y., Kim, Y. H., et al. (1999). *N*-Methyl-D-aspartate receptor blockade induces neuronal apoptosis in cortical culture. *Experimental Neurology, 159*, 124–130.

Jane, D. E., Olverman, H., et al. (1994). Agonists and competitive antagonists: Structure activity and molecular modelling studies. In G. L. Collingridge & J. Watkins (Eds.), *The NMDA receptor*. New York: Oxford University Press.

Javitt, D. C., & Zukin, S. (1990). The role of excitatory amino acids in neuropsychiatric illness. *The Journal of Neuropsychiatry and Clinical Neurosciences, 2*, 44–52.

Jhamandas, K. H., Boegman, R. J., et al. (2000). Excitotoxicity of quinolinic acid: Modulation by endogenous antagonists. *Neurotoxicity Research, 2*(2–3), 139–155.

Kato, T. (2004). Memantine: A therapeutic drug for Alzheimer's disease and the comparison with MK-801. *Folia Pharmacologica Japan, 124*, 145–151.

Kawamata, J., & Shimohama, S. (2002). Association of novel and established polymorphisms in neuronal nicotinic acetylcholine receptors with sporadic Alzheimer's disease. *Journal of Alzheimer's Disease, 4*(2), 71–76.

Kemp, J. A., & Leeson, P. (1993). The glycine site of the NMDA receptor – Five years on. *Trends in Pharmacological Sciences, 14*, 20–25.

Kemp, J. A., Foster, A. C., et al. (1988). 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the *N*-methyl-D-aspartate receptor. *Proceedings of the National Academy of Sciences of the United States of America, 85*, 6547–6550.

Kerchner, G., Wilding, T., et al. (2002). Kainate receptor subunits underlying presynaptic regulation of transmitter release in the dorsal horn. *The Journal of Neuroscience, 22*, 8010–8017.

Kew, J. N. C., & Kemp, J. A. (2005). Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology, 179*, 4–29.

Kew, J., Koester, A., et al. (2000). Functional consequences of reduction in NMDA receptor glycine affinity in mice carrying targeted point mutations in the glycine binding site. *The Journal of Neuroscience, 20*, 4037–4049.

Kloda, A., Clements, J., et al. (2004). Adenosine triphosphate acts as both a competitive antagonist and a positive allosteric modulator at recombinant *N*-methyl-D-aspartate receptors. *Molecular Pharmacology, 65*, 1386–1396.

Lazzaro, J., Paternain, A., et al. (2002). Functional characterisation of CP-465,022, a selective, non-competitive AMAP receptor antagonist. *Neuropharmacology, 42*, 143–153.

Lee, M., Ting, K., et al. (2011). Characterisation of the expression of NMDA receptors in human astrocytes. *PloS One, 5*(11), e14123.

Lerma, J., Paternain, A., et al. (2001). Molecular physiology of kainate receptors. *Physiological Reviews, 81*, 971–998.

Levine, M., Klapstein, G., et al. (1999). Enhanced sensitivity to *N*-methyl-D-aspartate receptor activation and knockdown mouse models of Huntington's disease. *Journal of Neuroscience Research, 58*, 515–532.

Liu, S., & Cull-Candy, S. (2002). Activity-dependent change in AMPA receptor properties in cerebellar stellate cells. *The Journal of Neuroscience, 22*, 3881–3889.

Liu, Q., Xu, Q., et al. (2004). Astrocyte-mediated activation of neuronal kainate receptors. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 3172–3177.

Loftis, J. M., & Janowsky, A. (2003). The *N*-methyl-D-aspartate receptor subunit NR2B: Localization, functional properties, regulation, and clinical implications. *Pharmacology & Therapeutics, 97*(1), 55–85.

Luqquin, M., Obeso, J., et al. (1993). The AMPA receptor antagonist NBQX does not alter the motor response induced by selective dopamine agonists in MPTP-treated monkeys. *European Journal of Pharmacology, 235*, 297–300.

Matsuda, K., Fletcher, M., et al. (2003). Specific assembly with the NMDA receptor 3B subunit controls surface expression and calcium permeability of NMDA receptors. *The Journal of Neuroscience, 23*(31), 10064–10073.

Mattson, M. P. (2004). Pathway towards and away from Azheimer's disease. *Nature, 430*, 630–639.

Mayer, M., & Armstrong, N. (2004). Structure and function of glutamate receptor ion channels. *Annual Review of Physiology, 66*, 161–181.

McBain, C., & Mayer, M. (1994). *N*-Methyl-D-aspartic acid receptor structure and function. *Physiological Reviews, 74*, 723–760.

Miller, R. G., Mitchell J. D. et al. (2007). Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *The Cochrane Database of Systematic Reviews*, (1), CD001447.

Monyer, H., Sprengel, R., et al. (1992). Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science, 256*(5060), 1217–1221.

Monyer, H., Burnashev, N., et al. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron, 12*(3), 529–540.

Mosley, R., Benner, E., et al. (2006). Neuroinflammation, oxidative stress, and the pathogenesis of Parkinson's disease. *Clinical Neuroscience Research, 6*(5), 261–281.

Nakai, M., Qin, Z., et al. (2000). NMDA and non-NMDA receptor-stimulated IkappaB-alpha degradation: Differential effects of the caspase-3 inhibitor DEVD.CHO, ethanol and free radical scavenger OPC-14117. *Brain Research, 859*(2), 207–216.

Nishi, M., Hinds, H., et al. (2001). Motoneuron-specific expression of NR3B, a novel NMDA-type glutamate receptor subunit that works in a dominant-negative manner. *The Journal of Neuroscience, 21*(23), RC185.

Olney, J. W. (1978). Neurotoxicity of excitatory amino acids. In E. G. McGeer, J. W. Olney, & P. L. McGeer (Eds.), *Kainic acid as a tool in neurobiology* (pp. 95–112). New York: Raven Press.

Parameshwaran, K., Dhanasekaran, M., et al. (2008). Amyloid beta peptides and glutamatergic synaptic dysregulation. *Experimental Neurology, 210*, 7–13.

Pierson, J., Norris, J., et al. (2004). Molecular profiling of experimental Parkinson's disease: Direct analysis of peptides and proteins on brain tissue sections by MALDI mass spectrometry. *Journal of Proteome Research, 3*(2), 289–295.

Priestley, T., Horne, A. L., et al. (1990). The effect of NMDA receptor glycine site antagonists on hypoxia-induced neurodegeneration of rate cortical cell cultures. *Brain Research, 531*, 183–188.

Priestley, T., Ochu, E., et al. (1994). Subtypes of NMDA receptor in neurons cultured from rat brain. *NeuroReport, 5*, 1763–1765.

Priestley, T., Laughton, P., et al. (1995). Pharmacological properties of recombinant human *N*-methyl-D-aspartate receptors comprising NR1a/NR2A and NR1a/NR2B subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Molecular Pharmacology, 48*(5), 841–848.

Rae, C., Moussa, C. E., et al. (2006). A metabolomic approach to ionotropic glutamate receptor subtype function: A nuclear magnetic resonance in vitro investigation. *Journal of Cerebral Blood Flow & Metabolism, 8*, 1005–1017.

Rahman, A., Ting, K., et al. (2009). The excitotoxin quinolinic acid induces tau phosphorylation in human neurons. *PloS One, 4*(7), e6344.

Roche, K. W., Standley, S., et al. (2001). Molecular determinants of NMDA receptor internalisation. *Nature Neuroscience, 4*, 794–802.

Rogawski, M. A., Gryder, D., et al. (2003). GluR5 kainate receptors, seizures, and the amygdala. *Annals of the New York Academy of Sciences, 985*, 150–162.

Rosenmund, C., Stern-Bach, Y., et al. (1998). The tetrameric structure of a glutamate receptor channel. *Science, 280*, 1596–1599.

Rothstein, J. D. (1995). Excitotoxicity and neurodegeneration in amyotrophic lateral sclerosis. *Clinical Neuroscience, 3*(6), 348–359.

Seeburg, P. H., & Hartner, J. (2003). Regulation of ion channel/neurotransmitter receptor function by RNA editing. *Current Opinion in Neurobiology, 13*, 279–283.

Seeburg, P. H., Burnashev, N., et al. (1995). The NMDA receptor channel: Molecular design of a coincidence detector. *Recent Progress in Hormone Research, 50*, 19–34.

Solyom, S., & Tarnawa, I. (2002). Non-competitive AMPA antagonists of 2,3-benzodiazepine type. *Current Pharmaceutical Design, 8*, 913–939.

Stensbol, T., Madsen, U., et al. (2002). The AMPA receptor binding site: Focus on agonists and competitive antagonists. *Current Pharmaceutical Design, 8*, 857–872.

Stewart, G. R., Olney, J. W., et al. (1991). Excitotoxicity in the embryonic chick spinal cord. *Annals of Neurology, 30*, 758–766.

Stone, T. W. (2001). Endogenous neurotoxins from tryptophan. *Toxicon, 39*(1), 61–73.

Sun, Y., Savanenin, A., et al. (2001). Polyglutamine-expanded huntingtin promotes sensitisation of *N*-methyl-D-aspartate receptors via post-synaptic density 95. *The Journal of Biological Chemistry, 276*, 24713–24718.

Tolle, T., Berthele, A., et al. (1993). The differential expression of NMDA and non-NMDA receptor subunits in the rat spinal cord and periaqueductal gray. *The Journal of Neuroscience, 13*, 5009–5028.

Turski, L., Huth, A., et al. (1998). ZK200775: A phosphonate quinoxalinedione AMPA antagonist for neuroprotection in stroke and trauma. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 10960–10965.

Williams, T., Day, N., et al. (1997). Calcium permeable AMPA receptors: A molecular basis for selective vulnerability in motor neurone disease. *Annals of Neurology, 42*, 200–207.

Wong, P., Cai, H., et al. (2002). Genetically engineered mouse models of neurodegenerative diseases. *Nature Neuroscience, 5*, 663–669.

Yu, W., Sun, Y., et al. (2011). The PINK1/Parkin pathway regulates mitochondrial dynamics and function in mammalian hippocampal and dopaminergic neurons. *Human Molecular Genetics, 20*(16), 3227–3240.

Zhang, H., Li, Q., et al. (2008). Full length mutant huntingtin is required for altered Ca^{2+} signaling and apoptosis of striatal neurons in the YAC mouse model of Huntington's disease. *Neurobiology of Disease, 2008*(31), 80–88.

Zoccolella, S., Beghi, E., et al. (2007). Riluzole and amyotrophic lateral sclerosis survival: A population-based study in southern Italy. *European Journal of Neurology, 14*(3), 262–268.

Glutamate Neurotoxicity, Transport and Alternate Splicing of Transporters

Aven Lee, Shannon Beasley, and David V. Pow

Contents

Abstract

Glutamate is the major excitatory neurotransmitter in the central nervous system and its levels in the synaptic cleft are tightly controlled by high affinity glutamate transporters (also known as *E*xcitatory *A*mino *A*cid *T*ransporters or EAATs). The EAAT family is comprised of five members (EAAT1-5), and these transporters are subject to alternative splicing. Alternative splicing of the EAAT genes is a fundamental mechanism that can give rise to multiple distinct

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mRNA transcripts, producing protein isoforms with potentially altered functions. Numerous splice variants of EAATs have been identified in humans, rodents, and other mammalian species. All splice variants of EAATs cloned to date are either exon-skipping and/or intron-retaining types. These modifications may impact upon protein structure, posttranslational modification, function, cellular localization, and trafficking. Message and protein for these splice variants are detectable in the normal brain and, in many instances, have been shown to be induced by pathophysiological insults such as hypoxia. In addition, aberrant expression of EAAT splice variants has been reported in neurodegenerative conditions such as amyotrophic lateral sclerosis, Alzheimer's disease, ischemic stroke, and age-related macular degeneration. These EAAT variants may represent therapeutic targets and thus require an improved understanding of their regulation. This chapter describes recent developments in investigating the molecular heterogeneity, localization, function, structure, and regulation of the EAATs and their splice variants.

Keywords

EAAT • GLAST • GLT1 • EAAC1 • Excitotoxicity • Splicing • Stroke • Schizophrenia • Motor neurone • ALS • Glaucoma

List of Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
AMD	Age-related macular degeneration
CNS	Central nervous system
EAAC1	Excitatory amino acid carrier 1
EAAT	Excitatory amino acid transporter
GABA	Gamma-aminobutyric acid
GLAST	Glutamate aspartate transporter
GLT-1	Glutamate transporter-1
GTRAP	Glutamate transporter associated protein
hnRNP	heterogeneous nuclear ribonucleoproteins
HSP70	70 kDa heat shock proteins
MAP2	Microtubule-associated protein 2
MLO-Y4	Murine long bone osteocyte Y4
mRNA	messenger ribonucleic acid
PDZ	Postsynaptic density (PSD), *Drosophila* disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (ZO-1)
SLC	Solute carrier
SOD	Superoxide dismutase
SR protein	Serine/arginine-rich protein
SRPK	Serine/arginine-rich protein kinase
UTR	Untranslated region

1 Introduction

1.1 Neurotransmission: A Regulated Process of Neurotransmitter Release and Removal

The brain is a highly regulated environment, protected by a blood-brain barrier and other homeostatic mechanisms, to ensure that events such as neurotransmission are able to occur without impediment. Neurotransmission is typically mediated by the evoked fusion of one or more synaptic vesicles containing around 10,000 molecules of neurotransmitter, each of which can potentially activate a receptor molecule. Thus, the timeframe for the neurotransmission signaling event is heavily dependent upon the kinetics of clearance of the neurotransmitter. Accordingly, an understanding of the biological events that influence the kinetics of neurotransmitter removal from the extracellular space is central to our understanding of brain function.

Many neurotransmitters are removed from the extracellular space using high affinity neurotransmitter transporters, and these are frequently pharmacological targets. Thus, the serotonin transporter is a target for selective serotonin reuptake inhibitors (SSRIs) used to treat disorders such as depression. This whole area of pharmacology depends upon the drug interacting with a defined transporter that is expressed in a predictable manner with predictable properties. However, alternately spliced forms of many high affinity neurotransmitter transporters have been identified, including the serotonin transporter (Sakai et al. 2003), dopamine transporter (Talkowski et al. 2010), noradrenalin transporter (Sogawa et al. 2007), betaine-GABA transporter (Takenaka et al. 1995), and glycine transporters (Ponce et al. 1998; Hanley et al. 2000). In the case of the serotonin transporter, there is evidence for differential transport capacity of serotonin by the variant forms, while alternately spliced dopamine transporter may influence relative expression of the functional form of the transporter and thus perturb extracellular levels of dopamine. These types of finding lead to the conclusion that alternate splicing might be a mechanism that differentially regulates clearance of many neurotransmitters under differing physiological and pathophysiological states.

This chapter focuses upon the most abundant excitatory neurotransmitter in the central nervous system (CNS), namely, glutamate because glutamate is a molecule that may be dysregulated in a wide variety of CNS disease states, ranging from Alzheimer's disease, schizophrenia, motor neuron disease, and glaucoma to depression (Cowburn et al. 1988; McCarthy et al. 2012; Moghaddam and Javitt 2011). Therefore, an understanding of the transporters that mediate glutamate removal and the potential ways in which the transporters might be modified by events, such as alternate splicing, are central to our understanding of brain pathophysiology and ultimately to the development of therapeutic paradigms that optimize brain glutamate homeostasis. Extracellular and intracellular levels of

glutamate are regulated principally a family of high affinity sodium-dependent Excitatory Amino Acid Transporters (EAATs), which are members of the Solute Carrier Family-1 (SLC1) (Danbolt 2001). In the mammalian CNS, five major subtypes of EAATs have been identified; they share considerable homology (50–60 %) at the amino acid level, but each member exhibits distinct and differential localizations at the subcellular, cellular, and regional levels. EAAT1 (SLC1A3), the human homologue of the rodent glutamate/aspartate transporter (GLAST), is present in glia throughout the CNS but is particularly abundant in the radial (Bergmann) glia of the cerebellum and the radial (Müller) glia of the retina; EAAT2 (SLC1A2), the human homologue of glutamate transporter 1 (GLT-1), is present mainly in glia, particularly astrocytes, and is widespread throughout the CNS; EAAT3 (SLC1A1), the human homologue of Excitatory Amino Acid Carrier 1 (EAAC1), is localized to subsets of neurons throughout the CNS; EAAT4 (SLC1A6) is primarily localized to cerebellar Purkinje cells but with some expression in astrocytes (Hu et al. 2003) and EAAT5 (SLC1A7) is abundant in the retina, particularly in photoreceptors and populations of bipolar cells (Reviewed by Beart and O'Shea 2007).

One mechanism that is likely to influence EAAT function or dysfunction is alternative splicing. Alternative splicing of RNA is a common mechanism that allows a single gene to produce multiple different protein products (Lopez 1998). This versatile mechanism can also regulate parameters such as the amount of mRNA expressed and the stability of such mRNA. Changes in encoded protein sequence can affect nearly all aspects of protein function, including protein stability, binding properties, posttranslational modifications, cellular localization, and trafficking (Stamm et al. 2005). In recent years, many splice variants of EAATs have been described in humans, rodents, and other mammalian species. Message and protein for these splice variants are detectable in the normal brain and in some instances, their expression can be induced by pathophysiological insults. This chapter reviews recent progress in investigating the molecular heterogeneity, localization, function, structure, and regulation of the EAATs and their splice variants.

2 Splicing of Glutamate Transporters

2.1 Mechanism of Splicing

Alternative splicing of EAATs arises from differential removal of exons from the pre-mRNA that was transcribed from the DNA, or in some instances the retention of introns from the pre-mRNA, to yield many distinct mRNA and protein isoforms. Exon skipping is a common mode of splicing in mammalian brain, generating, for instance, the four known forms of GLAST (EAAT1). By contrast, GLT-1 (EAAT2) is spliced in a combinatorial manner (exon skipping and/or intron retention) to produce many distinct protein isoforms (for review, see Lee and Pow 2010).

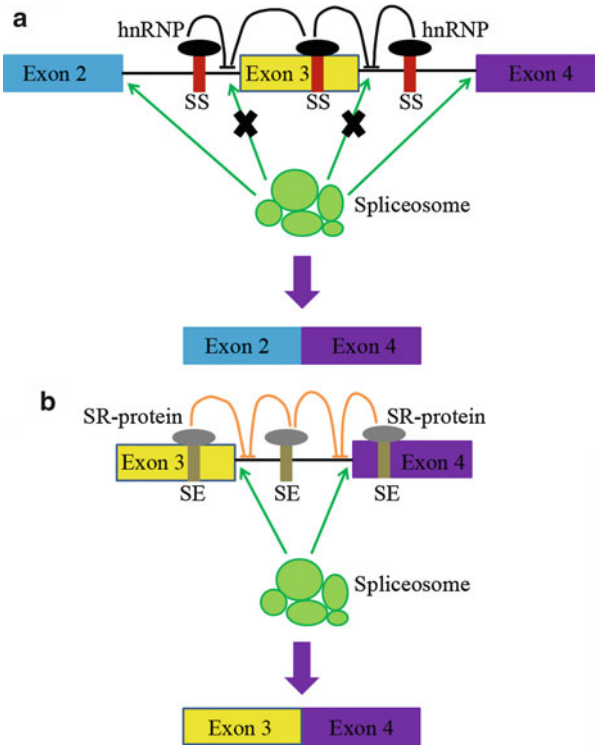

Fig. 1 (**a**) Model of splicing repression: hnRNPs bind to splicing silencers (*SS*) located in exons and introns of the pre-mRNA; this inhibits component of the spliceosome from binding to consensus splice site sequences at the ends of introns, resulting in skipping of the exon (*yellow*). (**b**) Model of splicing activation: SR proteins bind to splicing enhancers (*SE*) located in exons and introns; this facilitates binding of spliceosome components to consensus splice site sequences in the pre-mRNA that ultimately lead to excision of the targeted intron and ligation of flanking exons (Adapted from Wang and Burge 2008; Black 2003)

It is notable that alternate splice variants for EAAT3 and EAAT4 have not so far been described in the mammalian brain, though single nucleotide polymorphisms are undoubtedly present. However, given the limited attention that has been paid to these transporters to date, it is plausible that splice variants may exist but have not yet been detected, and this remains to be resolved. A similar situation has recently been resolved for EAAT5, which had until recently been described in mammals as a single form of the protein. Lee et al. (2012a) have recently demonstrated that it also exists as multiple splice variants, and that all splice variants cloned to date are either exon-skipping or intron-retaining types.

The regulation of EAAT splicing involves consensus splice site sequences in the pre-mRNA and cellular factors such as the "spliceosomes" which are dynamic complexes of proteins and RNA that act as ribozymes, to typically excise introns and ligate the flanking exons. In addition to the consensus splice site sequence on the pre-mRNA, auxiliary elements such as splicing enhancers and splicing silencers can strongly influence alternative splicing; such elements are variable in sequence and contain binding sites for serine/arginine-rich (SR)-proteins or heterogeneous nuclear ribonucleoproteins (hnRNPs), the binding of which can activate or repress the adjacent splice sites, respectively (Fig. 1). Therefore, the binding of

SR proteins and hnRNPs to such elements strongly influences the choice of splicing sites and regulate the formation of active spliceosomes in the pre-mRNA. Whether an exon is included in the mature transcript is determined by the concentration or activity of each type of regulators (SR proteins and hnRNPs). Concentration of SR proteins and hnRNPs can vary significantly between different cell types, and it is thought that such differential expression of SR proteins and hnRNPs affects the splicing of many pre-mRNAs (for reviews, see Chen and Manley 2009; Matlin et al. 2005).

The activity of SR proteins is strongly influenced by the phosphorylation state of these proteins. At least four different protein kinase families have been shown to phosphorylate SR proteins. The most specific of these is the SRPK family (e.g., SRPK1 and SRPK2), which are typically localized to the cytoplasm of cells, indicating that regulation of SR proteins is a cytoplasmic event. SR protein kinases are also regulated; SRPK1 binds to co-chaperones including heat shock proteins such as HSP40, in turn influencing interactions with HSP70 and HSP90. Accordingly, stress responses in cells such as astrocytes and neurons, including production of heat shock proteins, may modify pre-mRNA splicing for transporters such as GLT-1 (EAAT2). The activity of hnRNP proteins can also be modified by phosphorylation. For example, hnRNP A1 is directly phosphorylated by PKA, PKC, and Casein Kinase II (reviewed in Lynch 2007). Serine/threonine phosphorylation under hypoxia and stress conditions (van der Houven van Oordt et al. 2000; Daoud et al. 2002; Allemand et al. 2005) was previously shown to cause hyperphosphorylation and accumulation of hnRNPs in the cytosol. This could serve the purpose of removing such regulatory factors from pre-mRNA processing events. Indeed, a number of in vivo studies have clearly shown that splice site selection is altered following hypoxia and application of osmotic shock (van der Houven van Oordt et al. 2000; Daoud et al. 2002). Accordingly, stressors such as hypoxia are likely to affect the nuclear ratio of specific splicing regulators (SR proteins and hnRNPs) in cells such as astrocytes, thereby varying the regulation of alternative splicing of particular EAATs.

2.2 Splicing of 5′-Untranslated Region

Alternative splicing is a mechanism used for generating 5′-heterogeneity of transporters such as GLT-1 (EAAT2). Multiple alternative 5′-untranslated regions (UTR) exist for GLT-1 in human, rat, and mouse, with studies showing that mRNA levels for such variants change following pathological insults. Tian et al. (2007) showed that GLT-1 variants with longer 5′-UTRs (>310 nucleotides) were highly regulated at the translational level by factors such as corticosterone and retinol. In addition, some disease-associated insults, such as elevated glutamate levels and oxidative stress, were shown to affect the translational efficiency of such transcripts in primary astrocyte cultures. These observations suggest that expressions of such GLT-1 variants at the mRNA level are highly regulated under normal and disease state conditions and will thus influence GLT-1 protein expression.

2.3 Splicing of 3′-Untranslated Region

Splicing of the GLT-1 gene can produce transcripts with long 3′-UTR's; both human EAAT2 and mouse GLT-1a are known to contain unusually long 3′-UTRs (>9 Kb), suggesting that they contain potential elements that control mRNA localization, translational efficiency, or stability of mRNA (Kim et al. 2003; Kirschner et al. 1994). The 3′-UTR of human EAAT2 is reported to contain 23AU-rich elements (AUUUA) and 8 putative polyadenylation signals that could potentially be used to generate shorter EAAT2 transcripts (Kim et al. 2003); thus, Munch et al. (2000) have reported human EAAT2 transcripts of varying sizes (1.8, 2.4, 7, and 12 Kb) in human brain. Furthermore, sequences at the extreme end of the EAAT2/GLT-1a 3′-UTR is highly conserved between human, rat, mouse, and macaque, suggesting that this region has a regulatory function in posttranscriptional control (Kim et al. 2003). Computational analyses of the 3′-UTR of GLT-1a have also revealed the presence of putative K boxes, which are binding sites for microRNAs such as miR-218 (Lewis et al. 2005). Interestingly, this binding site is absent in the 3′-UTR of another alternately spliced form of GLT-1 called GLT-1b, suggesting that miR-218 may be a specific regulator of GLT-1a (for a detailed review, see Lauriat and McInnes 2007). Alignment analysis of 3′-UTRs between GLT-1a and GLT-1b revealed that there are sequences unique to each variant. These unique regions may contain potential regulatory elements that control the differential expression of the two GLT-1 variants. The complete sequence of the 3′-UTR of a third form called GLT-1c is unknown, and thus, it is not known if this variant contains any unique 3′-UTR sequences. However, it is interesting to note that expression levels of both GLT-1b and GLT-1c are much lower than that of GLT-1a in brain tissue homogenates, raising the possibility that in brain at least, their expression may be more tightly controlled by trans-acting factors (such as RNA-binding proteins) (Lauriat and McInnes 2007). Conversely, the highly abundant expression of GLT-1b and GLT-1c in the retina albeit in distinct populations of neurons, relative to GLT-1a (Pow et al. 2000; Rauen et al. 2004), suggests both a cell-specific enhancement of GLT-1b and GLT-1c expression and a co-associated suppression of expression of GLT-1a in this tissue.

2.4 Alternative Splicing and Generation of PDZ-Binding Motifs

Three distinct C-terminal regions of GLT-1 are generated from alternative splicing, and these variants are referred to as GLT-1a, GLT-1b, and GLT-1c. GLT-1a is the wild-type form of GLT-1 cloned by Pines et al. (1992) and contains 11 exons. GLT-1b lacks exon 11 and the coding region terminates in the intron after exon 10, generating a novel 11 amino acid sequence at the C-terminus (Chen et al. 2002; Schmitt et al. 2002). Similarly, GLT-1c includes exons 1 to 10 and terminates with a small exon spliced in from partial sequences of intron 10, again generating a novel 11 amino acid sequence at the C-terminus (Rauen et al. 2004).

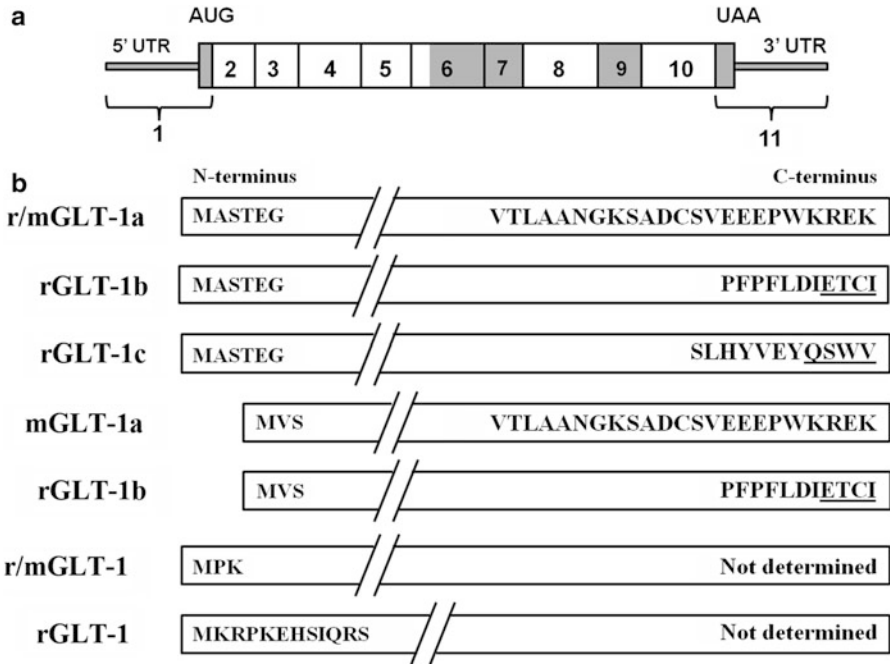

Fig. 2 (**a**) Exon structure of GLT-1 mRNA with exons subject to alternate splicing depicted in gray. Exons are depicted by *rectangles*; *UTR* untranslated region, *AUG* start codon, *UAA* stop codon. (**b**) Schematic illustration depicting "normally" spliced GLT-1 (referred to as GLT-1a), and known alternate N-terminal and C-terminal amino acid sequences, with PDZ motifs underlined. "m" prefix denotes sequences identified in mouse; "r" indicates sequences from rat

The last four amino acids in the C-termini of both GLT-1b (ETCI) and GLT-1c (QSWV) conform to the consensus sequence of a class I PDZ-binding motif (X-S/T-X-Φ_{COOH}), where X is any residue and Φ is a hydrophobic residue (Ivarsson 2012) (Fig. 2). These motifs are similar to the C-terminal PDZ motif (ETKM) present on GLAST that bind to the NHERF1 PDZ protein, which in turn interacts with ERM proteins and the astrocyte cytoskeletal protein GFAP (Lee et al. 2007; Sullivan et al. 2007b). Disruptions of these interactions were shown to perturb glutamate transport (Sullivan et al. 2007b). The PDZ proteins are particularly interesting, because they play key roles in anchoring transmembrane proteins to the cytoskeleton and hold together signaling complexes (Reviewed by Romero et al. 2011). Accordingly, the GLAST-NHERF1 interactions may limit the targeting of this transporter to central "core" zone of astrocytes where GFAP predominates (Sullivan et al. 2007b).

GLT-1b does not interact with NHERF1 (Lee et al. 2007) and potential PDZ-binding partners that associate with this transporter variant in astrocytes are still unclear. Bassan et al. (2008) have shown that GLT-1b can interact with the PDZ protein PICK1; however, very little PICK1 is expressed by astrocytes.

Similarly, Gonzalez-Gonzalez et al. (2008) demonstrated GLT-1b interactions with PSD95; however, this PDZ protein is mainly expressed in neurons, and anatomical co-localization of GLT-1b and PSD95 has not been demonstrated to indicate a functional relationship between the two proteins in brain.

No PDZ-binding partner has been identified for GLT-1c, which is abundantly expressed in photoreceptors of the retina. GLT-1c is also expressed in the brain at low levels, being primarily restricted to end-feet of astrocytes (Rauen et al. 2004). The differential expression of GLT-1c relative to GLT-1b may be indicative of interactions with different PDZ proteins and participation in different signaling pathways.

PDZ-binding motifs are also present on other EAATs, including EAAT3, EAAT4, and EAAT5. Neuronal EAAT4 was previously shown to interact with GTRAP48 (glutamate transporter-4 associated protein), a PDZ-domain-containing protein expressed predominantly in the cerebellum (Jackson et al. 2001). GTRAP48 specifically interacts with the C-terminal PDZ motif (ESAM) of EAAT4 and positively modulates its glutamate transport activity by enhancing the cell surface expression of EAAT4 (Jackson et al. 2001). Similarly, the cell surface density of EAAT3 is regulated via interaction of its C-terminal PDZ motif (TSQF) with the PDZK1 adaptor protein (D'Amico et al. 2010). The retinal EAAT5 has also been reported to contain a PDZ-binding motif (ETNV) at its C-terminus, based on findings from yeast two-hybrid screening, making it likely that the EAAT5 C-terminus may be involved in EAAT5-PDZ interactions (Arriza et al. 1997). The inclusion of PDZ-binding motifs in many of the EAATs suggests that interactions with PDZ-domain-containing proteins are common and probably important for creating differential targeting with consequential functional diversity. It is therefore plausible that splice variants differing in their C-terminal PDZ motif sequences may play distinct roles in regulation of neurotransmission or development.

2.5 Alternative Splicing and Generation of Exon-Skipping EAAT Variants

Several exon-skipping GLAST (EAAT1) variants are generated from alternative splicing. Huggett et al (2000) identified a splice variant of GLAST, lacking exon 3 (GLAST-1a), which is expressed in brain and bone. MLO-Y4 cells transfected with GFP-tagged GLAST and GLAST-1a appear to target the proteins to different cellular compartments; GLAST was targeted to the plasma membrane whereas GLAST-1a appeared to be expressed within internal vesicles (Huggett et al. 2002). When an antibody was later developed against GLAST-1a, expression of this protein was readily demonstrable in populations of astrocytes throughout the brain and retina (Macnab et al. 2006). There are notable differences between the distribution of GLAST-1a and wild-type GLAST in retina; GLAST-1a is preferentially localized to the outer end-feet of Muller cells, whereas GLAST is distributed throughout the cells, suggesting targeting and compartmentalization to different

parts of the cells. GLAST-1b is an exon 9–skipping form of GLAST and based on preliminary studies in transfected HEK293 cells was suggested to be a nonfunctional glutamate transporter which exerts a dominant negative effect on wild-type GLAST function (Vallejo-Illarramendi et al. 2005). Expression of GLAST-1b is highly upregulated at the protein level (albeit mainly in neurons), following insults such as hypoxia (Sullivan et al. 2007a). GLAST-1c is a splice variant of GLAST, lacking both exon 5 and exon 6 that was cloned from neonatal hypoxic pig brain (Lee et al. 2012b). GLAST-1c protein was shown to be abundantly expressed in astrocytes and oligodendrocytes in rat brain, pig brain, and human brain, including gray and white matter. Similarly, expression of GLAST-1c was observed in primary astrocyte cultures and in cultured oligodendrocytes. In astrocytes, GLAST-1c rapidly redistributed to the cell surface upon stimulation of protein kinase with phorbol esters, suggesting that phosphorylation plays a key role in the targeting of this protein.

Numerous exon-skipping variants of GLT-1 have been identified in human and rodent brains. These include a GLT-1 splice variant, missing exon 7, and a form, missing both exon 7 and exon 9 (Scott et al. 2011). Meyer et al. (1998, 1999) have discovered a form of GLT-1 missing exon 9 as well as more complex splice variants such as a form missing parts of exon 6 and exon 7 and a form that skips between part of exon 7 and part of exon 11. Little information is available on the expression of these proteins. Macnab and Pow (2007a) have generated antibodies against the splice site of the exon 9–skipping form of GLT-1 and demonstrated its expression in populations of white matter astrocytes. More recently, Lee et al. (2011) cloned an exon 4–skipping form of GLT-1 from the rodent brain, and an antibody that selectively recognized this variant revealed strong signals in gray matter astrocytes. Interestingly, the 5′-UTR of the exon 4–skipping GLT-1 is 306-nucleotides longer than that of regular GLT-1 (Pines et al. 1992). This is of interest because Tian et al. (2007) have shown that some GLT-1 transcripts bearing longer 5′-UTR are translationally regulated by factors such as corticosteroids and retinol as well as by several disease-associated insults in primary astrocyte cultures and in mice. Therefore, the possibility exists that translational regulation may play an important role in expression of exon 4–skipping GLT-1 under normal and disease conditions.

EAAT5 also exists as alternatively spliced forms in the retina. By PCR cloning and sequencing, Lee et al. (2012a) recently demonstrated the presence of five novel splice variant forms of EAAT5 which skip either partial or complete exons in the rat retina: Three of the splice variants skipped full exons, resulting in forms of EAAT5 that skipped exon 3, exon 9, or exon 10; another variant skipped a large portion of exon 7, while a fifth variant skipped part of exon 8 and all of exon 9 (Fig. 3). Each of the EAAT5 variants was shown to be expressed at the protein level by Western blotting using splice site–specific antibodies (Lee et al. 2012a). EAAT5 is particularly interesting since this protein exhibits a relatively large chloride conductance, associated with modest transport activity, suggesting a function more closely related to ligand-gated chloride channels than classic transporters. This has led to the finding that wild-type EAAT5 functions as an “inhibitory” presynaptic glutamate receptor in retinal bipolar cells (Wersinger et al. 2006).

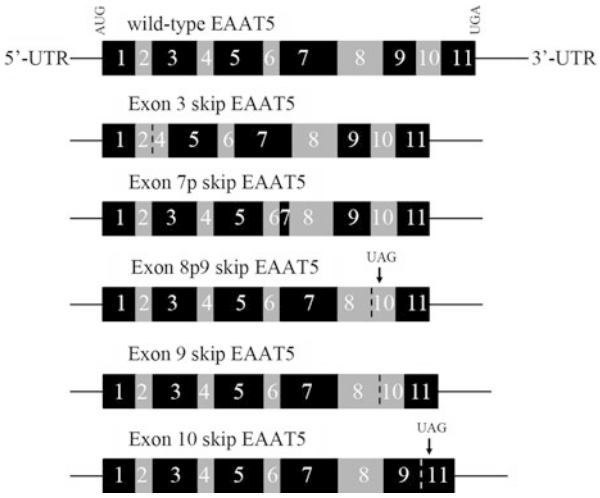

Fig. 3 Exon structure of wild-type EAAT5 mRNA and alternatively spliced forms of EAAT5. Exons are depicted by *rectangles*; *UTR* untranslated region, *AUG* START codon, *UGA* STOP codon, *arrow* indicates premature STOP codon (*UAG*) in the Exon8p9skip and Exon10skip variants of EAAT5

Currently, it is unknown whether the EAAT5 splice variants could function as transporters or receptors. These variants appear to be missing key structural features that may be important for chloride conductance or glutamate binding/transport. Thus, the functional characterization of such variants may require analysis beyond the determination of whether they conduct chloride or translocate glutamate.

2.6 Splicing of N- and C-Termini

At least, three 5′-variants of GLT-1 have been identified in brain which contain varying lengths of upstream open-reading frames encoding GLT-1 proteins with distinct N-terminal sequences: MASTEGANN, MPK, or MKRPKEHSIQRS (Rozyczka and Engele 2005; Münch et al. 2002; Peacey et al. 2009). A different 5′-variant of GLT-1 has also been identified in liver which contain the N-terminal sequence MVS (Utsunomiya-Tate et al. 1997); differences in the 5′-ends of the GLT-1 transcripts in brain and liver suggest the presence of tissue-specific promoters. In addition, at the 3′ end of the GLT-1 gene, there is extensive differential exon usage that can result in the generation of at least three distinct C-termini sequences (see Fig. 2b). Thus, at least 12 variant forms of GLT-1 protein potentially exist, depending on the splicing at the 5′ and 3′-ends of the GLT-1-coding sequence. Uptake studies in cultured cells and *Xenopus* oocytes have shown that functional and pharmacological properties of GLT-1 variants are not changed by alterations in their N- and C-termini sequences (Utsunomiya-Tate et al. 1997; Peacey et al. 2009). Therefore, N- and C-terminal splicing may be more important for differential expression or targeting of the transporters to particular cell types than in regulating the properties of glutamate uptake.

3 Are the EAAT Variants Generated by Alternative Splicing Functional?

While N- and C-terminal splicing can produce functional EAAT variants such as GLT-1b and GLT-1c, very little information is available on the functional properties of exon-skipping variants such as GLAST-1a, GLAST-1b, and GLAST-1c. The exon 9–skipping GLAST-1b has received little attention because when overexpressed in HEK293 cells, it was localized to the endoplasmic reticulum (ER) and thus is unable to act as a plasmalemmal glutamate transporter. When co-expressed with wild-type GLAST, it exerted a dominant negative effect over GLAST function and reduced GLAST insertion into the plasma membrane, suggesting that GLAST-1b could potentially act as an interfering subunit of the GLAST homo-oligomeric structure (Vallejo-Illarramendi et al. 2005). However, when a specific antibody was later developed to GLAST-1b (Macnab and Pow 2007b), it was shown that rather than being restricted to the ER, GLAST-1b was readily demonstrable in the plasma membranes of a very small subset of neurons. Not only was GLAST-1b targeted to the plasma membrane, which would be essential for its role as a transporter but it was also shown that the expression levels rise dramatically in neurons after a hypoxic insult, indicating that expression is physiologically regulated (Sullivan et al. 2007a). Similar issues have arisen for GLAST-1a, which is an exon 3–skipping variant of GLAST. Interestingly, some algorithms have predicted that the loss of exon 3 would produce a reversal of the membrane topology of GLAST-1a (Huggett et al. 2000), where the orientation of the glutamate-binding/-translocating region and the direction of glutamate transport may also be reversed (Fig. 4). This does not however imply a loss of function but rather may indicate a different function for GLAST-1a, such as acting as a glutamate "efflux" protein. Patch clamping experiments in brain slices have clearly demonstrated that glutamate transporters can operate in reverse, transporting glutamate from inside to outside of the cell under conditions such as anoxia (Billups and Attwell 1996; Rossi et al. 2000). Furthermore, alternative splicing of the bile acid transporter (ASBT) gene is known to generate an exon 2–skipping variant that functions as a bile acid efflux protein and localized to basolateral domains of cholangiocytes. This is in contrast to wild-type ASBT which functions to take up bile acids across the apical membranes of cholangiocytes (Lazaridis et al. 2000). Thus, alternative splicing can alter the cellular targeting and/or functional properties of transporters.

In the case of GLAST-1c, which skips exons 5 and 6, this variant has significant key features in common with the prototypical bacterial aspartate transporter GLTph (Yernool et al. 2004), which appears to be an ancestral form of glutamate transporter; these include the absence of glycosylation sites, and much of the same extracellular loop that is missing in GLAST-1c is also absent in GLTph. Such similarities between GLAST-1c and a functional prototypic transporter may support the view that GLAST-1c is plausibly a functional glutamate transporter and possibly represents a primitive form of GLAST. Accordingly, the possibility that GLAST-1a, GLAST-1b, and GLAST-1c are involved in either directly transporting glutamate or in regulating the trafficking of wild-type GLAST must remain a possibility at this stage.

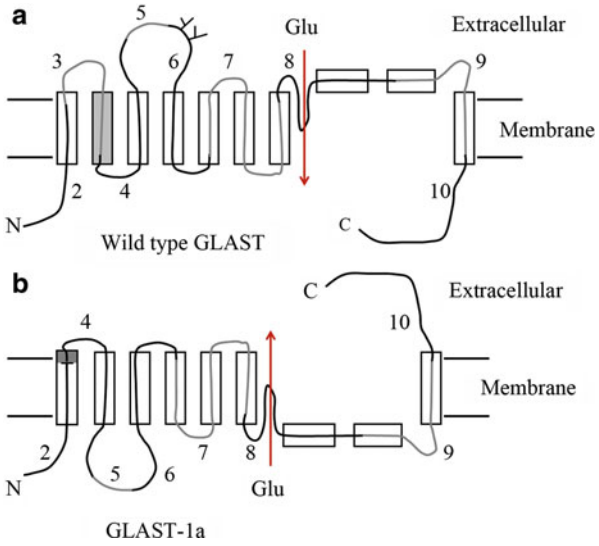

Fig. 4 Predicted transmembrane (*TM*) topologies of wild-type GLAST and GLAST-1a; the extracellular surface and the plasma membrane are indicated. Exon boundaries are overlaid onto the predicted topologies to demonstrate the predicted changes in topology that result from the skipping of exon 3. Deletion of exon 3 in GLAST-1a converts the first two TM domains into one and is predicted to reverse the membrane topology, resulting in an extracellular C-terminal region that may be associated with the reversal of glutamate transport (Adapted from Huggett et al. 2000)

4 Disease Association of EAAT Splice Variants

Much evidence exists for the differential regulation of EAAT2 and EAAT2b in amyotrophic lateral sclerosis (ALS) Meyer et al., 1999. Dramatic reduction in EAAT2 protein has been observed in motor cortex of ALS patients without alterations in EAAT2 (GLT-1) mRNA levels, suggesting posttranscriptional regulation may be involved (Bristol and Rothstein1996; Rothstein et al. 1995; Fray et al. 1998). This is in contrast to expression of EAAT2b (GLT-1b) protein, which is increased in motor cortex of ALS patients (Maragakis et al. 2004). A number of animal models have been used to study the potential role(s) of EAAT expression in ALS. Studies in mice where the animals are fed with cycads reveal behavior and histological features resembling ALS-PDS. In these animals, there is a significant downregulation of GLT-1a protein in spinal cord and primary somatosensory cortex; GLT-1b protein levels were significantly decreased in the spinal cord, in the motor, somatosensory, and piriform cortices, and in the striatum. Notably, GLT-1b expression was decreased in areas where GLT-1a expression was unaltered, suggesting differential regulation of the two transporter subtypes (Wilson et al. 2003). Similarly, Munch et al. (2002) have also shown that the N-terminal splicing of GLT-1a was altered in the spinal cord of SOD transgenic mice, suggesting a link between altered splicing of the 5′-end sequences of GLT-1

gene and ALS. Whether changes in expression or splicing of GLT-1 are aspects of early causation, or a consequence of ALS, remains open to interpretation. However, Guo et al. (2003) produced a double transgenic SOD mutant mice overexpressing GLT-1a which showed significant delays in grip-strength decline and a delay in the loss of motor neurons (Guo et al. 2003). However, these transgenic animals did not show decline in the onset of paralysis nor did they live longer. These results suggest that loss of GLT-1/EAAT2 may contribute to, but do not initially cause, motor neuron degeneration in ALS (Guo et al. 2003).

Aberrant expression of EAAT splice variants has been observed in Alzheimer's disease (AD). Scott et al. (2011) showed increased mRNA expression of an exon 9–skipping form of EAAT2 and protein for this variant has been observed in neurons of AD brain (Pow and Cook 2009). Similarly, protein for the exon 3–skipping form of EAAT1 has been observed in neurons of the AD cortex (Pow and Cook 2009). It is plausible that changes to the splicing of GLT-1 (EAAT2) and GLAST (EAAT1) may explain why there has been a paradoxical observation of an apparently decreased GLAST and GLT-1 expression but increased glutamate reuptake activity in cortex of AβPP23 mice (Schallier et al. 2011), suggesting possibly that modified forms of the transporters were present and functional but not detected with the tools used. Aberrant neuronal expression of EAAT splice variants has also been observed in other pathophysiological states. Thus, Sullivan et al. (2007a) have shown expression of an exon 9–skipping form of GLAST (GLAST-1b) in populations of neurons after a hypoxic insult. GLT-1b, which has an alternatively spliced C-terminal region, was also shown to be aberrantly expressed in neurons after hypoxic insult (Pow et al. 2004). In both scenarios, it has been proposed that an elevation of extracellular glutamate concentration may be driving the expression of the EAAT subtypes. Similar observations have been seen in disease states such as glaucoma and age-related macular degeneration (AMD) as well as in stroke where glutamate excitotoxicity has been implicated. For example, induced expression of a C-terminal splice variant of GLT-1 (GLT-1c) has been demonstrated in retinal ganglion cells in response to elevated intraocular pressure in rats or in human eyes afflicted by glaucoma (Sullivan et al. 2006), and this has been interpreted as being adaptive, to protect the ganglion cells that are vulnerable to glutamate excitotoxicity. Conversely, a complete loss of GLT-1c from photoreceptors in peri-macular regions was observed in AMD patients (Pow et al. 2005) and may explain why these cells lose their content of glutamate that acts both as a neurotransmitter and as an essential metabolic fuel for these cells, which subsequently die. In stroke, induced expression of the exon 3–skipping form of GLAST (GLAST-1a) has been observed in cerebral cortex of ischemic rats induced by middle cerebral artery occlusion (MCAo) (Fig. 5; Pow et al. Unpublished data). Thus, anomalies in local excitation (as a result of ischemic stroke) can induce expression of glial glutamate transporter splice variants such as GLAST-1a in affected neurons, possibly as a protective mechanism. This is in contrast to expression of GLT-1a mRNA and protein, which was reported to be decreased in cerebral cortex of ischemic rats induced by MCAo (Rao et al. 2001a). Interestingly, decreasing GLT-1a expression by antisense knockdown was shown to

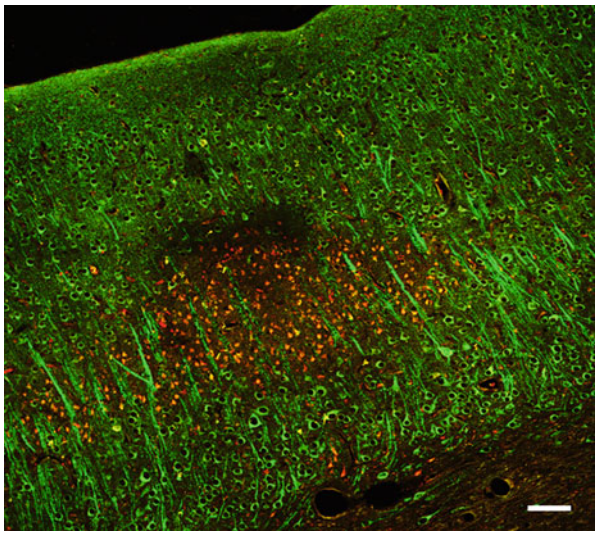

Fig. 5 Image of rat cortex after a stroke induced by transient middle cerebral artery occlusion, immunolabeled for MAP2 (*green*) which labels cell bodies and dendrites of healthy neurons. The large central area where the neurons have lost MAP2 labeling represents the incipient lesion area; GLAST-1a labeling (*red*) is upregulated in this area. Scale bar, 50 μm

exacerbate neuronal damage induced by MCAo (Rao et al. 2001b) while targeted overexpression of GLT-1a reduced ischemic brain injury caused by MCAo (Harvey et al. 2011), suggesting that increasing the capacity to remove extracellular glutamate may provide beneficial outcomes against ischemia-induced glutamate excitotoxicity.

In Schizophrenia, an ambiguous literature exists concerning the expression level of EAATs with increases and decreases being reported. Thus, Smith et al. (2001) examined postmortem tissues from Schizophrenia subjects by in situ hybridization and showed elevated expression of EAAT2 mRNA in thalamus. Similar studies by Ohnuma et al. (1998, 2000) have reported reduced expression of EAAT2 mRNA in prefrontal cortex and parahippocampal regions of Schizophrenia subjects. Conversely, Bauer et al. (2008) did not detect changes in transcript or protein expression of EAAT2 in postmortem prefrontal cortex tissues of Schizophrenia patients using in situ hybridization and Western blotting. One possible limitation of these studies is the putative effects of psychotropic medications on EAAT expression. Several studies have addressed this issue by examining expression of EAAT2 (GLT-1) in animals treated with such drugs. Melone et al. (2001) have shown that short-term (3 weeks) administration of oral clozapine (25–35 mg/kg per day) to rats reduced expression of GLT-1a in cerebral cortex, resulting in decreased glutamate transport and increased extracellular glutamate concentration. Suppression of GLT-1a expression was also observed in rat brain after long-term (6 months) chronic antipsychotic treatment with clozapine (45 mg/kg per day) (Schmitt et al. 2003). The current literature, therefore, suggests that GLT-1a expression is regulated by exposure to antipsychotic drugs. There is no data at present as to whether changes in GLT-1a expression are due to changes in splicing or to changes in overall transcription or translation of the mRNA.

5 Conclusion

Alternative splicing of EAATs is a common mechanism that allows for diversification of a single gene into different protein products. For EAAT2, many of the splice variants produced by N- and C-terminal splicing have been shown to be functional, however, with changes in tissue distribution. For other EAAT members such as EAAT1 and EAAT5, "exon skipping" is the most common mode of splicing whereby certain exons have been removed, therefore resulting in shorter protein products. This highly versatile mechanism can regulate the amount of mRNA and changes in protein sequence that is likely to affect many aspects of protein function such as substrate-binding properties, posttranslational modifications, cellular localization, and trafficking. Interestingly, some of the "exon skipping" EAAT variants cloned to date lack significant portions of the C-terminal regions, suggesting that critical interactions such as binding to the cytoskeleton of the cell would be differentially affected in such variants. Conversely, others lack portions of the N-terminal region and thus may exhibit deficiencies in parameters such as chloride conductances. Where key structural elements of a transporter are missing, it is often dismissed as "junk protein," but biological systems are extremely conservative and unlikely to waste energy making junk in physiological situations. Instead we suggest, by homology to more recent ideas on "junk DNA," that it is plausible that many of these novel proteins may instead have primary functions that are distinct from their initially described roles as glutamate transporters or receptors. In some cases, the splice variants have been shown to possess a dominant negative effect on the co-transporter function, suggesting an important role in the functional regulation of the wild-type transporter. Validating the function of such EAAT variants will require further experimental analysis. From a clinical perspective, however, these EAAT variants are of particular interest, given that their expression and/or regulatory pathways are associated with neurological disorders such as Alzheimer's disease, glaucoma, and macular degeneration as well as hypoxia. Determining whether EAAT changes in any of these disease states are causal or consequential and whether modulation of the activity for a specific EAAT variant might evolve as a therapeutic option are just two examples for the many questions waiting to be answered. Further studies of alternative splicing regulation of EAATs will no doubt reveal new opportunities.

References

Allemand, E., Guil, S., Myers, M., Moscat, J., Ca' ceres, J. F., & Krainer, A. R. (2005). Regulation of heterogenous nuclear ribonucleoprotein A1 transport by phosphorylation in cells stressed by osmotic shock. *Proceedings of the National Academy of Sciences of the United States of America, 102*(10), 3605–3610.

Arriza, J. L., Eliasof, S., Kavanaugh, M. P., & Amara, S. G. (1997). Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 4155–4160.

Bassan, M., Liu, H., Madsen, K. L., Armsen, W., Zhou, J., Desilva, T., Chen, W., Paradise, A., Brasch, M. A., Staudinger, J., Gether, U., Irwin, N., & Rosenberg, P. A. (2008). Interaction between the glutamate transporter GLT1b and the synaptic PDZ domain protein PICK1. *The European Journal of Neuroscience, 27*(1), 66–82.

Bauer, D., Gupta, D., Harotunian, V., Meador-Woodruff, J. H., & McCullumsmith, R. E. (2008). Abnormal expression of glutamate transporter and transporter interacting molecules in prefrontal cortex in elderly patients with schizophrenia. *Schizophrenia Research, 104*(1–3), 108–120.

Beart, P. M., & O'Shea, R. D. (2007). Transporters for L-glutamate: An update on their molecular pharmacology and pathological involvement. *British Journal of Pharmacology, 150*, 5–17.

Billups, B., & Attwell, D. (1996). Modulation of non-vesicular glutamate release by pH. *Nature, 379*(6561), 171–174.

Black, D. L. (2003). Mechanism of alternative pre-mRNA splicing. *Annual Review of Biochemistry, 72*, 291–336.

Bristol, L. A., & Rothstein, J. D. (1996). Glutamate transporter gene expression in amyotrophic lateral sclerosis motor cortex. *Annals of Neurology, 39*(5), 676–679.

Chen, C., & Manley, J. L. (2009). Mechanisms of alternative splicing regulation: Insights from molecular and genomics approaches. *Nature Reviews, 10*, 741–754.

Chen, W., Aoki, C., Mahadomrongkul, V., Gruber, C. E., Wang, G. J., Blitzblau, R., Irwin, N., & Rosenberg, P. A. (2002). Expression of a variant form of the glutamate transporter GLT1 in neuronal cultures and in neurons and astrocytes in the rat brain. *The Journal of Neuroscience, 22*(6), 2142–2152.

Cowburn, R., Hardy, J., Roberts, P., & Briggs, R. (1988). Presynaptic and postsynaptic glutamatergic function in Alzheimer's disease. *Neuroscience Letters, 86*(1), 109–113.

D'Amico, A., Soragna, A., Di Cairano, E., Panzeri, N., Anzai, N., Vellea Sacchi, F., & Perego, C. (2010). The surface density of the glutamate transporter EAAC1 is controlled by interactions with PDZK1 and AP2 adaptor complexes. *Traffic, 11*(11), 1455–1470.

Danbolt, N. C. (2001). Glutamate uptake. *Progress in Neurobiology, 65*, 1–105.

Daoud, R., Mies, G., Smialowska, A., Oláh, L., Hossmann, K. A., & Stamm, S. (2002). Ischemia induces a translocation of the splicing factor tra2-beta 1 and changes alternative splicing patterns in the brain. *The Journal of Neuroscience, 22*(14), 5889–5899.

Fray, A. E., Ince, P. G., Banner, S. J., Milton, I. D., Usher, P. A., Cookson, M. R., & Shaw, P. J. (1998). The expression of the glial glutamate transporter protein EAAT2 in motor neuron disease: An immunohistochemical study. *The European Journal of Neuroscience, 10*(8), 2481–2489.

González-González, I. M., García-Tardón, N., Cubelos, B., Giménez, C., & Zafra, F. (2008). The glutamate transporter GLT1b interacts with the scaffold protein PSD-95. *Journal of Neurochemistry, 105*(5), 1834–1848.

Guo, H., Lai, L., Butchbach, M. E., Stockinger, M. P., Shan, X., Bishop, G. A., & Lin, C. L. (2003). Increased expression of the glial glutamate transporter EAAT2 modulates excitotoxicity and delays the onset but not the outcome of ALS in mice. *Human Molecular Genetics, 12*(19), 2519–2532.

Hanley, J. G., Jones, E. M., & Moss, S. J. (2000). GABA receptor rho1 subunit interacts with a novel splice variant of the glycine transporter, GLYT-1. *The Journal of Biological Chemistry, 275*(2), 840–846.

Harvey, B. H., Airavaara, M., Hinzman, J., Wires, E. M., Chiocco, M. J., Howard, D. B., Shen, H., Gerhardt, G., Hoffer, B. J., & Wang, Y. (2011). Targeted over-expression of glutamate transporter 1 (GLT-1) reduces ischemic brain injury in a rat model of stroke. *PloS ONE, 6*(8), e22135–e22135.

Hu, W. H., Walters, W. M., Xia, X. M., Karmally, S. A., & Bethea, J. R. (2003). Neuronal glutamate transporter EAAT4 is expressed in astrocytes. *Glia, 44*(1), 13–25.

Huggett, J., Vaughan-Thomas, A., & Mason, D. (2000). The open reading frame of the Na(+)-dependent glutamate transporter GLAST-1 is expressed in bone and a splice variant of this molecule is expressed in bone and brain. *FEBS Letters, 485*(1), 13–18.

Huggett, J. F., Mustafa, A., O'Neal, L., & Mason, D. J. (2002). The glutamate transporter GLAST-1 (EAAT-1) is expressed in the plasma membrane of osteocytes and is responsive to extracellular glutamate concentration. *Biochemical Society Transactions, 30*(Pt 6), 890–893.

Ivarsson, Y. (2012). Plasticity of PDZ domains in ligand recognition and signaling. *FEBS Letters, 586*(17), 2638–2647.

Jackson, M., Song, W., Liu, M. Y., Jin, L., Dykes-Hoberg, M., Lin, C. I., Bowers, W. J., Federoff, H. J., Sternweis, P. C., & Rothstein, J. D. (2001). Modulation of the neuronal glutamate transporter EAAT4 by two interacting proteins. *Nature, 410*(6824), 89–93.

Kim, S., Chao, W., Choi, S., & Volsky, D. J. (2003). Cloning and characterization of the 3′-untranslated region of the human excitatory amino acid transporter 2 transcript. *Journal of Neurochemistry, 86*, 1458–1467.

Kirschner, M. A., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., & Amara, S. G. (1994). Mouse excitatory amino acid transporter EAAT2: Isolation, characterization, and proximity to neuroexcitability loci on mouse chromosome 2. *Genomics, 24*, 218–224.

Lauriat, T. L., & McInnes, L. A. (2007). EAAT2 regulation and splicing: Relevance to psychiatric and neurological disorders. *Molecular Psychiatry, 12*(12), 1065–1078.

Lazaridis, K. N., Tietz, P., Wu, T., Kip, S., Dawson, P. A., & LaRusso, N. F. (2000). Alternative splicing of the rat sodium/bile acid transporter changes its cellular localization and transport properties. *Proceedings of the National Academy of Sciences of the United States of America, 97*(20), 11092–11097.

Lee, A., & Pow, D. V. (2010). Astrocytes: Glutamate transport and alternate splicing of transporters. *The International Journal of Biochemistry & Cell Biology, 42*, 1901–1906.

Lee, A., Rayfield, A., Hryciw, D. H., Ma, T. A., Wang, D., Pow, D., Broer, S., Yun, C., & Poronnik, P. (2007). Na+-H+ exchanger regulatory factor 1 is a PDZ scaffold for the astroglial glutamate transporter GLAST. *Glia, 55*(2), 119–129.

Lee, A., Anderson, A. R., Stevens, M. G., & Pow, D. V. (2011). Exon 4-skipping GLT-1: A new form of an abundantly expressed glutamate transporter. *Neuroscience Letters, 504*(3), 228–231.

Lee, A., Anderson, A. R., Barnett, N. L., Stevens, M. G., & Pow, D. V. (2012a). Alternate splicing and expression of the glutamate transporter EAAT5 in the rat retina. *Gene, 506*(2), 283–288.

Lee, A., Anderson, A. R., Beasley, S. J., Barnett, N. L., Poronnik, P., & Pow, D. V. (2012b). A new splice variant of the glutamate-aspartate transporter: Cloning and immunolocalization of GLAST1c in rat, pig and human brains. *Journal of Chemical Neuroanatomy, 43*(1), 52–63.

Lewis, B. P., Burge, C. B., & Bartel, D. P. (2005). Conserved seed pairing, often flanked by adenosine, indicates that thousands of human genes are microRNA targets. *Cell, 120*, 15–20.

Lopez, A. J. (1998). Alternative splicing of pre-mRNA: Developmental consequences and mechanisms of regulation. *Annual Review of Genetics, 32*, 279–305.

Lynch, K. W. (2007). Regulation of alternative splicing by signal transduction pathways. In B. J. Blencowe & B. R. Graveley (Eds.), *Alternative splicing in the postgenomic era* (pp. 161–174). Austin/New York: Landes Bioscience/Springer Bioscience.

Macnab, L. T., & Pow, D. V. (2007a). Expression of the exon 9-skipping form of EAAT2 in astrocytes of rats. *Neuroscience, 150*(3), 705–711.

Macnab, L. T., & Pow, D. V. (2007b). Central nervous system expression of the exon 9 skipping form of the glutamate transporter GLAST. *Neuroreport, 18*(8), 741–745.

Macnab, L. T., Williams, S. M., & Pow, D. V. (2006). Expression of the exon 3 skipping form of GLAST, GLAST1a, in brain and retina. *Neuroreport, 17*(18), 1867–1870.

Maragakis, N. J., Dykes-Hoberg, M., & Rothstein, J. D. (2004). Altered expression of the glutamate transporter EAAT2b in neurological disease. *Annals of Neurology, 55*(4), 469–477.

Matlin, A. R., Clark, F., & Smith, C. W. J. (2005). Understanding alternative splicing: Towards a cellular code. *Nature Reviews, 6*, 386–298.

McCarthy, D. J., Alexander, R., Smith, M. A., Pathak, S., Kanes, S., Lee, C. M., & Sanacora, G. (2012). Glutamate-based depression GBD. *Medical Hypotheses, 78*(5), 675–681.

Melone, M., Vitellaro-Zuccarello, L., Vallejo-Illarramendi, A., Pérez-Samartin, A., Matute, C., Cozzi, A., Pellegrini-Giampietro, D. E., Rothstein, J. D., & Conti, F. (2001). The expression of glutamate transporter GLT-1 in the rat cerebral cortex is down-regulated by the antipsychotic drug clozapine. *Molecular Psychiatry, 6*(4), 380–386.

Meyer, T., Münch, C., Knappenberger, B., Liebau, S., Völkel, H., & Ludolph, A. C. (1998). Alternative splicing of the glutamate transporter EAAT2 (GLT-1). *Neuroscience Letters, 241*(1), 68–70.

Meyer, T., Fromm, A., Münch, C., Schwalenstöcker, B., Fray, A. E., Ince, P. G., Stamm, S., Grön, G., Ludolph, A. C., & Shaw, P. J. (1999). The RNA of the glutamate transporter EAAT2 is variably spliced in amyotrophic lateral sclerosis and normal individuals. *Journal of the Neurological Sciences, 170*(1), 45–50.

Moghaddam, B., & Javitt, D. (2011). From revolution to evolution: The glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology, 37*(1), 4–15.

Munch, C., Schwalenstocker, B., Hermann, C., Cirovic, S., Stamm, S., Ludolph, A. C., & Meyer, T. (2000). Differential RNA cleavage and polyadenylation of the glutamate transporter EAAT2 in the human brain. *Molecular Brain Research, 80*, 244–251.

Münch, C., Ebstein, M., Seefried, U., Zhu, B., Stamm, S., Landwehrmeyer, G. B., Ludolph, A. C., Schwalenstöcker, B., & Meyer, T. (2002). Alternative splicing of the 5′-sequences of the mouse EAAT2 glutamate transporter and expression in a transgenic model for amyotrophic lateral sclerosis. *Journal of Neurochemistry, 82*(3), 594–603.

Ohnuma, T., Augood, S. J., Arai, H., McKenna, P. J., & Emson, P. C. (1998). Expression of the human excitatory amino acid transporter 2 and metabotropic glutamate receptors 3 and 5 in the prefrontal cortex from normal individuals and patients with schizophrenia. *Brain Research. Molecular Brain Research, 56*(1–2), 207–217.

Ohnuma, T., Tessler, S., Arai, H., Faull, R. L., McKenna, P. J., & Emson, P. C. (2000). Gene expression of metabotropic glutamate receptor 5 and excitatory amino acid transporter 2 in the schizophrenic hippocampus. *Brain Research. Molecular Brain Research, 85*(1–2), 24–31.

Peacey, E., Miller, C. C., Dunlop, J., & Rattray, M. (2009). The four major N- and C-terminal splice variants of the excitatory amino acid transporter GLT-1 form cell surface homomeric and heteromeric assemblies. *Molecular Pharmacology, 75*(5), 1062–1073.

Pines, G., Danbolt, N. C., Bjørås, M., Zhang, Y., Bendahan, A., Eide, L., Koepsell, H., Storm-Mathisen, J., Seeberg, E., & Kanner, B. I. (1992). Cloning and expression of a rat brain L-glutamate transporter. *Nature*, 360(6403), 464–467. Erratum in: *Nature* (1992); 360(6406), 768.

Ponce, J., Poyatos, I., Aragón, C., Giménez, C., & Zafra, F. (1998). Characterization of the 5′ region of the rat brain glycine transporter GLYT2 gene: Identification of a novel isoform. *Neuroscience Letters, 242*(1), 25–28.

Pow, D. V., & Cook, D. G. (2009). Neuronal expression of splice variants of "glial" glutamate transporters in brains afflicted by Alzheimer's disease: Unmasking an intrinsic neuronal property. *Neurochemical Research, 34*(10), 1748–1757.

Pow, D. V., Barnett, N. L., & Penfold, P. (2000). Are neuronal transporters relevant in retinal glutamate homeostasis? *Neurochemistry International, 37*(2–3), 191–198.

Pow, D. V., Naidoo, T., Lingwood, B. E., Healy, G. N., Williams, S. M., Sullivan, R. K., O'Driscoll, S., & Colditz, P. B. (2004). Loss of glial glutamate transporters and induction of neuronal expression of GLT-1B in the hypoxic neonatal pig brain. *Brain Research. Developmental Brain Research, 153*(1), 1–11.

Pow, D. V., Sullivan, R. K., Williams, S. M., & WoldeMussie, E. (2005). Metabolic functions of Muller cells in the AMD retina. In P. L. Penfold & J. M. Provis (Eds.), *Macular degeneration* (pp. 141–144). Berlin/Heidelberg: Springer.

Rao, V. L., Bowen, K. K., & Dempsey, R. J. (2001a). Transient focal cerebral ischemia down-regulates glutamate tramsporters GLT-1 and EAAC1 expression in rat brain. *Neurochemical Research, 26*, 497–502.

Rao, V. L., Dogen, A., Bowen, K. K., Todd, K. G., & Dempsey, R. J. (2001b). Antisense knockdown of the glial glutamate transporter GLT-1 exacerbates hippocampal neuronal damage following traumatic injury to rat brain. *The European Journal of Neuroscience, 13*, 119–128.

Rauen, T., Wiessner, M., Sullivan, R., Lee, A., & Pow, D. V. (2004). A new GLT1 splice variant: Cloning and immunolocalization of GLT1c in the mammalian retina and brain. *Neurochemistry International, 45*(7), 1095–1106.

Romero, G., von Zastrow, M., & Friedman, P. A. (2011). Role of PDZ proteins in regulating trafficking, signaling, and function of GPCRs: Means, motif, and opportunity. *Advances in Pharmacology, 62*, 279–314.

Rossi, D. J., Oshima, T., & Attwell, D. (2000). Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature, 403*(6767), 316–321.

Rothstein, J. D., Van Kammen, M., Levey, A. I., Martin, L. J., & Kuncl, R. W. (1995). Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Annals of Neurology, 38*(1), 73–84.

Rozyczka, J., & Engele, J. (2005). Multiple 5′-splice variants of the rat glutamate transporter-1. *Brain Research. Molecular Brain Research, 133*(1), 157–161.

Sakai, K., Hasegawa, C., Okura, M., Morikawa, O., Ueyama, T., Shirai, Y., Sakai, N., & Saito, N. (2003). Novel variants of murine serotonin transporter mRNA and the promoter activity of its upstream site. *Neuroscience Letters, 342*(3), 175–178.

Schallier, A., Smolders, I., Van Dam, D., Loyens, E., De Deyn, P. P., Michotte, A., Michotte, Y., & Massie, A. (2011). Region and age-specific changes in glutamate transport in the AβPP23 mouse model for Alzheimer's disease. *Journal of Alzheimer's Disease, 4*(2), 287–300.

Schmitt, A., Asan, E., Lesch, K. P., & Kugler, P. (2002). A splice variant of glutamate transporter GLT1/EAAT2 expressed in neurons: Cloning and localization in rat nervous system. *Neuroscience, 109*(1), 45–61.

Schmitt, A., Zink, M., Petroianu, G., May, B., Braus, D. F., & Henn, F. A. (2003). Decreased gene expression of glial and neuronal glutamate transporters after chronic antipsychotic treatment in rat brain. *Neuroscience Letters, 347*(2), 81–84.

Scott, H. A., Gebhardt, F. M., Mitrovic, A. D., Vandenberg, R. J., & Dodd, P. R. (2011). Glutamate transporter variants reduce glutamate uptake in Alzheimer's disease. *Neurobiology of Aging, 32*(3), 553e.1–553e.11.

Smith, R. E., Haroutunian, V., Davis, K. L., & Meador-Woodruff, J. H. (2001). Expression of excitatory amino acid transporter transcripts in the thalamus of subjects with schizophrenia. *The American Journal of Psychiatry, 158*(9), 1393–1399.

Sogawa, C., Kumagai, K., Sogawa, N., Morita, K., Dohi, T., & Kitayama, S. (2007). C-terminal region regulates the functional expression of human noradrenaline transporter splice variants. *The Biochemical Journal, 401*(Pt 1), 185–195.

Stamm, S., Ben-Ari, S., Rafalska, I., Tang, Y., Zhang, Z., Toiber, D., Thanaraj, T. A., & Soreq, H. (2005). Function of alternative splicing. *Gene, 344*, 1–20.

Sullivan, R. K., Woldemussie, E., Macnab, L., Ruiz, G., & Pow, D. V. (2006). Evoked expression of the glutamate transporter GLT-1c in retinal ganglion cells in human glaucoma and in a rat model. *Investigative Ophthalmology & Visual Science, 47*(9), 3853–3859.

Sullivan, S. M., Macnab, L. T., Björkman, S. T., Colditz, P. B., & Pow, D. V. (2007a). GLAST1b, the exon-9 skipping form of the glutamate-aspartate transporter EAAT1 is a sensitive marker of neuronal dysfunction in the hypoxic brain. *Neuroscience, 149*(2), 434–445.

Sullivan, S. M., Lee, A., Björkman, S. T., Miller, S. M., Sullivan, R. K., Poronnik, P., Colditz, P. B., & Pow, D. V. (2007b). Cytoskeletal anchoring of GLAST determines susceptibility to brain damage: An identified role for GFAP. *The Journal of Biological Chemistry, 282*(40), 29414–29423.

Takenaka, M., Bagnasco, S. M., Preston, A. S., Uchida, S., Yamauchi, A., Kwon, H. M., & Handler, J. S. (1995). The canine betaine gamma-amino-n-butyric acid transporter gene: Diverse mRNA isoforms are regulated by hypertonicity and are expressed in a tissue-specific manner. *Proceedings of the National Academy of Sciences of the United States of America, 92*(4), 1072–1076.

Talkowski, M. E., McCann, K. L., Chen, M., McClain, L., Bamne, M., Wood, J., Chowdari, K. V., Watson, A., Prasad, K. M., Kirov, G., Georgieva, L., Toncheva, D., Mansour, H., Lewis, D. A., Owen, M., O'Donovan, M., Papasaikas, P., Sullivan, P., Ruderfer, D., Yao, J. K., Leonard, S., Thomas, P., Miyajima, F., Quinn, J., Lopez, A. J., & Nimgaonkar, V. L. (2010). Fine-mapping reveals novel alternative splicing of the dopamine transporter. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 153B*(8), 1434–1447.

Tian, G., Lai, L., Guo, H., Lin, Y., Butchbach, M. E. R., Chang, Y., & Lin, C. G. (2007). Translational control of glial glutamate transporter EAAT2 expression. *The Journal of Biological Chemistry, 282*(3), 1727–1737.

Utsunomiya-Tate, N., Endou, H., & Kanai, Y. (1997). Tissue specific variants of glutamate transporter GLT-1. *FEBS Letters, 416*(3), 312–316.

Vallejo-Illarramendi, A., Domercq, M., & Matute, C. (2005). A novel alternative splicing form of excitatory amino acid transporter 1 is a negative regulator of glutamate uptake. *Journal of Neurochemistry, 95*, 341–348.

van der Houven van Oordt, W., Diaz-Meco, M. T., Lozano, J., Krainer, A. R., Moscat, J., & Cáceres, J. F. (2000). The MKK(3/6)-p38-signaling cascade alters the subcellular distribution of hnRNP A1 and modulates alternative splicing regulation. *The Journal of Cell Biology, 149*(2), 307–316.

Wang, Z., & Burge, C. B. (2008). Splicing regulation: From a parts list of regulatory elements to an integrated splicing code. *RNA, 14*(5), 802–813.

Wersinger, E., Schwab, Y., Sahel, J. A., Rendon, A., Pow, D. V., Picaud, S., & Roux, M. J. (2006). The glutamate transporter EAAT5 works as a presynaptic receptor in mouse rod bipolar cells. *The Journal of Physiology, 577*, 221–234.

Wilson, J. M., Khabazian, I., Pow, D. V., Craig, U. K., & Shaw, C. A. (2003). Decrease in glial glutamate transporter variants and excitatory amino acid receptor down-regulation in a murine model of ALS-PDC. *Neuromolecular Medicine, 3*(2), 105–118.

Yernool, D., Boudker, O., Jin, Y., & Gouaux, E. (2004). Structure of a glutamate transporter homologue from *Pyrococcus horikoshii*. *Nature, 431*(7010), 811–818.

AIDS

M. Kaul

Contents

Abstract

Infection with human immunodeficiency virus (HIV)-1 not only targets and compromises cells of the immune system and eventually causes AIDS but also triggers a variety of neurological problems that can culminate in frank dementia. Several lines of evidence suggest that HIV-1 strikes at the brain in at least two ways: (i) by causing toxicity leading to neuronal injury and death and (ii) by impairing neurogenesis without killing neural precursors. Both of the pathogenic mechanisms share the involvement of neuroinflammatory mediators, and both processes seem to be influenced by host and viral factors, including the interferon response, age and viral subtypes, as well as treatment and comorbidity factors, such as drug abuse. Development of both HIV-associated neurocognitive disorders (HAND) and AIDS goes hand-in-hand, and despite

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viral control and life-prolonging effects of combination antiretroviral treatments (cART), neuroinflammation and concomitant activation of macrophages and microglia accompany the continued development of HAND in HIV patients. Besides intact HIV-1, six of its proteins have been linked to neuronal injury. Experimental evidence supports two different, but not exclusive, hypotheses of how HIV-1 causes neuronal damage in the brain. The "direct injury" hypothesis proposes that viral proteins directly compromise neurons, whereas the "indirect" or "bystander effect" hypothesis states virus infection and viral components trigger in macrophages and microglia and astrocytes the production of neurotoxins that injure neurons. HIV-1 and its six neurotoxic protein components can trigger a plethora of cellular responses and signaling mechanisms. However, it appears that the HIV-1 and its viral components cause neurotoxicity via converging mechanisms even if the virus and each component may start out their pathogenic mechanism by interacting with different host cell factors. Converging mechanisms in HIV neurotoxicity include neuroinflammation, MAPK signaling, altered gene expression, oxidative stress, interference with the cell cycle machinery and autophagy, excitotoxicity, synaptic injury, and, ultimately, neuronal apoptosis.

Keywords

HIV-1 • Infection • Inflammation • Brain • Cognition • Dementia • Neurotoxicity • Excitotoxicity • Neurogenesis • Neurons • Astrocytes • Macrophages • Microglia • Host-pathogen Interaction • Immune Response • Co-morbidity • Drug Abuse

List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
ANI	Asymptomatic neurocognitive impairment
ANT	Adenine nucleotide translocator
ApoE3	Apolipoprotein E3
BBB	Blood–brain barrier
cART	Combination antiretroviral therapy
CEBP	CCAAT-enhancer-binding protein
CNS	Central nervous system
COUP-TF	Chicken ovalbumin upstream promoter transcription factor
CSF	Cerebrospinal fluid
Dox	Doxycycline
EAAs	Excitatory amino acids
Env	Envelope protein
ER	Endoplasmic reticulum
ERK-1/ERK-2	Extracellularly regulated kinase-1 and kinase-2
GABA	γ-aminobutyric acid

GSK	Glucagon synthase kinase
HAART	Highly active antiretroviral therapy
HAD	HIV-associated dementia
HAND	HIV-associated neurocognitive disorders
HIV-1	Human immunodeficiency virus-1
HIVE	HIV encephalitis
HNE	Hydroxynonenal
IFN	Interferon
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IRIS	Immune reconstitution inflammatory syndrome
LPS	Lipopolysaccharide
LRP	Low-density lipoprotein receptor-related protein
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
MCMD	Minor cognitive motor disorder
MCP	Monocyte chemoattractant protein
mGluRs	Metabotropic glutamate receptors
miR	MicroRNAs
MLK	Mixed lineage kinase
MMPs	Metalloproteinases
MND	Mild neurocognitive disorder
Nef	Negative factor
NMDAR	*N*-methyl-*D*-aspartate-type glutamate receptor
Pol	Polymerase
PTPC	Permeability transition pore complex
Rb	Retinoblastoma protein
Rev	Regulatory for expression of viral proteins
RyR	Ryanodine receptors
SCID	Severe combined immunodeficiency
SIV	Simian immunodeficiency virus
Tat	Transactivator of transcription
tg	Transgenic
TLR	Toll-like receptors
TNF	Tumor necrosis factor
TNFR	TNFα receptor
TRAIL	TNF-related apoptosis-inducing ligand
TUNEL	Terminal-deoxynucleotidyl-transferase-mediated dUTP nick-end labeling
UPR	Unfolded protein response
Vif	Viral infectivity factor
Vpr	Viral protein r
Vpu	Viral protein u

1 Introduction

Human immunodeficiency virus-1 (HIV-1) is a retrovirus belonging to the subgroup of lentiviruses (Barre-Sinoussi et al. 1983; Alizon et al. 1984; Hahn et al. 1984; Wain-Hobson et al. 1985). HIV was discovered in 1983 using lymphoid tissue from one of a quickly increasing number of patients presenting with lymphadenopathy followed by an acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi et al. 1983; Hahn et al. 1984; Barre-Sinoussi 2003). In 2011, an estimated 34.2 million people were living with HIV infection or AIDS, and despite improvements in therapy and access to treatments, the epidemic caused 2.5 million deaths (UNAIDS; www.unaids.org).

Although HIV-1 primarily targets and compromises cells of the immune system and eventually causes AIDS (Stevenson 2003), it was observed early on in the epidemic that the virus can also cause a variety of neurological problems that can culminate in frank dementia (Loewenstein and Sharfstein 1983; Jordan et al. 1985; Petito et al. 1985). While AIDS-related opportunistic infections obviously affected the central nervous system (CNS), in particular in the absence of treatment, HIV-1 infection itself seemed also able to initiate a number of neurological syndromes (Jordan et al. 1985; Petito et al. 1985). Today it is widely accepted that HIV-1 can directly trigger neurological conditions, including peripheral neuropathies, vacuolar myelopathy, and a clinical syndrome of cognitive and motor dysfunction that has early on been designated AIDS dementia and later on HIV-associated dementia (HAD) (Navia et al. 1986a, b; Petito et al. 1986). A milder and, in the presence of antiviral treatment, more common form of HAD is termed minor cognitive motor disorder (MCMD) (Ellis et al. 1997; Kaul et al. 2001; Gendelman et al. 2005; Power et al. 2009). In recognition of a changing clinical presentation, neurocognitive complications of HIV-1 infection have more recently been categorized as HIV-associated neurocognitive disorders (HAND) (Antinori et al. 2007). HAND defines three categories of disorders with increasing severity according to standardized measures of dysfunction: (i) asymptomatic neurocognitive impairment (ANI), (ii) mild neurocognitive disorder (MND), and (iii) HAD.

The mechanisms contributing to the development of the various forms of HAND remain incompletely understood, although it is known that anemia early in HIV-1 infection appears to presage later neuropsychological impairment (McArthur et al. 1993). In order to learn how HIV infection can lead to dementia or the milder forms of HAND, scientists have employed a variety of strategies. Besides neurological and neuropsychological assessments, which are essential to identify the clinical domains of HAND, the current knowledge is based on multiple methodological approaches, including analyses of cerebrospinal fluid (CSF), neuroimaging, histopathological studies on *postmortem* brain specimens, animal models ranging from monkeys to rodents, and in vitro models using neuronal, glial, and immune cells from humans, monkeys, and rodents (Maung et al. 2012). New and sometimes surprising insights into the ways HIV-1 compromises the CNS came from the

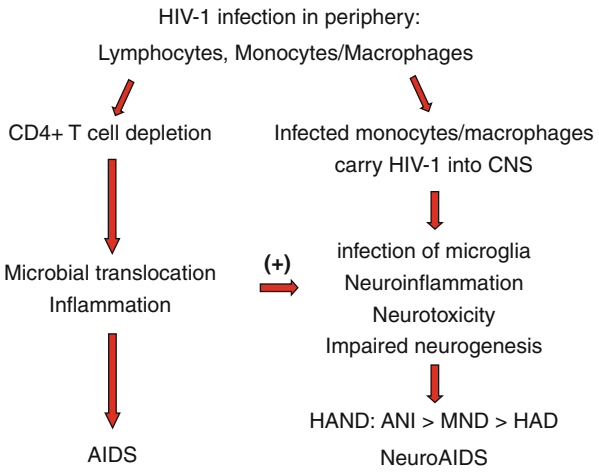

Fig. 1 *Schematic outline of key pathological processes and events associated with HIV-1 infection and the development of HAND, neuroAIDS, and AIDS*. HIV-1 infection occurs in the periphery, but soon afterwards the virus arrives in the brain, presumably carried by infected infiltrating macrophages and possibly lymphocytes. In the brain, the virus can infect microglia. Infected macrophages and microglia release viral proteins and host factors that can stimulate uninfected cells contributing to the development of neuroinflammation, neurotoxicity, and impaired neurogenesis. The latter two processes are presumed to underlie the development of HAND. In the periphery, HIV infection most often leads to the loss and eventual depletion of $CD4^+$ T cells, including those in the intestinal tract. The weakened immune surveillance seems to enable microbial translocation, which further contributes to inflammation. Depletion of $CD4^+$ T cells will eventually result in the development of AIDS. Peripheral inflammation also seems to promote neuroinflammation and the development of HAND and neuroAIDS

discovery of HIV-1 coreceptors, the chemokine receptors CCR5 and CXCR4, in the brain as well as from progress in understanding neuroinflammation and neural stem cell biology (Lavi et al. 1998; Miller and Meucci 1999; Kaul et al. 2001; Kramer-Hammerle et al. 2005; Minghetti 2005; Kaul et al. 2005; Gonzalez-Scarano and Martin-Garcia 2005; Jones and Power 2006). Currently, there is strong evidence suggesting that HIV-1 may strike at the brain in at least two ways: (i) by causing toxicity leading to neuronal injury and death and (ii) by impairing neurogenesis without killing neural precursors (Fig. 1) (Kaul 2008). Both of the two mechanisms appear to share the involvement of inflammatory processes, and both seem to be influenced by host and viral factors, including aging and viral subtypes, as well as treatment and comorbidity factors, such as drug abuse (Kaul 2008; Kaul 2009; Gannon et al. 2011). While we cannot yet reliably determine how much impairment of neurogenesis contributes to HAND, based on currently available information, this chapter will discuss what may be the most destructive mechanism for the CNS during HIV-1 infection: neurotoxicity.

2 HIV-1 Infection, AIDS, and HIV-Associated Neurocognitive Disorders

HAD as the most severe manifestation of HAND occurred at the beginning of the AIDS epidemic primarily in patients with advanced HIV disease and $CD4^+$ T cell counts below 200 μl^{-1} (McArthur et al. 1993). The introduction in the mid-1990 of combination antiretroviral therapy (cART), which was also called "highly active antiretroviral therapy" (HAART), was a major advance in the treatment of HIV infection that in most cases significantly delayed the progression to AIDS. Thus, cART increased the life span of people infected with HIV-1 and resulted in a temporary decrease in the incidence of HAD from 20 % to 30 % to as low as 10.5 % (McArthur et al. 2003). Control of peripheral viral replication and the treatment of opportunistic infections continue to extend survival times, and HIV infection has become a chronic rather than acute disease. Unfortunately, however, cART fails so far to prevent the eventual development of MND or HAD or to reverse HAND (Cunningham et al. 2000; Cysique et al. 2006; Giancola et al. 2006; Nath and Sacktor 2006; Ellis et al. 1997; Antinori et al. 2007; Liner et al. 2008; Boisse et al. 2008; Brew et al. 2008; Heaton et al. 2010, 2011; Gannon et al. 2011). In the era of cART, MND may be more prevalent than overt dementia, but as an AIDS-defining illness, the incidence of dementia has increased in recent years and HAD still constitutes a significant independent risk factor for death due to AIDS (Ellis et al. 1997; Liner et al. 2008). In a group of 669 HIV patients who died between 1996 and 2001, more than 90 % had been diagnosed with HAD as an AIDS-defining condition within the last 12 months of life (Welch and Morse 2002). In addition, the proportion of new cases of HAD displaying a $CD4^+$ T cell count greater than 200 μl^{-1} has been growing (McArthur et al. 2003). Of note, recent therapeutic guidelines for AIDS suggest to start cART only once the number of $CD4^+$ T cells begins to decline. Since this might occur up to years after initial peripheral infection, cART most likely has no chance to forestall the entry of HIV-1 into the CNS (Kramer-Hammerle et al. 2005). Furthermore, distinct patterns of viral drug resistance in plasma and CSF compartments have been ascribed to limited penetration of certain antiretroviral drugs, such as HIV protease inhibitors and several of the nucleoside analogues, into the CNS (Cunningham et al. 2000; Kaul et al. 2005; Kramer-Hammerle et al. 2005). On the other hand, several studies have found clear evidence that cART can benefit the brain and neurocognitive performance, in particular if the therapeutic compounds reach the CNS in concentrations sufficient to suppress virus (Smurzynski et al. 2011; Letendre et al. 2004; Patel et al. 2009; Winston et al. 2010; Gannon et al. 2011). At the same time, some studies suggest that long-term exposure to cART poses a potential toxicological problem, which may contribute to neurocognitive deterioration on its own (Liner et al. 2008, 2010; Letendre et al. 2008; Cardenas et al. 2009; Marra et al. 2009; Schweinsburg et al. 2005).

Early studies during the AIDS epidemic indicated that HIV penetrates into the CNS soon after infection in the periphery and then resides primarily in perivascular macrophages and microglia (Ho et al. 1985; Gartner 2000; Gonzalez-Scarano and Martin-Garcia 2005). Therefore it is not very surprising that despite cART the

prevalence of dementia may be rising as people live longer and age with HIV-1 infection (Lipton 1997; Jones and Power 2006; Cunningham et al. 2000; McArthur et al. 2003; Kaul et al. 2005, 2001; Kramer-Hammerle et al. 2005; Brew et al. 2009; Lindl et al. 2010; Gannon et al. 2011).

The blood–brain barrier (BBB) seems to play an important role in the acute and chronic HIV infection of the CNS as it potentially controls the infiltration of virus-infected and uninfected peripheral immune cells, such as monocytes and macrophages (Gartner 2000; Nottet et al. 1996; Gras and Kaul 2010). Astrocytes and microglia produce chemokines – cell migration-/chemotaxis-inducing cytokines – such as monocyte chemoattractant protein (MCP)-1, which appear to attract peripheral blood mononuclear cells across the BBB into the brain parenchyma (Asensio and Campbell 1999). In fact, an increased risk of HAD has recently been connected to a mutant MCP-1 allele that causes increased infiltration of monocytes and macrophages into tissues which may enhance the transfer of HIV-1 into the brain and promote neuroinflammation (Gonzalez et al. 2002; Gras and Kaul 2010). Alternatively, it has been suggested that the proinflammatory cytokine TNFα promotes a paracellular route for free virus across the BBB (Fiala et al. 1997).

Importantly, infectious events in the periphery and the associated immune response and inflammatory processes influence all cell types in the CNS (Turrin and Rivest 2004; Chakravarty and Herkenham 2005). Potentially important for the understanding of all aspects of HIV disease are recent findings strongly suggesting that the pathogenic or nonpathogenic course of HIV/simian immunodeficiency virus (SIV) infection may be determined by the reaction of plasmacytoid dendritic cells to the virus, in particular the time and amount of interferon (IFN)-α production (Mandl et al. 2008). Signaling via Toll-like receptors (TLR)-7 and −9 and the extent of downstream activation of interferon regulatory factor (IRF)-7 appears to control how much IFNα is being produced. While IFNs are important for an antiviral immune response, the lasting production of IFNα in HIV/SIV infection is suspected to result in erroneous and exhaustive immune activation which in turn may lead to immune suppression and progression to SIV/AIDS (Mandl et al. 2008). Besides immune suppression, the extensive production of IFNα can be of direct detrimental consequence to the brain, as IFNα production in the HIV-infected CNS directly correlates with neurocognitive impairment (Sas et al. 2007). In contrast, IFNβ induces a dominant-negative form of the transcription factor CCAAT-enhancer-binding protein (CEBP)-β and suppresses SIV in macaque macrophages (Dudaronek et al. 2007). Moreover, IFNβ triggers in macrophages and microglia the expression of the antiviral and neuroprotective β-chemokines CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES (Kitai et al. 2000; Garzino-Demo et al. 1998; Kaul and Lipton 1999; Kaul et al. 2007).

HIV-1/SIV infection also affects the intestinal tract and can cause leakage of bacteria or bacterial products into the bloodstream (Brenchley et al. 2006). Such microbial translocation is reflected by elevated plasma levels of lipopolysaccharide (LPS), which is associated with increased monocyte activation and dementia in HIV-infected patients (Ancuta et al. 2008). Thus, immune activation and inflammation are prime suspects regarding a contribution to neurotoxicity, impairment of

neurogenesis, and the development of HAND, but the biological effects of IFNβ suggest that innate immunity can also protect.

3 Neuropathology of HIV-1 Infection Before and After Introduction of cART

Development of HAND and in particular HAD in patients during their lifetime has been correlated to a *postmortem* neuropathological diagnosis that has often been referred to as HIV encephalitis (HIVE) (Petito et al. 1986; Masliah et al. 1992). The salient pathological features include microglial nodules; activated resident microglia; HIV-infected multinucleated giant cells; infiltration predominantly by monocytoid cells, including blood-derived macrophages; widespread reactive astrocytosis; myelin pallor; and decreased synaptic and dendritic density, combined with selective neuronal loss (Petito et al. 1986; Masliah et al. 1997). The designation and hallmarks of HIVE indicate that signs of neuroinflammation have commonly been found in HIV patients since the beginning of the AIDS epidemic. Surprisingly, however, *antemortem* measures of cognitive dysfunction did not correlate well with numbers of HIV-infected cells, multinucleated giant cells, or viral antigens in CNS tissue (Glass et al. 1995; Achim et al. 1994; Wiley et al. 1994; Masliah et al. 1997). Instead, the pathological characteristics showing the best correlation with clinical signs of HAD were increased numbers of microglia (Glass et al. 1995), decreased synaptic and dendritic density, selective neuronal loss (Masliah et al. 1997; Achim et al. 1994; Wiley et al. 1994), elevated tumor necrosis factor (TNF)-α mRNA in microglia and astrocytes (Wesselingh et al. 1997), and evidence of excitatory neurotoxins in CSF and serum (Heyes et al. 1991). Thus, several lines of evidence pointed toward an important role of inflammatory factors, macrophages/microglia, and neurotoxins in the development of HAD. In addition, two recent studies also suggested a crucial role of mononuclear phagocytes because the findings showed that the amount of proviral HIV DNA in circulating monocytes and macrophages correlated well with HAD, whereas viral load was a less reliable indicator (Shiramizu et al. 2005, 2006).

Neuronal damage and loss has been observed *postmortem* in distinct brain regions of HIV and AIDS patients, including frontal cortex (Ketzler et al. 1990; Everall et al. 1991), substantia nigra (Reyes et al. 1991), cerebellum (Graus et al. 1990), and putamen (Everall et al. 1993). Focal neuronal necrosis appeared to occur in HAD brains early in the AIDS epidemic (Ho et al. 1989), whereas neuronal apoptosis has more recently been linked to HAD (Petito and Roberts 1995; Adle-Biassette et al. 1999). The localization of apoptotic neurons was correlated with signs of structural atrophy and microglial activation, in particular within subcortical deep gray structures (Adle-Biassette et al. 1999). Surprisingly, however, neuronal death was not clearly associated with viral burden or a history of dementia. On the other hand, a separate study found that cellular DNA fragmentation detected by terminal-deoxynucleotidyl-transferase-mediated dUTP nick-end labeling (TUNEL) in basal ganglia correlated well with HAD (Rostasy et al. 2000).

To be sure, the neuropathology of HIV infection and AIDS has shifted following the introduction of cART (Anthony and Bell 2008; Boisse et al. 2008; Brew et al. 2008). Although the number of opportunistic infections declined, two *postmortem* studies found evidence of increased infiltration and activation of macrophages and microglia in hippocampus and basal ganglia of cART-experienced patients in comparison to cases from the time before cART and a higher prevalence of HIV-associated encephalitis at the time of autopsy (Anthony et al. 2005; Bell 2004; Langford et al. 2003). Even more encephalitis and severe leukoencephalopathy was observed in *postmortem* specimens from AIDS patients who had failed cART (Anthony et al. 2005). In line with these findings, more recent neuropathological descriptions also reported various forms with severe HIVE and white matter injury, extensive perivascular lymphocytic infiltration, "burnt-out" forms of HIVE, and aging-associated β-amyloid accumulation with Alzheimer's-like neuropathology (Everall et al. 2005; Green et al. 2005; Achim et al. 2009).

Signs of neuroinflammation were commonly found in HIV patients since the beginning of the AIDS epidemic (HIVE) (Masliah et al. 1992), and neuroinflammation usually developed with the progression of HIV patients from the latent, asymptomatic stage of the disease to AIDS and HAD. Therefore, inflammation itself was generally seen as a pathological mechanism, and improved therapy was expected to ameliorate neuroinflammation. Surprisingly, autopsy cases of HIV-related death collected since the advent of cART rather suggested the opposite (Bell 2004; Everall et al. 2005; Anthony et al. 2005). However, the predominant localization of neuroinflammation had changed. Specimen from pre-cART patients showed strong involvement of basal ganglia, whereas post-cART cases displayed extensive signs of inflammation in hippocampus and adjacent regions of entorhinal and temporal cortex (Anthony and Bell 2008). Interestingly, cART seemed to have limited or prevented lymphocyte infiltration, with the exception of occasional, distinct instances, termed "immune reconstitution inflammatory syndrome" (IRIS), where massive lymphocytosis was associated with pronounced demyelination and white matter damage (Anthony and Bell 2008; Boisse et al. 2008). Thus, despite viral control and life-prolonging effects of cART, neuroinflammation and concomitant activation of macrophages and microglia accompany the continued development of HAND in HIV patients.

4 Chemokine Receptors and HIV-1 Infection of the Brain

Chemokine receptors are members of the large family of G-protein-coupled seven transmembrane-spanning domain receptors. Their ligands are primarily small proteins, the chemokines, which trigger leukocyte trafficking and participate intimately in the inflammatory and anti-infection responses of the immune system (Locati and Murphy 1999; Dragic et al. 1996; Oberlin et al. 1996; Tran and Miller 2003). Beyond the immune defense, chemokines and their receptors contribute to organogenesis, including hematopoiesis, angiogenesis, and development of heart and

brain (Tachibana et al. 1998; Zou et al. 1998; Ma et al. 1998; Locati and Murphy 1999), and are essential for maintenance, maturation, and migration of hematopoietic and neural stem cells (Lapidot and Petit 2002; Tran and Miller 2003). However, some pathogens are able to hijack certain chemokine receptors for their purposes, such as the mediation of HIV-1 infection (Dragic et al. 1996; Oberlin et al. 1996; Locati and Murphy 1999; Sodhi et al. 2004).

HIV-1 infects macrophages and lymphocytes in the periphery and microglia in the brain after binding of the viral envelope protein gp120 to CD4 in conjunction with one of several possible chemokine receptors. CD4 and HIV-1/gp120 coreceptors are likely the first sites of host-virus interaction. Different HIV-1 strains may use CCR5 (CD195) – and CCR3 on microglia – or CXCR4 (CD184) or a combination of these chemokine receptors to enter target cells, depending on specific properties of their gp120 (Dragic et al. 1996; Oberlin et al. 1996; He et al. 1997a). Generally, "T-tropic" viruses predominantly infect T lymphocytes via the α-chemokine receptor CXCR4 and/or the β-chemokine receptor CCR5. In contrast, "M-tropic" HIV-1 primarily enter macrophages and microglia via CCR5 and CCR3, although CXCR4 may also be involved (He et al. 1997a; Michael and Moore 1999; Ohagen et al. 1999; Chen et al. 2002). Of note, usage of CCR5 is neither necessary nor sufficient for macrophage tropism, and CXCR4-preferring viruses can infect macrophages (Gorry et al. 2005; Li et al. 1999; Yi et al. 1998, 2003). In any case, chemokine receptors play crucial roles on several levels in HIV-1 disease (Verani and Lusso 2002). Usually, CCR5-preferring HIV-1 strains (R5-tropic) are transmitted between humans, and individuals lacking CCR5 are highly resistant to primary HIV infection (Liu et al. 1996). CXCR4-using viruses (X4-tropic) occur in about 50 % of infected individuals later in the course of HIV-1 disease and generally indicate progression to AIDS (Michael and Moore 1999). These X4-tropic viruses can easily be transmitted via blood transfusion or shared needles in intravenous drug users. However, in many HIV/AIDS patients, R5-tropic viruses evolve over time into more cytopathic variants possessing higher CCR5 affinity combined with diminished CD4 dependence, and a switch in coreceptor usage does not occur (Gorry et al. 2005).

In the brain, neurons and astrocytes express among other chemokine receptors also CCR5 and CXCR4 (Asensio and Campbell 1999; Miller and Meucci 1999), although these cell types, in contrast to microglia, are not thought to harbor productive HIV-1 infection under in vivo conditions. However, HIV infection of astrocytes seems to occur and require the help of a mannose receptor (Liu et al. 2004). Contrasting earlier reports indicating nonproductive infection, recent studies suggest that under certain conditions of immune activation, astrocytes may be able to produce virus (Li et al. 2011). In any case, several in vitro studies strongly suggest that CXCR4 is prominently involved in HIV-associated neuronal damage, whereas CCR5 may play a dual role by mediating either a toxic or protective effect depending on the kind and concentration of its available ligands (Hesselgesser et al. 1998; Meucci et al. 1998, 2000; Kaul and Lipton 1999; Kaul et al. 2007).

5 HIV-1-Induced Neurotoxicity

While progress is being made in unraveling the pathological processes, it remains a controversial issue how exactly HIV-1 infection provokes neuronal injury and death as well as neurocognitive and motor impairment (Kaul et al. 2005; Mattson et al. 2005; Gonzalez-Scarano and Martin-Garcia 2005; Kramer-Hammerle et al. 2005). There is general agreement that HIV does not infect postmitotic, mature neurons, but the mechanism of neuronal damage is in question.

Recent evidence suggests that viral clade may influence the occurrence of HIV-associated neuronal injury and development of HAND (reviewed in Gannon et al. (2011)). However, the HIV-1 genome of all clades encodes nine gene products which serve three major biological functions: (i) structure (Gag, polymerase (Pol), envelope protein (Env, gp120 and gp41)), (ii) regulation (transactivator of transcription (Tat), regulatory for expression of viral proteins (Rev)), or iii) accessory functions (viral protein u (Vpu), viral protein r (Vpr), viral infectivity factor (Vif), negative factor (Nef); recently reviewed in Ellis et al. (2007)). Most of these viral factors have been linked to distinct functions during infection and in the viral life cycle. Thus, whereas various HIV proteins affect the course of viral infection and disease, the envelope gp120 seems to play a major role in the induction of apoptosis in infected and uninfected bystander lymphocytes (Perfettini et al. 2005a, b). On the other hand, six HIV proteins have been reported to directly or indirectly affect neurons and glia (Ellis et al. 2007), and there exists ample evidence for neuronal injury by the five viral proteins Tat, Nef, Vpr, and the Env proteins gp120 and gp41 (Brenneman et al. 1988; New et al. 1997; Adamson et al. 1996; Piller et al. 1998; Koedel et al. 1999; Kaul et al. 2001, 2005; Mattson et al. 2005).

Human macrophages (and lymphocytes) provide suitable models to investigate HIV infection and potential neurotoxic effects in vitro and also in vivo, when injected into immunocompromised mice. However, using the intact virus and infection does not permit to study the contribution of a particular virus component to CNS injury. In order to study the injurious potential of HIV components in the brain in vivo, a number of transgenic mouse models have been generated (Toggas and Mucke 1996; Maung et al. 2012; Power et al. 2012; Gorantla et al. 2012). Transgenic mice expressing HIV/gp120 or Tat in their brains manifested several neuropathological features observed in AIDS brains, such as decreased synaptic and dendritic density, increased numbers of activated microglia, and pronounced astrocytosis (Toggas et al. 1994; Kim et al. 2003; Fitting et al. 2010). HIV/gp120 transgenic mice also developed significant physiological and behavioral changes, such as reduced escape latency, swimming velocity, and spatial retention at 12 months of age (Mucke et al. 1995; Krucker et al. 1998; Asensio et al. 2001; D'hooge et al. 1999). Following induction of Tat expression with doxycycline (Dox), inducible HIV-/Tat-transgenic (iTat-tg) mice displayed failure to thrive, impaired movement, and compromised spatial learning and memory (Kim et al. 2003; Carey et al. 2012; Fitting et al. 2012). HIV/Vpr-transgenic mice showed injury and loss of neurons and astrocytes but surprisingly lacked overt signs of neuroinflammation (Jones et al. 2007;

Power et al. 2012). Nevertheless, Vpr-tg mice developed learning and memory impairment. The relevance of the transgenic models was supported by findings obtained in two mouse models using HIV-infected human immune cells. In the first model, HIV-infected human macrophages were intracerebrally administered into mice with severe combined immunodeficiency (SCID) (Tyor et al. 1993; Persidsky et al. 1996), and the second model employed mice with a humanized hematopoietic system (Gorantla et al. 2010; Dash et al. 2011). The chimeric mice in the second model were infected with HIV-1 and followed for 15 weeks. The neuropathology in these latter two models using intact HIV-1 also resembled numerous pathological features of human AIDS brains and was in particular comparable to the brain injury observed in both HIV/gp120tg and HIV/iTat-tg mice.

Over time investigations using in vitro and in vivo models have generated evidence for at least two different hypotheses on how HIV-1 initiates neuronal damage in the brain. The hypotheses can be described as the "direct injury" hypothesis and the "indirect" or "bystander effect" hypothesis. These two hypotheses are by no means mutually exclusive, and while the available data support a role for both, an indirect form of neurotoxicity may predominate in a setting where monocytic, glial, and neuronal cells are present (Fig. 2) (Gartner 2000; Kaul et al. 2001, 2005; Mattson et al. 2005; Gonzalez-Scarano and Martin-Garcia 2005).

5.1 Direct Neuronal Toxicity

The hypothesis that HIV proteins can directly injure neurons without any contribution of nonneuronal cells (microglia and/or astrocytes) is supported by experiments showing that several viral proteins, including envelope protein gp120, are toxic in serum-free primary neuronal cultures (Meucci et al. 1998, 2000) and in neuroblastoma cell lines (Hesselgesser et al. 1998). The impact of neurotoxic factors secreted by non-neuronal cells is minimized in these experimental paradigms because serum-free neuronal cultures contain few if any nonneuronal cells and neuroblastoma or other neuron-type cell lines do not contain cells of other phenotypes.

In case of HIV envelope protein gp120, which interacts with several members of the chemokine receptor family (see above), the direct form of HIV-induced neuronal injury may be mediated by chemokine receptor signaling in the absence of CD4 (Hesselgesser et al. 1998). Indeed, experiments employing an inhibitor of CXCR4 can in some cases prevent HIV/gp120-induced neuronal apoptosis (Meucci et al. 1998; Kaul and Lipton 1999; Zheng et al. 1999; Meucci et al. 2000). In a different study, gp120 was found to interact at nanomolar concentrations with the glycine binding site of the *N*-methyl-*D*-aspartate-type glutamate receptor (NMDAR) (Fontana et al. 1997), suggesting an alternative mechanism by which HIV/gp120 may directly interfere with neuronal function and viability.

Interestingly, gp120 of the CXCR4-preferring HIV-1 strain IIIB can be endocytosed by neuronal axons and subsequently disturb axonal transport

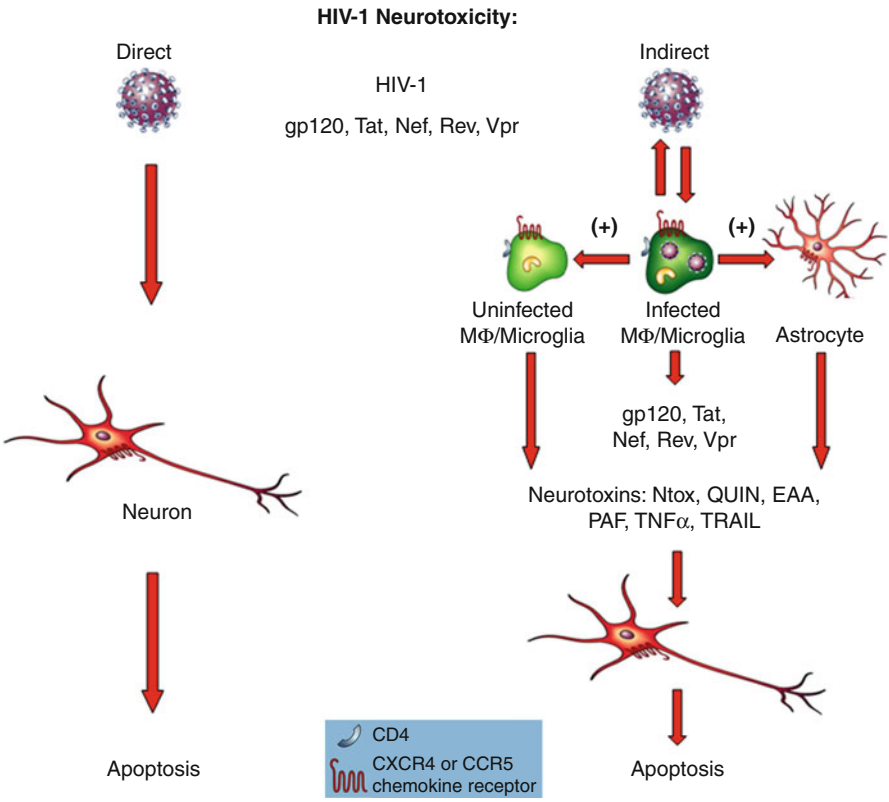

Fig. 2 *Schematic representation of direct and indirect HIV-1-induced neurotoxicity*. Studies using in vitro and in vivo models have generated evidence for at least two different hypotheses on how HIV-1 initiates neuronal damage in the brain: the "direct injury" hypothesis and the "indirect" or "bystander effect" hypothesis. These two hypotheses are by no means mutually exclusive. The "direct injury" hypothesis proposes that HIV and its proteins can directly damage and kill neurons without any contribution of nonneuronal cells, in particular microglia and/or astrocytes. The "indirect" or "bystander effect" hypothesis suggests that activation of macrophages and microglia by HIV infection or viral components and subsequent release of neurotoxins, such as excitotoxins and/or proinflammatory cytokines and mediators, provide the predominant trigger for neuronal injury and death. Macrophages (MΦ) and microglia are the major productively HIV-infected cell type in the brain and as such produce and release the virus and its components as well as cytokines and other factors that can stimulate uninfected MΦ/microglia and astrocytes. It is important to note that toxic viral proteins among factors released from microglia and excitatory glutamate and cytokines set free by astrocytes may all act in concert to provoke neurodegeneration and HAND, even in the absence of extensive viral invasion of the brain. Final neuronal loss seems to occur through apoptosis

(Mocchetti et al. 2012). Since neurotrophic support is critically dependent on this mode of transport, gp120 may not only compromise the endocytotic compartment but also neurotrophin function, and thus promote neuronal injury and demise. A follow-up study by the same group provided evidence that gp120 in addition

alters the neuronal processing of brain-derived neurotrophic factor (BDNF) in a fashion that facilitates neuronal deterioration (Bachis et al. 2012).

The viral regulatory protein Tat can freely diffuse through membranes and therefore interact with cellular binding partners on the surface as well as inside a cell (Fawell et al. 1994). Moreover, Tat can in principle interact with all cells although the consequences may vary between the individual cell types and depending on what Tat-interacting cellular factors are present. Tat can be taken up into neuron-like PC12 cells by a receptor-mediated mechanism (Liu et al. 2000) and may also have a direct effect on primary neurons by potentiating the response to excitotoxic stimuli (reviewed in (Mattson et al. 2005)). The various cell surface receptors that can interact with Tat include chemokine receptors, such as the CCR2, CCR3, and the HIV coreceptor CXCR4 (CD184), the low-density lipoprotein receptor-related protein (LRP), group I metabotropic glutamate receptors (mGluRs), and NMDA-type ionotropic glutamate receptors (Albini et al. 1998; Ghezzi et al. 2000; Xiao et al. 2000; Eugenin et al. 2007; Neri et al. 2007; Li et al. 2008). Inside a cell, Tat can affect glucagon synthase kinase (GSK) 3β and various transcription factors, such as nuclear receptor chicken ovalbumin upstream promoter transcription factor (COUP-TF) and AFF4 (Maggirwar et al. 1999; Rohr et al. 2000; He et al. 2010). A more recent study linked for the first time Tat to dysregulation of neuronal microRNAs (miR), including miR-128. The upregulation of miR-128 inhibited expression of the presynaptic protein SNAP25 (Eletto et al. 2008) and indicated a new way of how Tat may compromise synaptic function. Another report described a Tat-induced pathway to neurite retraction that required p73 and p53 and also led to accumulation of the pro-apoptotic Bcl-2 family protein Bax (Mukerjee et al. 2008). Finally, another direct pathway to neuronal injury initiated by Tat seems to involve activation of neuronal ryanodine receptors (RyR) by Tat, which causes a rapid loss of calcium (Ca^{2+}) from the endoplasmic reticulum (ER) and induces the unfolded protein response (UPR) and mitochondrial hyperpolarization (Norman et al. 2008). The latter is associated with a compromised capability to buffer intracellular Ca^{2+} and thus may affect neuronal excitability and synaptic activity.

Experiments using cultured hippocampal neurons suggested that the HIV protein Vpr may cause direct neurotoxicity through formation of a cation-permeable channel (Piller et al. 1998; Huang et al. 2000). Other studies found that HIV-1 Vpr induced apoptosis via a direct interaction with the mitochondrial adenine nucleotide translocator (ANT) and an effect on the permeability transition pore complex (PTPC) (Jacotot et al. 2000). However, the compromise of mitochondrial membrane potential and nuclear apoptosis triggered by Vpr were blocked in the presence of Bcl-2 (Jacotot et al. 2001). In human NT2 cell-derived neurons, Vpr was found to activate caspase-8 and trigger apoptosis (Pomerantz 2004). In a separate study, Vpr caused neuronal apoptosis accompanied by cytochrome c release, p53 induction, activation of caspase-9, and a diminishing effect on whole-cell currents. The same study found in Vpr-transgenic (tg) animals that the viral protein affected γ-aminobutyric acid (GABA)-ergic and cholinergic neurons in the basal ganglia besides a decreased immunoreactivity for subcortical synaptophysin and an

increase in activated caspase-3 (Jones et al. 2007). Of note, the in vivo neuropathology caused by Vpr in the tg mouse model may have resulted from direct and indirect effects (Power et al. 2012). Vpr was also reported to affect neuronal development by inducing mitochondrial dysfunction, reduced intracellular ATP concentration, compromised axonal transport of mitochondria, and consequent inhibition of axonal outgrowth (Kitayama et al. 2008).

HIV/Nef was also found to compromise neuronal survival, but the mechanism of the reported direct effect remained unknown (Trillo-Pazos et al. 2000). Of note, other studies found that Nef was present in astrocytes of HAD patients and induced apoptosis in astrocytes and neurons (He et al. 1997b). However, Nef triggered neuronal death indirectly because neurotoxicity required the presence of astrocytes and depended on their release of the CXCR3 ligand CXCL10/IP10 (van Marle et al. 2004).

A study investigating the potential neurotoxicity of HIV/Rev in vivo observed a lethal effect in mice that was linked to possible disturbance of cell membranes (Mabrouk et al. 1991). However, an in vitro study using nonneuronal cells found that Rev disturbed the structure of nucleoli and slowed the cell cycle during mitosis (Miyazaki et al. 1995). Thus, one may wonder whether Rev can affect besides HIV-infected, activated lymphocytes producing virus, also proliferating neural precursor cells.

While the absence of nonneuronal cells permits the investigation of potential direct effects of viral proteins on neurons, one ought to bear in mind that because of the absence of nonneuronal cells, a predominantly indirect effect cannot be detected. Notably, in the pathophysiological setting of the brain, neurons always encounter the potential toxins in the presence of glial and sometimes immune cells. Therefore, direct interaction of viral proteins with neurons, such as gp120 with neuronal chemokine receptors (Hesselgesser et al. 1997, 1998), may contribute to neuronal injury, but numerous additional studies suggest that activation of macrophage HIV coreceptors and subsequent release of neurotoxins, such as excitotoxins, chemokines, and/or proinflammatory cytokines, provide the predominant trigger for neuronal injury and death (Giulian et al. 1993; Kaul and Lipton 1999; Porcheray et al. 2006; O'Donnell et al. 2006; Cheung et al. 2008; Eggert et al. 2010; Medders et al. 2010).

5.2 Indirect Neuronal Toxicity

Intact HIV-1, as well as picomolar concentrations of isolated viral envelope gp120, can induce neuronal death via CXCR4 and CCR5 receptors in cerebrocortical and hippocampal neurons and neuronal cell lines from humans and rodents (Hesselgesser et al. 1998; Iskander et al. 2004; Walsh et al. 2004; Meucci et al. 1998; Ohagen et al. 1999; Kaul and Lipton 1999; Chen et al. 2002; Garden et al. 2004; Kaul et al. 2007). In mixed neuronal/glial cerebrocortical or hippocampal cell cultures which model the cellular composition of brain regions affected by HIV, virus and gp120-induced apoptotic death appears to be mediated predominantly via the release of toxins from microglia and macrophages rather than by direct neuronal

damage (Brenneman et al. 1988; Iskander et al. 2004; Walsh et al. 2004; Giulian et al. 1990; Kaul and Lipton 1999; Chen et al. 2002; Garden et al. 2004; Medders et al. 2010). In fact, both the absence or inactivation of macrophages and microglia basically abrogates the neurotoxicity of HIV-1 and gp120 in mixed neuronal/glial cultures (Giulian et al. 1990; Giulian et al. 1993; Kaul and Lipton 1999; Chen et al. 2002; Garden et al. 2004; O'Donnell et al. 2006; Medders et al. 2010). Since at least in vitro inhibition of microglial activation suffices to prevent neuronal death after gp120 exposure, it seems likely that stimulation of CXCR4 or CCR5 in macrophages and microglia is a prerequisite for the neurotoxicity of HIV-1 and gp120 (Ohagen et al. 1999; Kaul and Lipton 1999). This hypothesis is further supported by our observation that introduction of HIV coreceptor-expressing macrophages sufficed to restore neurotoxicity of gp120 in cerebrocortical cultures genetically deficient in both CXCR4 and CCR5 (Medders et al. 2010).

Chemokine receptors, including HIV coreceptors, are highly conserved between species (Maung et al. 2012). Therefore, our group recently studied the role of chemokine receptors in the neurotoxicity of gp120 using mixed neuronal/glial cerebrocortical cell cultures from rat and mouse. We observed that gp120 from CXCR4 (X4)-preferring as well as CCR5 (R5)-preferring and dual tropic HIV-1 strains all were able to trigger neuronal death, thus suggesting that both HIV coreceptors can mediate neurotoxicity, each alone and in combination (Kaul et al. 2007).

Experiments with cerebrocortical cell cultures from chemokine receptor knockout and wild-type mice addressed the role of each HIV coreceptor separately. Neurotoxicity of X4-preferring gp120 was strongly reduced or absent in CXCR4-deficient cerebrocortical cultures, while gp120 from a R5-preferring HIV-1 strain showed no longer neurotoxicity in the absence of CCR5. Surprisingly, however, gp120 of the dual tropic HIV-1_{SF2} exerted even greater neurotoxicity in CCR5 knockout cultures compared to wild-type or CXCR4-deficient cerebrocortical cells. Nevertheless, gp120 of HIV-1_{SF2}, like all other tested gp120s, was no longer toxic to double KO cerebrocortical cultures lacking both chemokine receptors (Kaul et al. 2007). The finding that the CCR5 or CXCR4 knockout significantly decreased neurotoxicity by CCR5- or CXCR4-preferring gp120s, respectively, resembles the specificity of HIV coreceptors in human cells and thus confirmed the rodent system as a suitable model.

In addition, we observed earlier that the CCR5 ligands CCL4/MIP-1β and CCL5/RANTES protected neurons against gp120-induced toxicity (Kaul and Lipton 1999). Interestingly, this protective effect was strictly dependent on CCR5 but occurred no matter if gp120 preferred CXCR4 or CCR5 or both coreceptors. While the β-chemokines may have prevented CCR5-preferring gp120 from interacting with its receptor by competitive displacement, the blockade of CXCR4-mediated toxicity apparently involved heterologous cross-desensitization of this receptor via stimulation of CCR5 (Kaul et al. 2007). These findings were consistent with a primarily neurotoxic activation of CXCR4 by gp120. In contrast, CCR5 may at least in part have stimulated cytoprotective signals depending on the HIV-1 strain from which a given envelope protein originated and depending on

whether or not antiviral and cytoprotective β-chemokines were present (Cocchi et al. 1995; Kaul et al. 2007).

Interestingly, in macrophages, HIV-1 infection and exposure to just the envelope protein gp120 seem to trigger a similar neurotoxic phenotype (Giulian et al. 1990, 1993). Tat and Nef each alone also causes activation of mononuclear phagocytes and release of cytokines as it can occur upon viral infection (Federico et al. 2001; Olivetta et al. 2003; Sui et al. 2006). In contrast, Vpr seems to promote a rather anti- than proinflammatory phenotype despite evidence that this viral protein plays an important role in HIV production in macrophages (Herbein et al. 2010; Power et al. 2012).

In any case, uninfected macrophages and microglia can be stimulated by factors released from infected cells. These factors include cytokines, such as IL-1β, IL-6, and TNFα, but also TNF-related apoptosis-inducing ligand (TRAIL), matrix metalloproteinases (MMPs), arachidonic acid metabolites, and platelet-activating factor (PAF), and shed viral proteins, such as gp120, Tat, Vpr, and Nef (Wahl et al. 1989; Achim et al. 1993; Gelbard et al. 1994; Miura et al. 2003a, b; Ryan et al. 2004; Conant et al. 1998; Johnston et al. 2000; Herbein et al. 2010). Variations of the HIV-1 gp120, in particular in its V1, V2, and V3 loop sequences, have been implicated in modulating the neurotoxicity of macrophages and microglia (Power et al. 1994, 1998). Activated microglia affect all cell types in the CNS, resulting in further upregulation of cytokines, chemokines, and endothelial adhesion molecules within the brain (Gartner 2000; Kaul et al. 2001, 2005; Gonzalez-Scarano and Martin-Garcia 2005; Kramer-Hammerle et al. 2005).

Some of the secreted factors from activated microglia or macrophages and astrocytes may directly or indirectly contribute to neuronal damage and apoptosis as discussed above for viral proteins. Directly neurotoxic host factors released from HIV-infected or activated microglia and macrophages include excitatory amino acids (EAAs) and related substances, such as quinolinate, a not completely characterized amine compound named "Ntox" but potentially also TRAIL (Giulian et al. 1990; Kramer-Hammerle et al. 2005; Lipton et al. 1991; Heyes et al. 1991; Kaul et al. 2001, 2005; Gonzalez-Scarano and Martin-Garcia 2005; Yeh et al. 2000; Miura et al. 2003a, b; Ryan et al. 2004). EAAs and quinolinic acid can trigger neuronal apoptosis through a process known as excitotoxicity. This detrimental process involves excessive Ca^{2+} influx and free radical (nitric oxide and superoxide anion) formation by overstimulation of glutamate receptors (Lipton et al. 1991), activation of stress-associated protein kinases and caspases, and production of proinflammatory lipids (Kaul et al. 2001; Mattson et al. 2005; Gonzalez-Scarano and Martin-Garcia 2005; Kramer-Hammerle et al. 2005; Jones and Power 2006; Nicotera et al. 1997).

It is important to note that toxic viral proteins among factors released from microglia and glutamate and cytokines set free by astrocytes may all act in concert to provoke neurodegeneration and HAND, even in the absence of extensive viral invasion of the brain. Furthermore, HAND and HAD might share the critical involvement of neuroinflammation and microglial activation with several other neurodegenerative diseases, such as Alzheimer's disease,

multiple sclerosis, Parkinson's disease, and even frontotemporal lobe dementia (Block and Hong 2005).

5.3 Downstream Cellular and Molecular Mechanisms in HIV-1 Neurotoxicity

Beyond infection by intact HIV-1, six of the viral protein components directly or indirectly affect immune cells, neurons, and glia (Ellis et al. 2007), and ample evidence exists for neuronal injury by the five viral proteins Tat, Nef, Vpr, and the Env proteins gp120 and gp41 (Brenneman et al. 1988; New et al. 1997; Adamson et al. 1996; Piller et al. 1998; Koedel et al. 1999; Kaul et al. 2001, 2005; Mattson et al. 2005). Therefore, it is not surprising that the available information suggests that HIV can trigger a plethora of cellular responses and signaling mechanisms. However, it appears that the various viral components that exert cyto- or neurotoxicity do so, at least in part, by converging mechanisms even if each component may start out its contribution to pathogenesis by interacting with different host cell factors. Currently recognized converging mechanisms include neuroinflammation, oxidative stress, interference with the cell cycle machinery, excitotoxicity, and apoptosis.

Recently, we and others found that p38 mitogen-activated protein kinase (MAPK) plays an essential role in the induction of neuronal injury and apoptotic death by HIV-1 gp120, Tat, and the intact virus (Kaul and Lipton 1999; Kaul et al. 2007; Choi et al. 2007; Medders et al. 2010; Singh et al. 2005; Sui et al. 2006; Eggert et al. 2010). Activation of microglia and macrophages by HIVgp120 via CD4, chemokine coreceptors, and downstream p38 MAPK is crucial to neurotoxin production (Kaul and Lipton 1999; Medders et al. 2010). Similarly, others reported that HIV-1 infection as well as Tat and gp120 all activate p38 MAPK and upstream, the mixed lineage kinase (MLK) 3 in infected or stimulated, neurotoxic monocytes and macrophages (Sui et al. 2006; Eggert et al. 2010). Moreover, we have observed that p38 MAPK signaling is essential upon exposure to HIV gp120 for both the neurotoxic phenotype of macrophages and microglia and the subsequent induction of neuronal apoptosis by macrophage toxins (Medders et al. 2010). The requirement of p38 MAPK activity in both macrophages and neurons for the occurrence of HIV gp120 neurotoxicity therefore parallels the indispensability previously reported for the transcription factor p53 (Garden et al. 2004).

Increased activity of p38 MAPK has been linked to inflammation of the brain, such as that seen in HIVE patients and Alzheimer's disease (AD) (Kaminska et al. 2009). Furthermore, neurons showed increased activity of p38 MAPK when they were cocultured with LPS-stimulated microglia, and this kinase signaling was essential to induction of neuronal death (Xie et al. 2004). Amyloid-β-induced activation of p38 MAPK resulted in upregulation of proinflammatory cytokines TNFα, immunologic, inducible nitric oxide synthase (iNOS), and interleukin (IL)-1β and subsequent neuronal death (Delgado et al. 2008). Our and other groups showed that inhibition of p38 MAPK activity in vitro with pharmacological

inhibitor SB203580 or dominant-negative p38αAF (TGY substituted with AGF) prevented neuronal death induced by HIV-1 gp120 or glutamate (Kaul and Lipton 1999; Singh et al. 2005; Kaul et al. 2007; Choi et al. 2007; Chaparro-Huerta et al. 2008). HIV-1 Tat and gp120 both were found to activate MLK 3, which controls p38 MAPK and JNK pathways, in neurons (Sui et al. 2006; Gallo and Johnson 2002). Inhibition or MLK 3 protected neurons from toxicity of gp120, Tat, and HIV-1-infected macrophages (Sui et al. 2006; Eggert et al. 2010). Of note, while pharmacological inhibition of p38 MAPK or JNK can both independently ameliorate the toxicity of Tat in cerebellar granular neurons, only blockade of p38 MAPK, but not JNK, could prevent Tat-induced death in striatal neurons (Singh et al. 2005; Sui et al. 2006).

In neurons, p38α MAPK activity is regulated by the intracellular Ca^{2+} concentration and the small GTPase Rho. The concentration of intracellular Ca^{2+} in turn is linked to the activity of the NMDAR-regulated ion channel and excitotoxicity (Semenova et al. 2007). A number of studies have suggested that excitotoxicity itself constitutes one important mechanism of HIV-1-induced neurotoxicity (Dreyer et al. 1990; O'Donnell et al. 2006; Kaul et al. 2001; Lindl et al. 2010). The link between glutamate receptors, influx of Ca^{2+}, Rho, and activation of p38α certainly suggests that in neurons synaptic activity may be a major regulator of the kinase's baseline activity. Our studies indicate that inhibition of p38 MAPK prevents neuronal death in the context of HIV/gp120-induced, macrophage-mediated neurotoxicity, but surprisingly, neurons surviving in the absence of kinase inhibition display a higher level of active p38 MAPK compared to control cells not exposed to neurotoxins (Medders et al. 2010).

Macrophages and microglia infected with HIV-1 or stimulated by viral gp120, Tat, or Nef release neurotoxins, some of which stimulate the NMDAR, an ionotropic glutamate and neurotransmitter receptor (Dreyer et al. 1990; Chen et al. 2002; Smith et al. 2001; Sui et al. 2007). NMDAR antagonists can ameliorate neuronal cell death in vitro due to HIV-infected macrophages or purified recombinant gp120 (Dreyer et al. 1990; Chen et al. 2002) and in vivo in gp120 transgenic mice (Toggas et al. 1996). Under physiological conditions, activation of ionotropic glutamate receptors in neurons initiates a transient depolarization and excitation. However, extended or excessive NMDAR activation results in excitotoxicity because the prolonged channel opening leads to a sustained elevation of the intracellular Ca^{2+} concentration and thus compromises mitochondrial function and cellular energy metabolism, which in turn causes the generation of free radicals (Lipton and Rosenberg 1994; Doble 1999; Olney 1969). In case the initial excitotoxic insult is fulminant, for example, in the ischemic core of a stroke, the neurons die early from loss of ionic homeostasis, resulting in acute swelling and cell lysis (necrosis). If the insult is milder, as seen in several neurodegenerative disorders, including HAND/HAD, neurons may enter a delayed death pathway known as programmed cell death or apoptosis (Nicotera et al. 1997; Petito and Roberts 1995). Neuronal apoptosis following exposure to gp120-induced macrophage toxins or after a direct excitotoxic insult involves Ca^{2+} overload, activation of p38 MAPK and p53, release of cytochrome c and other molecules such as apoptosis-inducing

factor (AIF) from mitochondria, activation of caspases, free radical formation, lipid peroxidation, and chromatin condensation (Tenneti et al. 1998; Kaul and Lipton 1999; Asensio and Campbell 1999; Garden et al. 2002, 2004; Kaul et al. 2007). The increase of intracellular Ca^{2+} has also been linked to the detrimental activation of the Ca^{2+}-dependent protease Calpain and the activating, proteolytic cleavage of the cell cycle-dependent kinase (Cdk)-5 (Wang et al. 2007). Excessive activation of Ca^{2+}-dependent proteolysis has also been implicated in synaptic injury where the scaffolding inside of pre- and postsynaptic endings may be compromised and thus disturb timely trafficking and localization of vesicles as well as receptor signaling (Lindl et al. 2010). In line with Ca^{2+}-dependent oxidative stress is a different study reporting that in human neurons, CXCR4 mediates the toxic effect of gp120 via a process involving the synthesis of ceramide and NADPH-dependent production of superoxide radicals (Jana and Pahan 2004).

Oxidative stress is a well-recognized cellular response triggered by infections, inflammation, as well as excitotoxicity and occurs in the context for HIV infection or exposure to viral proteins (Roederer et al. 1992; Walmsley et al. 1997; Shi et al. 1998; Corasaniti et al. 2000; Mollace et al. 2001; Boven et al. 1999; Haughey et al. 2004; Louboutin et al. 2007; Agrawal et al. 2009). Oxidative damage occurs in proteins, lipids, and nuclear DNA and can therefore contribute in several ways to neuronal deterioration (Louboutin et al. 2010; Haughey et al. 2004; Bonfoco et al. 1995; Zhang et al. 2012). Interestingly, gp41 in infected cells was found to correlate with the expression of iNOS in AIDS brains and cause neurotoxicity in vitro in an NO-dependent fashion (Adamson et al. 1996). Oxidative stress alters proteins by causing carbonylation and affects lipid metabolism. The latter leads to generation of distinct patterns of ceramide, sphingomyelin, and hydroxynonenal (HNE) detectable in brain tissue and CSF of HAND patients (Turchan et al. 2003; Haughey et al. 2004; Mattson et al. 2005). In line with oxidative stress is the upregulation of the transcription factor ATF-6ß and of the ER chaperone BiP indicating ER stress in neurons and astrocytes of brains from neurocognitively impaired HIV patients (Lindl et al. 2007). In a more recent follow-up study, the same group linked ATF-6ß and BiP to an "integrated stress response," which brains of HAND/HAD patients may share with other neurodegenerative diseases (Akay et al. 2012).

Autophagy allows cells to remove exhausted or damaged organelles or to replenish their resources under starvation conditions and appears to be an essential mechanism to maintain cellular homeostasis. Recent studies strongly suggest that autophagy is disturbed in HIV infection and neurodegenerative diseases, including HIVE and HAND (Espert et al. 2006; Alirezaei et al. 2008; Zhou et al. 2011; Hui et al. 2012). Another mechanistic link to HIV neurotoxicity is suggested by the observation that both TNFα and excitotoxic glutamate concentrations seem to compromise neuronal autophagy and in this way also promote cell death (Alirezaei et al. 2008).

HIV-infected or gp120-activated microglia also release inflammatory cytokines, such as TNFα (Achim et al. 1993; Wesselingh et al. 1997; Persidsky et al. 1997). TNFα directly affects glutamate neurotransmission in a p38 MAPK-dependent

manner by increasing the synaptic expression of certain glutamate receptors (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; AMPAR) and inhibiting long-term potentiation (LTP) (Butler et al. 2004; Pickering et al. 2005). Interestingly, a significant change in the expression at the RNA level of a number of ion channels was reported for brains of HIV patients with various degrees of HAND in comparison to uninfected controls (Gelman et al. 2004). Those disturbances of the ion channel repertoire were likely to affect excitability of neuronal membranes, neurotransmission, as well as the propagation of action potentials in neurons. Therefore, it was proposed that a channelopathy could be underlying the development of HAND and its most severe form, HAD. In any case, TNFα can inhibit astroglial glutamate transporters and thus promote neurotoxicity by facilitating glutamate excitotoxicity (Pickering et al. 2005) or even provoke neurotransmitter release from glial cells (Benveniste and Benos 1995; Bezzi et al. 2001). Interestingly, TNFα can synergize with HIV/Tat to initiate neuronal death, but antioxidants block the process (Ohagen et al. 1999). Finally, it remains possible that TNFα activates caspases in neurons via TNFα receptor-1 (TNFR1). TNFR1 is expressed on at least some neurons, and it can initiate caspase-8 activation, which in turn may activate caspase-3, leading to apoptosis. We found that neutralization of TNFα with antibody or pharmacological inhibition of caspase-8 prevented the neurotoxicity of HIV/gp120 in cultured cerebrocortical neurons (Garden et al. 2002).

Supporting a prominent role of neuronal apoptosis in the development of HAND and HAD, activated caspase-3 and p53 were prominently detected in neurons of brains from neurocognitively impaired HIV patients (Garden et al. 2002). Furthermore, in vitro, neuronal caspase-3, caspase-8, and caspase-9 were found to be necessary for HIV-1/gp120 to cause neurotoxicity, and p53 was essential not only in neurons but also in microglia (Garden et al. 2002, 2004; Tun et al. 2007). Separate studies suggested that CXCR4 and p53 are connected through signaling pathways that mediate toxic or protective mechanisms depending on whether gp120 or SDF-1, the receptor's natural binding partner, serves as the ligand (Khan et al. 2005). Contrary to gp120 of $HIV1_{IIIB}$, CXCL12/SDF-1 was neuroprotective in this experimental paradigm. The α-chemokine activated Akt and MAPKs (Khan et al. 2004) and regulated the expression and localization of cell cycle proteins (Khan et al. 2003, 2005). CXCL12/SDF-1 increased acetylation of p53 and p21 as well as the expression of retinoblastoma protein (Rb) while reducing the amount of phosphorylated Rb in the nucleus. Together with a reduction of the activity of the transcription factor E2F1, an overall anti-apoptotic effect was observed. In contrast, envelope protein of $HIV\text{-}1_{IIIB}$ triggered activation of Apaf-1 and promoted cell death. Besides these in vitro findings, a role for a disturbed cell cycle machinery in the development of HAND was supported by abnormal changes in the expression pattern of the same cell cycle proteins in *postmortem* brains of nonhuman primates with SIV encephalitis and humans with HIVE (Jordan-Sciutto et al. 2002). Interestingly, these changes in cell cycle proteins also correlated with the presence of activated microglia and macrophages. Moreover, cell cycle inhibition has been shown to protect neurons in excitotoxic circumstances from induction of apoptosis (Park et al. 2000).

6 Effects of HIV-1 on Neurogenesis

Compared to the discovery of HIV-induced neurotoxicity, the appreciation of the virus' effect on neurogenesis is a relatively recent development. Notably, HIV primarily seems to compromise the proliferation of neural progenitors but not kill the cells (Krathwohl and Kaiser 2004a, b; Okamoto et al. 2007; Peng et al. 2008). Therefore, HIV does not exert a lethal cytotoxic effect on neural precursors, but rather exacerbates the effects of the above-discussed toxicity in postmitotic and terminally differentiated neurons by impairing neurogenesis and brain homeostasis.

It is widely accepted that HIV-1 fails to productively infect neurons, but it has been reported that neural progenitor cells are permissive to the virus (Lawrence et al. 2004; Mattson et al. 2005; Schwartz and Major 2006). However, infection seems dispensable as HIV-1 gp120 and chemokines suffice to disturb human or rodent neural progenitor cells (Krathwohl and Kaiser 2004a, b; Okamoto et al. 2007). One study showed that chemokines promoted the quiescence and survival of human neural progenitor cells via stimulation of CXCR4 and CCR3 and a mechanism that involved downregulation of extracellularly regulated kinase-1 and kinase-2 (ERK-1/ERK-2) with simultaneous upregulation of the neuronal glycoprotein reelin (Krathwohl and Kaiser 2004a). Exposure to intact HIV-1 also caused quiescence of neural progenitors through engagement of CXCR4 and CCR3. In contrast to the chemokines, the viral envelope protein gp120 downregulated ERK-1/ERK-2 but had no effect on reelin (Krathwohl and Kaiser 2004b). Interestingly, the effects of both the chemokines and HIV-1/gp120 were reversible and could be inhibited with recombinant apolipoprotein E3 (ApoE3), but not ApoE4. Finally, fewer adult neural progenitor cells were detected in the dentate gyrus of the hippocampus in *postmortem* brain specimen from HAD patients than from non-demented and uninfected controls (Krathwohl and Kaiser 2004b).

Cultures of primary rodent and human neural progenitor cells obtained from fetal or adult tissue express CXCR4 and CCR5 besides the neural progenitor marker nestin and readily undergo cell division. After several rounds of proliferation, the progenitors exit the cell cycle and begin to express neuronal markers such as βIII-tubulin (TuJ1). We found that exposure to HIV-1/gp120 in vitro reduced the proliferation of adult progenitors without producing cell death. Others observed a virtually identical effect brought about by HIV Tat (Mishra et al. 2010). Similar to what others observed in brain specimen from HAD patients, we found a reduction of proliferating neural progenitors in the hippocampal dentate gyrus of transgenic mice that express HIV/gp120 in the brain in comparison to non-transgenic controls (Okamoto et al. 2007). Accounting for these effects, we found that gp120 inhibited proliferation of neural progenitor cells through activation of a pathway that involved p38MAPK, MAPK-activated protein kinase-2, a cell cycle checkpoint kinase, and Cdc25B/C which in turn caused a delay of the cell cycle in the G1 phase. The decrease in neural progenitor proliferation caused by gp120 constituted an impairment in neurogenesis because there were fewer progenitor cells available to differentiate into neurons (Okamoto et al. 2007). Neurogenesis in the dentate gyrus of the hippocampus has been implicated in learning and memory formation,

neurocognitive functions which are compromised in HAND. The apparent ability of HIV-1 or its component gp120 and Tat to interfere with the normal function of neural progenitor cells suggested the possibility that HAND might develop as a consequence not only of injury and death of existing neurons but also due to virus-induced disturbance of brain homeostasis and renewal mechanisms.

7 Comorbidity Factors of HAND and HIV-1 Neurotoxicity

HIV infection, HAND, and AIDS are frequently associated with the use of recreational drugs, such as methamphetamine, cocaine, and opiates (Rippeth et al. 2004; Urbina and Jones 2004; Kapadia et al. 2005; Mitchell et al. 2006; Nath 2010; Beyrer et al. 2010; Gannon et al. 2011). Intravenous drug use not only is a risk factor for contracting HIV through needle sharing but also promotes coinfection with hepatitis C virus (HCV) (Gatignol et al. 2007; Clifford et al. 2009; Cherner et al. 2005). The scientific literature provides ample evidence that both abused drugs and infectious agents, such as HIV and HCV, compromise the immune and nervous system, but this still-expanding topic is beyond the scope of this chapter. However, there exist excellent reviews addressing the apparent intersection of HAND and HIV-induced neurotoxicity and impaired neurogenesis with drug abuse (Hauser et al. 2006; Ferris et al. 2008; Nath 2010).

HIV infection has become a chronic rather than acute disease due to efficient control of peripheral viral replication by cART and the treatment of opportunistic infections. With the improved survival times, the population of HIV patients has begun to age. While aging is a normal physiological process, evidence is accumulating that HIV infection may lead to earlier occurrence of aging symptoms physically, immunologically, and mentally (Deeks 2009; Brew et al. 2009). Thus, aging is becoming a comorbidity factor associated with long survival of HIV infection. In reverse, the virus can also be seen as a comorbidity factor of aging that accelerates an otherwise normal but later ensuing increase of frailty.

8 Conclusion

HIV-1 infection continues to trigger neurological problems that can culminate in frank dementia, despite efficient virological control by cART. Currently, strong evidence suggests that HIV-1 can strike at the brain in at least two ways: (i) by causing toxicity leading to neuronal injury and death and (ii) by impairing neurogenesis without killing neural precursors. Both of the pathogenic mechanisms share the involvement of neuroinflammatory mediators, and both processes seem to be influenced by host and viral factors, including the interferon response, age, and viral subtypes, as well as treatment and comorbidity factors, such as drug abuse. Microbial translocation may enhance systemic and central inflammation during HIV-1 infection and thus promote the development of HAND. Thus, despite viral control and life-prolonging effects of cART, neuroinflammation and concomitant

activation of macrophages and microglia accompany the continued development of HAND in HIV patients. Besides intact HIV-1, six viral proteins have been reported to directly or indirectly affect neurons and glia and cause neurotoxicity: Tat, Nef, Vpr, Rev, and the Env proteins gp120 and gp41. Experimental evidence generated at least two different hypotheses on how HIV-1 initiates neuronal damage in the brain: (i) the "direct injury" hypothesis and (ii) the "indirect" or "bystander effect" hypothesis. These two hypotheses are by no means mutually exclusive. However, macrophages and microglia constitute the primary productively infected cell type in the brain, suggesting that an indirect form of neurotoxicity may predominate in a setting where monocytic, glial, and neuronal cells are present. Considering that beyond infection by intact HIV-1, six of the viral proteins trigger neuronal injury, it is not surprising that the available information suggests a plethora of cellular responses and signaling mechanisms. However, it appears that the various viral components that exert cyto- or neurotoxicity do so, at least in part, by converging mechanisms even if each component may start out its contribution to pathogenesis by interacting with different host cell factors. Currently recognized converging mechanisms in HIV neurotoxicity include neuroinflammation, MAPK signaling, altered gene expression, oxidative stress, interference with the cell cycle machinery, excitotoxicity, synaptic injury, and neuronal apoptosis.

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References

Achim, C. L., Adame, A., Dumaop, W., Everall, I. P., & Masliah, E. (2009). Increased accumulation of intraneuronal amyloid beta in HIV-infected patients. *Journal of Neuroimmune Pharmacology, 4*, 190–199.

Achim, C. L., Heyes, M. P., & Wiley, C. A. (1993). Quantitation of human immunodeficiency virus, immune activation factors, and quinolinic acid in AIDS brains. *Journal of Clinical Investigation, 91*, 2769–2775.

Achim, C. L., Wang, R., Miners, D. K., & Wiley, C. A. (1994). Brain viral burden in HIV infection. *Journal of Neuropathology and Experimental Neurology, 53*, 284–294.

Adamson, D. C., Wildemann, B., Sasaki, M., Glass, J. D., McArthur, J. C., Christov, V. I., et al. (1996). Immunologic NO synthase: Elevation in severe AIDS dementia and induction by HIV-1 gp41. *Science, 274*, 1917–1921.

Adle-Biassette, H., Chrétien, F., Wingertsmann, L., Héry, C., Ereau, T., Scaravilli, F., et al. (1999). Neuronal apoptosis does not correlate with dementia in HIV infection but is related to microglial activation and axonal damage. *Neuropathology and Applied Neurobiology, 25*, 123–133.

Agrawal, L., Maxwell, C. R., Peters, P. J., Clapham, P. R., Liu, S. M., Mackay, C. R., et al. (2009). Complexity in human immunodeficiency virus type 1 (HIV-1) co-receptor usage: Roles of CCR3 and CCR5 in HIV-1 infection of monocyte-derived macrophages and brain microglia. *Journal of General Virology, 90*, 710–722.

Akay, C., Lindl, K. A., Shyam, N., Nabet, B., Goenaga-Vazquez, Y., Ruzbarsky, J., et al. (2012). Activation status of integrated stress response pathways in neurones and astrocytes of HIV-associated neurocognitive disorders (HAND) cortex. *Neuropathology and Applied Neurobiology, 38*, 175–200.

Albini, A., Ferrini, S., Benelli, R., Sforzini, S., Giunciuglio, D., Aluigi, M. G., et al. (1998). HIV-1 Tat protein mimicry of chemokines. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 13153–13158.

Alirezaei, M., Kiosses, W. B., Flynn, C. T., Brady, N. R., & Fox, H. S. (2008). Disruption of neuronal autophagy by infected microglia results in neurodegeneration. *PLoS One, 3*, e2906.

Alizon, M., Sonigo, P., Barre-Sinoussi, F., Chermann, J. C., Tiollais, P., Montagnier, L., et al. (1984). Molecular cloning of lymphadenopathy-associated virus. *Nature, 312*, 757–760.

Ancuta, P., Kamat, A., Kunstman, K. J., Kim, E. Y., Autissier, P., Wurcel, A., et al. (2008). Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PLoS One, 3*, e2516.

Anthony, I. C., & Bell, J. E. (2008). The Neuropathology of HIV/AIDS. *International Review of Psychiatry, 20*, 15–24.

Anthony, I. C., Ramage, S. N., Carnie, F. W., Simmonds, P., & Bell, J. E. (2005). Influence of HAART on HIV-related CNS disease and neuroinflammation. *Journal of Neuropathology and Experimental Neurology, 64*, 529–536.

Antinori, A., Arendt, G., Becker, J. T., Brew, B. J., Byrd, D. A., Cherner, M., et al. (2007). Updated research nosology for HIV-associated neurocognitive disorders. *Neurology, 69*, 1789–1799.

Asensio, V. C., & Campbell, I. L. (1999). Chemokines in the CNS: Plurifunctional mediators in diverse states. *Trends in Neurosciences, 22*, 504–512.

Asensio, V. C., Maier, J., Milner, R., Boztug, K., Kincaid, C., Moulard, M., et al. (2001). Interferon-independent, human immunodeficiency virus type 1 gp120-mediated induction of CXCL10/IP-10 gene expression by astrocytes in vivo and in vitro. *Journal of Virology, 75*, 7067–7077.

Bachis, A., Avdoshina, V., Zecca, L., Parsadanian, M., & Mocchetti, I. (2012). Human immunodeficiency virus type 1 alters brain-derived neurotrophic factor processing in neurons. *Journal of Neuroscience, 32*, 9477–9484.

Barre-Sinoussi, F. (2003). The early years of HIV research: Integrating clinical and basic research. *Nature Medicine, 9*, 844–846.

Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., et al. (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science, 220*, 868–871.

Bell, J. E. (2004). An update on the neuropathology of HIV in the HAART era. *Histopathology, 45*, 549–559.

Benveniste, E. N., & Benos, D. J. (1995). TNF-alpha- and IFN-gamma-mediated signal transduction pathways: Effects on glial cell gene expression and function. *The FASEB Journal, 9*, 1577–1584.

Beyrer, C., Wirtz, A. L., Baral, S., Peryskina, A., & Sifakis, F. (2010). Epidemiologic links between drug use and HIV epidemics: An international perspective. *Journal of Acquired Immune Deficiency Syndromes, 55*(Suppl 1), S10–S16.

Bezzi, P., Domercq, M., Brambilla, L., Galli, R., Schols, D., De Clercq, E., et al. (2001). CXCR4-Activated astrocyte glutamate release via TNFalpha: Amplification by microglia triggers neurotoxicity. *Nature Neuroscience, 4*, 702–710.

Block, M. L., & Hong, J. S. (2005). Microglia and inflammation-mediated neurodegeneration: Multiple triggers with a common mechanism. *Progress in Neurobiology, 76*, 77–98.

Boisse, L., Gill, M. J., & Power, C. (2008). HIV infection of the central nervous system: Clinical features and neuropathogenesis. *Neurologic Clinics, 26*, 799–819, X.

Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P., & Lipton, S. A. (1995). Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 7162–7166.

Boven, L. A., Gomes, L., Hery, C., Gray, F., Verhoef, J., Portegies, P., et al. (1999). Increased peroxynitrite activity in AIDS dementia complex: Implications for the neuropathogenesis of HIV-1 infection. *Journal of Immunology, 162*, 4319–4327.

Brenchley, J. M., Price, D. A., Schacker, T. W., Asher, T. E., Silvestri, G., Rao, S., et al. (2006). Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nature Medicine, 12*, 1365–1371.

Brenneman, D. E., Westbrook, G. L., Fitzgerald, S. P., Ennist, D. L., Elkins, K. L., Ruff, M. R., et al. (1988). Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature, 335*, 639–642.

Brew, B. J., Crowe, S. M., Landay, A., Cysique, L. A., & Guillemin, G. (2008). Neurodegeneration and ageing in the HAART era. *Journal of Neuroimmune Pharmacology*. doi:10.1007/s11481-008-9143-1.

Brew, B. J., Crowe, S. M., Landay, A., Cysique, L. A., & Guillemin, G. (2009). Neurodegeneration and ageing in the HAART era. *Journal of Neuroimmune Pharmacology, 4*, 163–174.

Butler, M. P., O'Connor, J. J., & Moynagh, P. N. (2004). Dissection of tumor-necrosis factor-alpha inhibition of long-term potentiation (LTP) reveals a p38 mitogen-activated protein kinase-dependent mechanism which maps to early-but not late-phase LTP. *Neuroscience, 124*, 319–326.

Cardenas, V. A., Meyerhoff, D. J., Studholme, C., Kornak, J., Rothlind, J., Lampiris, H., et al. (2009). Evidence for ongoing brain injury in human immunodeficiency virus-positive patients treated with antiretroviral therapy. *Journal of Neurovirology, 15*, 324–333.

Carey, A. N., Sypek, E. I., Singh, H. D., Kaufman, M. J., & McLaughlin, J. P. (2012). Expression of HIV-Tat protein is associated with learning and memory deficits in the mouse. *Behavioral Brain Research, 229*, 48–56.

Chakravarty, S., & Herkenham, M. (2005). Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. *Journal of Neuroscience, 25*, 1788–1796.

Chaparro-Huerta, V., Flores-Soto, M. E., Gudiño-Cabrera, G., Rivera-Cervantes, M. C., Bitzer-Quintero, O. K., & Beas-Zárate, C. (2008). Role of p38 MAPK and pro-inflammatory cytokines expression in glutamate-induced neuronal death of neonatal rats. *International Journal of Developmental Neuroscience, 26*, 487–495.

Chen, W., Sulcove, J., Frank, I., Jaffer, S., Ozdener, H., & Kolson, D. L. (2002). Development of a human neuronal cell model for human immunodeficiency virus (HIV)-infected macrophage-induced neurotoxicity: Apoptosis induced by HIV type 1 primary isolates and evidence for involvement of the Bcl-2/Bcl-xL-sensitive intrinsic apoptosis pathway. *Journal of Virology, 76*, 9407–9419.

Cherner, M., Letendre, S., Heaton, R. K., Durelle, J., Marquie-Beck, J., Gragg, B., et al. (2005). Hepatitis C augments cognitive deficits associated with HIV infection and methamphetamine. *Neurology, 64*, 1343–1347.

Cheung, R., Ravyn, V., Wang, L., Ptasznik, A., & Collman, R. G. (2008). Signaling mechanism of HIV-1 gp120 and virion-induced IL-1beta release in primary human macrophages. *Journal of Immunology, 180*, 6675–6684.

Choi, W. T., Kaul, M., Kumar, S., Wang, J., Kumar, I. M., Dong, C. Z., et al. (2007). Neuronal apoptotic signaling pathways probed and intervened by synthetically and modularly modified (SMM) chemokines. *Journal of Biological Chemistry, 282*, 7154–7163.

Clifford, D. B., Smurzynski, M., Park, L. S., Yeh, T. M., Zhao, Y., Blair, L., et al. (2009). Effects of active HCV replication on neurologic status in HIV RNA virally suppressed patients. *Neurology, 73*, 309–314.

Cocchi, F., Devico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C., & Lusso, P. (1995). Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science, 270*, 1811–1815.

Conant, K., Garzino-Demo, A., Nath, A., McArthur, J. C., Halliday, W., Power, C., et al. (1998). Induction of monocyte chemoattractant protein-1 in HIV-1 Tat- stimulated astrocytes and elevation in AIDS dementia. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 3117–3121.

Corasaniti, M. T., Strongoli, M. C., Piccirilli, S., Nistico, R., Costa, A., Bilotta, A., et al. (2000). Apoptosis induced by gp120 in the neocortex of rat involves enhanced expression of

cyclooxygenase type 2 and is prevented by NMDA receptor antagonists and by the 21-aminosteroid U-74389G. *Biochemical and Biophysical Research Communications, 274*, 664–669.

Cunningham, P. H., Smith, D. G., Satchell, C., Cooper, D. A., & Brew, B. (2000). Evidence for independent development of resistance to HIV-1 reverse transcriptase inhibitors in the cerebrospinal fluid. *AIDS, 14*, 1949–1954.

Cysique, L. A., Maruff, P., & Brew, B. J. (2006). Variable benefit in neuropsychological function in HIV-infected HAART-treated patients. *Neurology, 66*, 1447–1450.

D'hooge, R., Franck, F., Mucke, L., & De Deyn, P. P. (1999). Age-related behavioural deficits in transgenic mice expressing the HIV-1 coat protein gp120. *European Journal of Neuroscience, 11*, 4398–4402.

Dash, P. K., Gorantla, S., Gendelman, H. E., Knibbe, J., Casale, G. P., Makarov, E., et al. (2011). Loss of neuronal integrity during progressive HIV-1 infection of humanized mice. *Journal of Neuroscience, 31*, 3148–3157.

Deeks, S. G. (2009). Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. *Topics in HIV Medicine, 17*, 118–123.

Delgado, M., Varela, N., & Gonzalez-Rey, E. (2008). Vasoactive intestinal peptide protects against beta-amyloid-induced neurodegeneration by inhibiting microglia activation at multiple levels. *Glia, 56*, 1091–1103.

Doble, A. (1999). The role of excitotoxicity in neurodegenerative disease: Implications for therapy. *Pharmacology and Therapeutics, 81*, 163–221.

Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., et al. (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature, 381*, 667–673.

Dreyer, E. B., Kaiser, P. K., Offermann, J. T., & Lipton, S. A. (1990). HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. *Science, 248*, 364–367.

Dudaronek, J. M., Barber, S. A., & Clements, J. E. (2007). CUGBP1 is required for IFNbeta-mediated induction of dominant-negative CEBPbeta and suppression of SIV replication in macrophages. *Journal of Immunology, 179*, 7262–7269.

Eggert, D., Dash, P. K., Gorantla, S., Dou, H., Schifitto, G., Maggirwar, S. B., et al. (2010). Neuroprotective activities of CEP-1347 in models of neuroAIDS. *Journal of Immunology, 184*, 746–756.

Eletto, D., Russo, G., Passiatore, G., Del, V. L., Giordano, A., Khalili, K., et al. (2008). Inhibition of SNAP25 expression by HIV-1 Tat involves the activity of mir-128a. *Journal of Cellular Physiology, 216*, 764–770.

Ellis, R., Langford, D., & Masliah, E. (2007). HIV and antiretroviral therapy in the brain: Neuronal injury and repair. *Nature Reviews Neuroscience, 8*, 33–44.

Ellis, R. J., Deutsch, R., Heaton, R. K., Marcotte, T. D., McCutchan, J. A., Nelson, J. A., et al. (1997). Neurocognitive impairment is an independent risk factor for death in HIV infection. San Diego HIV Neurobehavioral Research Center Group. *Archives of Neurology, 54*, 416–424.

Espert, L., Denizot, M., Grimaldi, M., Robert-Hebmann, V., Gay, B., Varbanov, M., et al. (2006). Autophagy is involved in T cell death after binding of HIV-1 envelope proteins to CXCR4. *Journal of Clinical Investigation, 116*, 2161–2172.

Eugenin, E. A., King, J. E., Nath, A., Calderon, T. M., Zukin, R. S., Bennett, M. V., et al. (2007). HIV-tat induces formation of an LRP-PSD-95- NMDAR-nNOS complex that promotes apoptosis in neurons and astrocytes. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 3438–3443.

Everall, I., Luthert, P., & Lantos, P. (1993). A review of neuronal damage in human immunodeficiency virus infection: Its assessment, possible mechanism and relationship to dementia. *Journal of Neuropathology and Experimental Neurology, 52*, 561–566.

Everall, I. P., Hansen, L. A., & Masliah, E. (2005). The shifting patterns of HIV encephalitis neuropathology. *Neurotoxicity Research, 8*, 51–61.

Everall, I. P., Luthert, P. J., & Lantos, P. L. (1991). Neuronal loss in the frontal cortex in HIV infection. *Lancet, 337*, 1119–1121.

Fawell, S., Seery, J., Daikh, Y., Moore, C., Chen, L. L., Pepinsky, B., et al. (1994). Tat-mediated delivery of heterologous proteins into cells. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 664–668.

Federico, M., Percario, Z., Olivetta, E., Fiorucci, G., Muratori, C., Micheli, A., et al. (2001). HIV-1 Nef activates STAT1 in human monocytes/macrophages through the release of soluble factors. *Blood, 98*, 2752–2761.

Ferris, M. J., Mactutus, C. F., & Booze, R. M. (2008). Neurotoxic profiles of HIV, psychostimulant drugs of abuse, and their concerted effect on the brain: Current status of dopamine system vulnerability in NeuroAIDS. *Neuroscience and Biobehavioral Reviews, 32*, 883–909.

Fiala, M., Looney, D. J., Stins, M., Way, D. D., Zhang, L., Gan, X., et al. (1997). TNF-alpha opens a paracellular route for HIV-1 invasion across the blood–brain barrier. *Molecular Medicine, 3*, 553–564.

Fitting, S., Scoggins, K. L., Xu, R., Dever, S. M., Knapp, P. E., Dewey, W. L., et al. (2012). Morphine efficacy is altered in conditional HIV-1 Tat transgenic mice. *European Journal of Pharmacology, 689*, 96–103.

Fitting, S., Xu, R., Bull, C., Buch, S. K., El-Hage, N., Nath, A., et al. (2010). Interactive comorbidity between opioid drug abuse and HIV-1 Tat: Chronic exposure augments spine loss and sublethal dendritic pathology in striatal neurons. *American Journal of Pathology, 177*, 1397–1410.

Fontana, G., Valenti, L., & Raiteri, M. (1997). gp120 can revert antagonism at the glycine site of NMDA receptors mediating GABA release from cultured hippocampal neurons. *Journal of Neuroscience Research, 49*, 732–738.

Gallo, K. A., & Johnson, G. L. (2002). Mixed-lineage kinase control of JNK and p38 MAPK pathways. *Nature Reviews Molecular Cell Biology, 3*, 663–672.

Gannon, P., Khan, M. Z., & Kolson, D. L. (2011). Current understanding of HIV-associated neurocognitive disorders pathogenesis. *Current Opinion in Neurology, 24*, 275–283.

Garden, G. A., Budd, S. L., Tsai, E., Hanson, L., Kaul, M., D'Emilia, D. M., et al. (2002). Caspase cascades in human immunodeficiency virus-associated neurodegeneration. *Journal of Neuroscience, 22*, 4015–4024.

Garden, G. A., Guo, W., Jayadev, S., Tun, C., Balcaitis, S., Choi, J., et al. (2004). HIV associated neurodegeneration requires p53 in neurons and microglia. *The FASEB Journal, 18*, 1141–1143.

Gartner, S. (2000). HIV infection and dementia. *Science, 287*, 602–604.

Garzino-Demo, A., Devico, A. L., Cocchi, F., & Gallo, R. C. (1998). Beta-chemokines and protection from HIV type 1 disease. *AIDS Research and Human Retroviruses, 14*(Suppl 2), S177–S184.

Gatignol, A., Dubuisson, J., Wainberg, M. A., Cohen, E. A., & Darlix, J. L. (2007). New pandemics: HIV and AIDS, HCV and chronic hepatitis, influenza virus and flu. *Retrovirology, 4*(8), 8.

Gelbard, H. A., Nottet, H. S., Swindells, S., Jett, M., Dzenko, K. A., Genis, P., et al. (1994). Platelet-activating factor: A candidate human immunodeficiency virus type 1-induced neurotoxin. *Journal of Virology, 68*, 4628–4635.

Gelman, B. B., Soukup, V. M., Schuenke, K. W., Keherly, M. J., Holzer, C., III, Richey, F. J., et al. (2004). Acquired neuronal channelopathies in HIV-associated dementia. *Journal of Neuroimmunology, 157*, 111–119.

Gendelman, H. E., Grant, I., Lipton, S. A., Everall, I., & Swindells, S. (2005). *The neurology of AIDS* (2nd ed.). London: Oxford University Press.

Ghezzi, S., Noonan, D. M., Aluigi, M. G., Vallanti, G., Cota, M., Benelli, R., et al. (2000). Inhibition of CXCR4-dependent HIV-1 infection by extracellular HIV-1 Tat. *Biochemical and Biophysical Research Communications, 270*, 992–996.

Giancola, M. L., Lorenzini, P., Balestra, P., Larussa, D., Baldini, F., Corpolongo, A., et al. (2006). Neuroactive antiretroviral drugs do not influence neurocognitive performance in less advanced HIV-infected patients responding to highly active antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes, 41*, 332–337.

Giulian, D., Vaca, K., & Noonan, C. A. (1990). Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. *Science, 250*, 1593–1596.

Giulian, D., Wendt, E., Vaca, K., & Noonan, C. A. (1993). The envelope glycoprotein of human immunodeficiency virus type 1 stimulates release of neurotoxins from monocytes. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 2769–2773.

Glass, J. D., Fedor, H., Wesselingh, S. L., & McArthur, J. C. (1995). Immunocytochemical quantitation of human immunodeficiency virus in the brain: Correlations with dementia. *Annals of Neurology, 38*, 755–762.

Gonzalez, E., Rovin, B. H., Sen, L., Cooke, G., Dhanda, R., Mummidi, S., et al. (2002). HIV-1 infection and AIDS dementia are influenced by a mutant MCP-1 allele linked to increased monocyte infiltration of tissues and MCP-1 levels. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 13795–13800.

Gonzalez-Scarano, F., & Martin-Garcia, J. (2005). The neuropathogenesis of AIDS. *Nature Reviews Immunology, 5*, 69–81.

Gorantla, S., Makarov, E., Finke-Dwyer, J., Castanedo, A., Holguin, A., Gebhart, C. L., et al. (2010). Links between progressive HIV-1 infection of humanized mice and viral neuropathogenesis. *American Journal of Pathology, 177*, 2938–2949.

Gorantla, S., Poluektova, L., & Gendelman, H. E. (2012). Rodent models for HIV-associated neurocognitive disorders. *Trends in Neurosciences, 35*, 197–208.

Gorry, P. R., Churchill, M., Crowe, S. M., Cunningham, A. L., & Gabuzda, D. (2005). Pathogenesis of macrophage tropic HIV-1. *Current HIV Research, 3*, 53–60.

Gras, G., & Kaul, M. (2010). Molecular mechanisms of neuroinvasion by monocytes-macrophages in HIV-1 infection. *Retrovirology, 7*, 30.

Graus, F., Ribalta, T., Abos, J., Alom, J., Cruz-Sanchez, F., Mallolas, J., et al. (1990). Subacute cerebellar syndrome as the first manifestation of AIDS dementia complex. *Acta Neurologica Scandinavica, 81*, 118–120.

Green, D. A., Masliah, E., Vinters, H. V., Beizai, P., Moore, D. J., & Achim, C. L. (2005). Brain deposition of beta-amyloid is a common pathologic feature in HIV positive patients. *AIDS, 19*, 407–411.

Hahn, B. H., Shaw, G. M., Arya, S. K., Popovic, M., Gallo, R. C., & Wong-Staal, F. (1984). Molecular cloning and characterization of the HTLV-III virus associated with AIDS. *Nature, 312*, 166–169.

Haughey, N. J., Cutler, R. G., Tamara, A., McArthur, J. C., Vargas, D. L., Pardo, C. A., et al. (2004). Perturbation of sphingolipid metabolism and ceramide production in HIV-dementia. *Annals of Neurology, 55*, 257–267.

Hauser, K. F., El-Hage, N., Buch, S., Nath, A., Tyor, W. R., Bruce-Keller, A. J., et al. (2006). Impact of opiate-HIV-1 interactions on neurotoxic signaling. *Journal of Neuroimmune Pharmacology, 1*, 98–105.

He, J., Chen, Y., Farzan, M., Choe, H., Ohagen, A., Gartner, S., et al. (1997a). CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature, 385*, 645–649.

He, J., deCastro, C. M., Vandenbark, G. R., Busciglio, J., & Gabuzda, D. (1997b). Astrocyte apoptosis induced by HIV-1 transactivation of the c-kit protooncogene. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 3954–3959.

He, N., Liu, M., Hsu, J., Xue, Y., Chou, S., Burlingame, A., et al. (2010). HIV-1 Tat and host AFF4 recruit two transcription elongation factors into a bifunctional complex for coordinated activation of HIV-1 transcription. *Molecular Cell, 38*, 428–438.

Heaton, R. K., Clifford, D. B., Franklin, D. R., Jr., Woods, S. P., Ake, C., Vaida, F., et al. (2010). HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER study. *Neurology, 75*, 2087–2096.

Heaton, R. K., Franklin, D. R., Ellis, R. J., McCutchan, J. A., Letendre, S. L., Leblanc, S., et al. (2011). HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: Differences in rates, nature, and predictors. *Journal of Neurovirology, 17*, 3–16.

Herbein, G., Gras, G., Khan, K. A., & Abbas, W. (2010). Macrophage signaling in HIV-1 infection. *Retrovirology, 7*, 34.

Hesselgesser, J., Halks-Miller, M., DelVecchio, V., Peiper, S. C., Hoxie, J., Kolson, D. L., et al. (1997). CD4-Independent association between HIV-1 gp120 and CXCR4: Functional chemokine receptors are expressed in human neurons. *Current Biology, 7*, 112–121.

Hesselgesser, J., Taub, D., Baskar, P., Greenberg, M., Hoxie, J., Kolson, D. L., et al. (1998). Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1 alpha is mediated by the chemokine receptor CXCR4. *Current Biology, 8*, 595–598.

Heyes, M. P., Brew, B. J., Martin, A., Price, R. W., Salazar, A. M., Sidtis, J. J., et al. (1991). Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: Relationship to clinical and neurological status. *Annals of Neurology, 29*, 202–209.

Ho, D. D., Bredesen, D. E., Vinters, H. V., & Daar, E. S. (1989). The acquired immunodeficiency syndrome (AIDS) dementia complex. *Annals of Internal Medicine, 111*, 400–410.

Ho, D. D., Rota, T. R., Schooley, R. T., Kaplan, J. C., Allan, J. D., Groopman, J. E., et al. (1985). Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. *The New England Journal of Medicine, 313*, 1493–1497.

Huang, M. B., Weeks, O., Zhao, L. J., Saltarelli, M., & Bond, V. C. (2000). Effects of extracellular human immunodeficiency virus type 1 vpr protein in primary rat cortical cell cultures. *Journal of Neurovirology, 6*, 202–220.

Hui, L., Chen, X., Haughey, N. J., & Geiger, J. D. (2012). Role of endolysosomes in HIV-1 Tat-induced neurotoxicity. *ASN Neuro, 4*, 243–252.

Iskander, S., Walsh, K. A., & Hammond, R. R. (2004). Human CNS cultures exposed to HIV-1 gp120 reproduce dendritic injuries of HIV-1-associated dementia. *Journal of Neuroinflammation, 1*, 7.

Jacotot, E., Ferri, K. F., El, H. C., Brenner, C., Druillennec, S., Hoebeke, J., et al. (2001). Control of mitochondrial membrane permeabilization by adenine nucleotide translocator interacting with HIV-1 viral protein rR and Bcl-2. *The Journal of Experimental Medicine, 193*, 509–519.

Jacotot, E., Ravagnan, L., Loeffler, M., Ferri, K. F., Vieira, H. L., Zamzami, N., et al. (2000). The HIV-1 viral protein R induces apoptosis via a direct effect on the mitochondrial permeability transition pore. *The Journal of Experimental Medicine, 191*, 33–46.

Jana, A., & Pahan, K. (2004). Human immunodeficiency virus type 1 gp120 induces apoptosis in human primary neurons through redox-regulated activation of neutral sphingomyelinase. *Journal of Neuroscience, 24*, 9531–9540.

Johnston, J. B., Jiang, Y., van Marle, G., Mayne, M. B., Ni, W., Holden, J., et al. (2000). Lentivirus infection in the brain induces matrix metalloproteinase expression: Role of envelope diversity. *Journal of Virology, 74*, 7211–7220.

Jones, G., & Power, C. (2006). Regulation of neural cell survival by HIV-1 infection. *Neurobiology of Disease, 21*, 1–17.

Jones, G. J., Barsby, N. L., Cohen, E. A., Holden, J., Harris, K., Dickie, P., et al. (2007). HIV-1 Vpr causes neuronal apoptosis and in vivo neurodegeneration. *Journal of Neuroscience, 27*, 3703–3711.

Jordan, B. D., Navia, B. A., Petito, C., Cho, E. S., & Price, R. W. (1985). Neurological syndromes complicating AIDS. *Frontiers of Radiation Therapy and Oncology, 19*, 82–87.

Jordan-Sciutto, K. L., Wang, G., Murphey-Corb, M., & Wiley, C. A. (2002). Cell cycle proteins exhibit altered expression patterns in lentiviral-associated encephalitis. *Journal of Neuroscience, 22*, 2185–2195.

Kaminska, B., Gozdz, A., Zawadzka, M., Ellert-Miklaszewska, A., & Lipko, M. (2009). MAPK signal transduction underlying brain inflammation and gliosis as therapeutic target. *Anatomical Record (Hoboken), 292*, 1902–1913.

Kapadia, F., Vlahov, D., Donahoe, R. M., & Friedland, G. (2005). The role of substance abuse in HIV disease progression: Reconciling differences from laboratory and epidemiologic investigations. *Clinical Infectious Diseases, 41*, 1027–1034.

Kaul, M. (2008). HIV's Double strike at the brain: Neuronal toxicity and compromised neurogenesis. *Frontiers in Bioscience, 13*, 2484–2494.

Kaul, M. (2009). HIV-1 associated dementia: Update on pathological mechanisms and therapeutic approaches. *Current Opinion in Neurology, 22*, 315–320.

Kaul, M., Garden, G. A., & Lipton, S. A. (2001). Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature, 410*, 988–994.

Kaul, M., & Lipton, S. A. (1999). Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 8212–8216.

Kaul, M., Ma, Q., Medders, K. E., Desai, M. K., & Lipton, S. A. (2007). HIV-1 coreceptors CCR5 and CXCR4 both mediate neuronal cell death but CCR5 paradoxically can also contribute to protection. *Cell Death and Differentiation, 14*, 296–305.

Kaul, M., Zheng, J., Okamoto, S., Gendelman, H. E., & Lipton, S. A. (2005). HIV-1 infection and AIDS: Consequences for the central nervous system. *Cell Death and Differentiation, 12*(Suppl 1), 878–892.

Ketzler, S., Weis, S., Haug, H., & Budka, H. (1990). Loss of neurons in the frontal cortex in AIDS brains. *Acta Neuropathologica (Berlin), 80*, 92–94.

Khan, M. Z., Brandimarti, R., Musser, B. J., Resue, D. M., Fatatis, A., & Meucci, O. (2003). The chemokine receptor CXCR4 regulates cell-cycle proteins in neurons. *Journal of Neurovirology, 9*, 300–314.

Khan, M. Z., Brandimarti, R., Patel, J. P., Huynh, N., Wang, J., Huang, Z., et al. (2004). Apoptotic and antiapoptotic effects of CXCR4: Is it a matter of intrinsic efficacy? Implications for HIV neuropathogenesis. *AIDS Research and Human Retroviruses, 20*, 1063–1071.

Khan, M. Z., Shimizu, S., Patel, J. P., Nelson, A., Le, M. T., Mullen-Przeworski, A., et al. (2005). Regulation of neuronal P53 activity by CXCR4. *Molecular and Cellular Neuroscience, 30*, 58–66.

Kim, B. O., Liu, Y., Ruan, Y., Xu, Z. C., Schantz, L., & He, J. J. (2003). Neuropathologies in transgenic mice expressing human immunodeficiency virus type 1 Tat protein under the regulation of the astrocyte-specific glial fibrillary acidic protein promoter and doxycycline. *American Journal of Pathology, 162*, 1693–1707.

Kitai, R., Zhao, M. L., Zhang, N., Hua, L. L., & Lee, S. C. (2000). Role of MIP-1beta and RANTES in HIV-1 infection of microglia: Inhibition of infection and induction by IFNbeta. *Journal of Neuroimmunology, 110*, 230–239.

Kitayama, H., Miura, Y., Ando, Y., Hoshino, S., Ishizaka, Y., & Koyanagi, Y. (2008). Human immunodeficiency virus type 1 Vpr inhibits axonal outgrowth through induction of mitochondrial dysfunction. *Journal of Virology, 82*, 2528–2542.

Koedel, U., Kohleisen, B., Sporer, B., Lahrtz, F., Ovod, V., Fontana, A., et al. (1999). HIV type 1 Nef protein is a viral factor for leukocyte recruitment into the central nervous system. *Journal of Immunology, 163*, 1237–1245.

Kramer-Hammerle, S., Rothenaigner, I., Wolff, H., Bell, J. E., & Brack-Werner, R. (2005). Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. *Virus Research, 111*, 194–213.

Krathwohl, M. D., & Kaiser, J. L. (2004a). Chemokines promote quiescence and survival of human neural progenitor cells. *Stem Cells, 22*, 109–118.

Krathwohl, M. D., & Kaiser, J. L. (2004b). HIV-1 promotes quiescence in human neural progenitor cells. *Journal of Infectious Diseases, 190*, 216–226.

Krucker, T., Toggas, S. M., Mucke, L., & Siggins, G. R. (1998). Transgenic mice with cerebral expression of human immunodeficiency virus type-1 coat protein gp120 show divergent changes in short- and long-term potentiation in CA1 hippocampus. *Neuroscience, 83*, 691–700.

Langford, T. D., Letendre, S. L., Larrea, G. J., & Masliah, E. (2003). Changing patterns in the neuropathogenesis of HIV during the HAART era. *Brain Pathology, 13*, 195–210.

Lapidot, T., & Petit, I. (2002). Current understanding of stem cell mobilization: The roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. *Experimental Hematology, 30*, 973–981.

Lavi, E., Kolson, D. L., Ulrich, A. M., Fu, L., & Gonzalez-Scarano, F. (1998). Chemokine receptors in the human brain and their relationship to HIV infection. *Journal of Neurovirology, 4*, 301–311.

Lawrence, D. M., Durham, L. C., Schwartz, L., Seth, P., Maric, D., & Major, E. O. (2004). Human immunodeficiency virus type 1 infection of human brain-derived progenitor cells. *Journal of Virology, 78*, 7319–7328.

Letendre, S., Marquie-Beck, J., Capparelli, E., Best, B., Clifford, D., Collier, A. C., et al. (2008). Validation of the CNS Penetration-Effectiveness rank for quantifying antiretroviral penetration into the central nervous system. *Archives of Neurology, 65*, 65–70.

Letendre, S. L., McCutchan, J. A., Childers, M. E., Woods, S. P., Lazzaretto, D., Heaton, R. K., et al. (2004). Enhancing antiretroviral therapy for human immunodeficiency virus cognitive disorders. *Annals of Neurology, 56*, 416–423.

Li, S., Juarez, J., Alali, M., Dwyer, D., Collman, R., Cunningham, A., et al. (1999). Persistent CCR5 utilization and enhanced macrophage tropism by primary blood human immunodeficiency virus type 1 isolates from advanced stages of disease and comparison to tissue-derived isolates. *Journal of Virology, 73*, 9741–9755.

Li, W., Henderson, L. J., Major, E. O., & al-Harthi, L. (2011). IFN-gamma mediates enhancement of HIV replication in astrocytes by inducing an antagonist of the beta-catenin pathway (DKK1) in a STAT 3-dependent manner. *Journal of Immunology, 186*, 6771–6778.

Li, W., Huang, Y., Reid, R., Steiner, J., Malpica-Llanos, T., Darden, T. A., et al. (2008). NMDA receptor activation by HIV-Tat protein is clade dependent. *Journal of Neuroscience, 28*, 12190–12198.

Lindl, K. A., Akay, C., Wang, Y., White, M. G., & Jordan-Sciutto, K. L. (2007). Expression of the endoplasmic reticulum stress response marker, BiP, in the central nervous system of HIV-positive individuals. *Neuropathology and Applied Neurobiology, 33*, 658–669.

Lindl, K. A., Marks, D. R., Kolson, D. L., & Jordan-Sciutto, K. L. (2010). HIV-associated neurocognitive disorder: Pathogenesis and therapeutic opportunities. *Journal of Neuroimmune Pharmacology, 5*, 294–309.

Liner, K. J., Hall, C. D., & Robertson, K. R. (2008). Effects of antiretroviral therapy on cognitive impairment. *Current HIV/AIDS Reports, 5*, 64–71.

Liner, K. J., Ro, M. J., & Robertson, K. R. (2010). HIV, antiretroviral therapies, and the brain. *Current HIV/AIDS Reports, 7*, 85–91.

Lipton, S. A. (1997). Treating AIDS dementia. *Science, 276*, 1629–1630 (letter; comment).

Lipton, S. A., & Rosenberg, P. A. (1994). Excitatory amino acids as a final common pathway for neurologic disorders. *The New England Journal of Medicine, 330*, 613–622 (see comments).

Lipton, S. A., Sucher, N. J., Kaiser, P. K., & Dreyer, E. B. (1991). Synergistic effects of HIV coat protein and NMDA receptor-mediated neurotoxicity. *Neuron, 7*, 111–118.

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., et al. (1996). Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell, 86*, 367–377.

Liu, Y., Jones, M., Hingtgen, C. M., Bu, G., Laribee, N., Tanzi, R. E., et al. (2000). Uptake of HIV-1 Tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nature Medicine, 6*, 1380–1387.

Liu, Y., Liu, H., Kim, B. O., Gattone, V. H., Li, J., Nath, A., et al. (2004). CD4-Independent infection of astrocytes by human immunodeficiency virus type 1: Requirement for the human mannose receptor. *Journal of Virology, 78*, 4120–4133.

Locati, M., & Murphy, P. M. (1999). Chemokines and chemokine receptors: Biology and clinical relevance in inflammation and AIDS. *Annual Review of Medicine, 50*, 425–440.

Loewenstein, R. J., & Sharfstein, S. S. (1983). Neuropsychiatric aspects of acquired immune deficiency syndrome. *International Journal of Psychiatry in Medicine, 13*, 255–260.

Louboutin, J. P., Agrawal, L., Reyes, B. A., Van Bockstaele, E. J., & Strayer, D. S. (2007). Protecting neurons from HIV-1 gp120-induced oxidant stress using both localized intracerebral and generalized intraventricular administration of antioxidant enzymes delivered by SV40-derived vectors. *Gene Therapy, 14*, 1650–1661.

Louboutin, J. P., Agrawal, L., Reyes, B. A., Van Bockstaele, E. J., & Strayer, D. S. (2010). HIV-1 gp120-induced injury to the blood–brain barrier: Role of metalloproteinases 2 and 9 and relationship to oxidative stress. *Journal of Neuropathology and Experimental Neurology, 69*, 801–816.

Ma, Q., Jones, D., Borghesani, P. R., Segal, R. A., Nagasawa, T., Kishimoto, T., et al. (1998). Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 9448–9453.

Mabrouk, K., Van Rietschoten, J., Vives, E., Darbon, H., Rochat, H., & Sabatier, J. M. (1991). Lethal neurotoxicity in mice of the basic domains of HIV and SIV Rev proteins. Study of these regions by circular dichroism. *FEBS Letters, 289*, 13–17.

Maggirwar, S. B., Tong, N., Ramirez, S., Gelbard, H. A., & Dewhurst, S. (1999). HIV-1 Tat-mediated activation of glycogen synthase kinase-3beta contributes to Tat-mediated neurotoxicity. *Journal of Neurochemistry, 73*, 578–586.

Mandl, J. N., Barry, A. P., Vanderford, T. H., Kozyr, N., Chavan, R., Klucking, S., et al. (2008). Divergent TLR7 and TLR9 signaling and type I interferon production distinguish pathogenic and nonpathogenic AIDS virus infections. *Nature Medicine, 14*, 1077–1087.

Marra, C. M., Zhao, Y., Clifford, D. B., Letendre, S., Evans, S., Henry, K., et al. (2009). Impact of combination antiretroviral therapy on cerebrospinal fluid HIV RNA and neurocognitive performance. *AIDS, 23*, 1359–1366.

Masliah, E., Ge, N., Morey, M., DeTeresa, R., Terry, R. D., & Wiley, C. A. (1992). Cortical dendritic pathology in human immunodeficiency virus encephalitis. *Laboratory Investigation, 66*, 285–291.

Masliah, E., Heaton, R. K., Marcotte, T. D., Ellis, R. J., Wiley, C. A., Mallory, M., et al. (1997). Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. HNRC group. The HIV Neurobehavioral Research Center. *Annals of Neurology, 42*, 963–972.

Mattson, M. P., Haughey, N. J., & Nath, A. (2005). Cell death in HIV dementia. *Cell Death and Differentiation, 12*(Suppl 1), 893–904.

Maung, R., Medders, K. E., Sejbuk, N. E., Desai, M. K., Russo, R., & Kaul, M. (2012). Genetic knockouts suggest a critical role for HIV co-receptors in models of HIV gp120-induced brain injury. *Journal of Neuroimmune Pharmacology, 7*, 306–318.

McArthur, J. C., Haughey, N., Gartner, S., Conant, K., Pardo, C., Nath, A., et al. (2003). Human immunodeficiency virus-associated dementia: An evolving disease. *Journal of Neurovirology, 9*, 205–221.

McArthur, J. C., Hoover, D. R., Bacellar, H., Miller, E. N., Cohen, B. A., Becker, J. T., et al. (1993). Dementia in AIDS patients: Incidence and risk factors. Multicenter AIDS Cohort Study. *Neurology, 43*, 2245–2252.

Medders, K. E., Sejbuk, N. E., Maung, R., Desai, M. K., & Kaul, M. (2010). Activation of p38 MAPK is required in monocytic and neuronal cells for HIV glycoprotein 120-induced neurotoxicity. *Journal of Immunology, 185*, 4883–4895.

Meucci, O., Fatatis, A., Simen, A. A., Bushell, T. J., Gray, P. W., & Miller, R. J. (1998). Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 14500–14505.

Meucci, O., Fatatis, A., Simen, A. A., & Miller, R. J. (2000). Expression of CX3CR1 chemokine receptors on neurons and their role in neuronal survival. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 8075–8080.

Michael, N. L., & Moore, J. P. (1999). HIV-1 entry inhibitors: Evading the issue [news] [see comments]. *Nature Medicine, 5*, 740–742.

Miller, R. J., & Meucci, O. (1999). AIDS and the brain: Is there a chemokine connection? *Trends in Neurosciences, 22*, 471–479.

Minghetti, L. (2005). Role of inflammation in neurodegenerative diseases. *Current Opinion in Neurology, 18*, 315–321.

Mishra, M., Taneja, M., Malik, S., Khalique, H., & Seth, P. (2010). Human immunodeficiency virus type 1 Tat modulates proliferation and differentiation of human neural precursor cells: Implication in NeuroAIDS. *Journal of Neurovirology, 16*, 355–367.

Mitchell, S. J., Morris, S. R., Kent, C. K., Stansell, J., & Klausner, J. D. (2006). Methamphetamine use and sexual activity among HIV-infected patients in care–San Francisco, 2004. *AIDS Patient Care and STDs, 20*, 502–510.

Miura, Y., Koyanagi, Y., & Mizusawa, H. (2003a). TNF-related apoptosis-inducing ligand (TRAIL) induces neuronal apoptosis in HIV-encephalopathy. *Journal of Medical and Dental Sciences, 50*, 17–25.

Miura, Y., Misawa, N., Kawano, Y., Okada, H., Inagaki, Y., Yamamoto, N., et al. (2003b). Tumor necrosis factor-related apoptosis-inducing ligand induces neuronal death in a murine model of HIV central nervous system infection. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 2777–2782.

Miyazaki, Y., Takamatsu, T., Nosaka, T., Fujita, S., Martin, T. E., & Hatanaka, M. (1995). The cytotoxicity of human immunodeficiency virus type 1 Rev: Implications for its interaction with the nucleolar protein B23. *Experimental Cell Research, 219*, 93–101.

Mocchetti, I., Bachis, A., & Avdoshina, V. (2012). Neurotoxicity of human immunodeficiency virus-1: Viral proteins and axonal transport. *Neurotoxicity Research, 21*, 79–89.

Mollace, V., Nottet, H. S., Clayette, P., Turco, M. C., Muscoli, C., Salvemini, D., et al. (2001). Oxidative stress and neuroAIDS: Triggers, modulators and novel antioxidants. *Trends in Neurosciences, 24*, 411–416.

Mucke, L., Abraham, C. R., Ruppe, M. D., Rockenstein, E. M., Toggas, S. M., Mallory, M., et al. (1995). Protection against HIV-1 gp120-induced brain damage by neuronal expression of human amyloid precursor protein. *The Journal of Experimental Medicine, 181*, 1551–1556.

Mukerjee, R., Deshmane, S. L., Fan, S., Del, V. L., White, M. K., Khalili, K., et al. (2008). Involvement of the p53 and p73 transcription factors in neuroAIDS. *Cell Cycle, 7*, 2682–2690.

Nath, A. (2010). Human immunodeficiency virus-associated neurocognitive disorder: Pathophysiology in relation to drug addiction. *Annals of the NewYork Academy of Sciences, 1187*, 122–128.

Nath, A., & Sacktor, N. (2006). Influence of highly active antiretroviral therapy on persistence of HIV in the central nervous system. *Current Opinion in Neurology, 19*, 358–361.

Navia, B. A., Cho, E. S., Petito, C. K., & Price, R. W. (1986a). The AIDS dementia complex: II Neuropathology. *Annals of Neurology, 19*, 525–535.

Navia, B. A., Jordan, B. D., & Price, R. W. (1986b). The AIDS dementia complex: I Clinical features. *Annals of Neurology, 19*, 517–524.

Neri, E., Musante, V., & Pittaluga, A. (2007). Effects of the HIV-1 viral protein TAT on central neurotransmission: Role of group I metabotropic glutamate receptors. *International Review of Neurobiology, 82*, 339–356.

New, D. R., Ma, M., Epstein, L. G., Nath, A., & Gelbard, H. A. (1997). Human immunodeficiency virus type 1 tat protein induces death by apoptosis in primary human neuron cultures. *Journal of Neurovirology, 3*, 168–173.

Nicotera, P., Ankarcrona, M., Bonfoco, E., Orrenius, S., & Lipton, S. A. (1997). Neuronal necrosis and apoptosis: Two distinct events induced by exposure to glutamate or oxidative stress. *Advances in Neurology, 72*, 95–101.

Norman, J. P., Perry, S. W., Reynolds, H. M., Kiebala, M., De Mesy Bentley, K. L., Trejo, M., et al. (2008). HIV-1 Tat activates neuronal ryanodine receptors with rapid induction of the unfolded protein response and mitochondrial hyperpolarization. *PLoS One, 3*, e3731.

Nottet, H. S., Persidsky, Y., Sasseville, V. G., Nukuna, A. N., Bock, P., Zhai, Q. H., et al. (1996). Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. *Journal of Immunology, 156*, 1284–1295.

O'Donnell, L. A., Agrawal, A., Jordan-Sciutto, K. L., Dichter, M. A., Lynch, D. R., & Kolson, D. L. (2006). Human immunodeficiency virus (HIV)-induced neurotoxicity: Roles for the NMDA receptor subtypes. *Journal of Neuroscience, 26*, 981–990.

Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J. L., Arenzana-Seisdedos, F., et al. (1996). The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature, 382*, 833–835.

Ohagen, A., Ghosh, S., He, J., Huang, K., Chen, Y., Yuan, M., et al. (1999). Apoptosis induced by infection of primary brain cultures with diverse human immunodeficiency virus type 1 isolates: Evidence for a role of the envelope. *Journal of Virology, 73*, 897–906.

Okamoto, S., Kang, Y. J., Brechtel, C. W., Siviglia, E., Russo, R., Clemente, A., et al. (2007). HIV/gp120 decreases adult neural progenitor cell proliferation via checkpoint kinase-mediated cell-cycle withdrawal and G1 arrest. *Cell Stem Cell, 1*, 230–236.

Olivetta, E., Percario, Z., Fiorucci, G., Mattia, G., Schiavoni, I., Dennis, C., et al. (2003). HIV-1 Nef induces the release of inflammatory factors from human monocyte/macrophages: Involvement of Nef endocytotic signals and NF-kappa B activation. *Journal of Immunology, 170*, 1716–1727.

Olney, J. W. (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science, 164*, 719–721.

Park, D. S., Obeidat, A., Giovanni, A., & Greene, L. A. (2000). Cell cycle regulators in neuronal death evoked by excitotoxic stress: Implications for neurodegeneration and its treatment. *Neurobiology of Aging, 21*, 771–781.

Patel, K., Ming, X., Williams, P. L., Robertson, K. R., Oleske, J. M., & Seage, G. R., III. (2009). Impact of HAART and CNS-penetrating antiretroviral regimens on HIV encephalopathy among perinatally infected children and adolescents. *AIDS, 23*, 1893–1901.

Peng, H., Whitney, N., Wu, Y., Tian, C., Dou, H., Zhou, Y., et al. (2008). HIV-1-infected and/or immune-activated macrophage-secreted TNF-alpha affects human fetal cortical neural progenitor cell proliferation and differentiation. *Glia, 56*, 903–916.

Perfettini, J. L., Castedo, M., Nardacci, R., Ciccosanti, F., Boya, P., Roumier, T., et al. (2005a). Essential role of p53 phosphorylation by p38 MAPK in apoptosis induction by the HIV-1 envelope. *The Journal of Experimental Medicine, 201*, 279–289.

Perfettini, J. L., Castedo, M., Roumier, T., Andreau, K., Nardacci, R., Piacentini, M., et al. (2005b). Mechanisms of apoptosis induction by the HIV-1 envelope. *Cell Death and Differentiation, 12*(Suppl 1), 916–923.

Persidsky, Y., Buttini, M., Limoges, J., Bock, P., & Gendelman, H. E. (1997). An analysis of HIV-1-associated inflammatory products in brain tissue of humans and SCID mice with HIV-1 encephalitis. *Journal of Neurovirology, 3*, 401–416.

Persidsky, Y., Limoges, J., McComb, R., Bock, P., Baldwin, T., Tyor, W., et al. (1996). Human immunodeficiency virus encephalitis in SCID mice. *American Journal of Pathology, 149*, 1027–1053.

Petito, C. K., Cho, E. S., Lemann, W., Navia, B. A., & Price, R. W. (1986). Neuropathology of acquired immunodeficiency syndrome (AIDS): An autopsy review. *Journal of Neuropathology and Experimental Neurology, 45*, 635–646.

Petito, C. K., Navia, B. A., Cho, E. S., Jordan, B. D., George, D. C., & Price, R. W. (1985). Vacuolar myelopathy pathologically resembling subacute combined degeneration in patients with the acquired immunodeficiency syndrome. *The New England Journal of Medicine, 312*, 874–879.

Petito, C. K., & Roberts, B. (1995). Evidence of apoptotic cell death in HIV encephalitis. *American Journal of Pathology, 146*, 1121–1130.

Pickering, M., Cumiskey, D., & O'Connor, J. J. (2005). Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system. *Experimental Physiology, 90*, 663–670.

Piller, S. C., Jans, P., Gage, P. W., & Jans, D. A. (1998). Extracellular HIV-1 virus protein R causes a large inward current and cell death in cultured hippocampal neurons: Implications for AIDS pathology. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 4595–4600.

Pomerantz, R. J. (2004). Effects of HIV-1 Vpr on neuroinvasion and neuropathogenesis. *DNA and Cell Biology, 23*, 227–238.

Porcheray, F., Samah, B., Leone, C., Dereuddre-Bosquet, N., & Gras, G. (2006). Macrophage activation and human immunodeficiency virus infection: HIV replication directs macrophages towards a pro-inflammatory phenotype while previous activation modulates macrophage susceptibility to infection and viral production. *Virology, 349*, 112–120.

Power, C., Boisse, L., Rourke, S., & Gill, M. J. (2009). NeuroAIDS: An evolving epidemic. *Canadian Journal of Neurological Sciences, 36*, 285–295.

Power, C., Hui, E., Vivithanaporn, P., Acharjee, S., & Polyak, M. (2012). Delineating HIV-associated neurocognitive disorders using transgenic models: The neuropathogenic actions of Vpr. *Journal of Neuroimmune Pharmacology, 7*, 319–331.

Power, C., McArthur, J. C., Johnson, R. T., Griffin, D. E., Glass, J. D., Perryman, S., et al. (1994). Demented and nondemented patients with AIDS differ in brain-derived human immunodeficiency virus type 1 envelope sequences. *Journal of Virology, 68*, 4643–4649.

Power, C., McArthur, J. C., Nath, A., Wehrly, K., Mayne, M., Nishio, J., et al. (1998). Neuronal death induced by brain-derived human immunodeficiency virus type 1 envelope genes differs between demented and nondemented AIDS patients. *Journal of Virology, 72*, 9045–9053.

Reyes, M. G., Faraldi, F., Senseng, C. S., Flowers, C., & Fariello, R. (1991). Nigral degeneration in acquired immune deficiency syndrome (AIDS). *Acta Neuropathologica (Berlin), 82*, 39–44.

Rippeth, J. D., Heaton, R. K., Carey, C. L., Marcotte, T. D., Moore, D. J., Gonzalez, R., et al. (2004). Methamphetamine dependence increases risk of neuropsychological impairment in HIV infected persons. *Journal of International Neuropsychological Society, 10*, 1–14.

Roederer, M., Ela, S. W., Staal, F. J., Herzenberg, L. A., & Herzenberg, L. A. (1992). N-acetylcysteine: A new approach to anti-HIV therapy. *AIDS Research and Human Retroviruses, 8*, 209–217.

Rohr, O., Schwartz, C., Hery, C., Aunis, D., Tardieu, M., & Schaeffer, E. (2000). The nuclear receptor chicken ovalbumin upstream promoter transcription factor interacts with HIV-1 Tat and stimulates viral replication in human microglial cells. *Journal of Biological Chemistry, 275*, 2654–2660.

Rostasy, K., Monti, L., Yiannoutsos, C., Wu, J., Bell, J., Hedreen, J., et al. (2000). NFkappaB activation, TNF-alpha expression, and apoptosis in the AIDS-Dementia-Complex. *Journal of Neurovirology, 6*, 537–543.

Ryan, L. A., Peng, H., Erichsen, D. A., Huang, Y., Persidsky, Y., Zhou, Y., et al. (2004). TNF-related apoptosis-inducing ligand mediates human neuronal apoptosis: Links to HIV-1-associated dementia. *Journal of Neuroimmunology, 148*, 127–139.

Sas, A. R., Bimonte-Nelson, H. A., & Tyor, W. R. (2007). Cognitive dysfunction in HIV encephalitic SCID mice correlates with levels of Interferon-alpha in the brain. *AIDS, 21*, 2151–2159.

Schwartz, L., & Major, E. O. (2006). Neural progenitors and HIV-1-associated central nervous system disease in adults and children. *Current HIV Research, 4*, 319–327.

Schweinsburg, B. C., Taylor, M. J., Alhassoon, O. M., Gonzalez, R., Brown, G. G., Ellis, R. J., et al. (2005). Brain mitochondrial injury in human immunodeficiency virus-seropositive (HIV+) individuals taking nucleoside reverse transcriptase inhibitors. *Journal of Neurovirology, 11*, 356–364.

Semenova, M. M., Maki-Hokkonen, A. M., Cao, J., Komarovski, V., Forsberg, K. M., Koistinaho, M., et al. (2007). Rho mediates calcium-dependent activation of p38alpha and subsequent excitotoxic cell death. *Nature Neuroscience, 10*, 436–443.

Shi, B., Raina, J., Lorenzo, A., Busciglio, J., & Gabuzda, D. (1998). Neuronal apoptosis induced by HIV-1 Tat protein and TNF-alpha: Potentiation of neurotoxicity mediated by oxidative stress and implications for HIV-1 dementia. *Journal of Neurovirology, 4*, 281–290.

Shiramizu, B., Gartner, S., Williams, A., Shikuma, C., Ratto-Kim, S., Watters, M., et al. (2005). Circulating proviral HIV DNA and HIV-associated dementia. *AIDS, 19*, 45–52.

Shiramizu, B., Ratto-Kim, S., Sithinamsuwan, P., Nidhinandana, S., Thitivichianlert, S., Watt, G., et al. (2006). HIV DNA and dementia in treatment-naive HIV-1-infected individuals in Bangkok, Thailand. *International Journal of Medical Sciences, 4*, 13–18.

Singh, I. N., El-Hage, N., Campbell, M. E., Lutz, S. E., Knapp, P. E., Nath, A., et al. (2005). Differential involvement of p38 and JNK MAP kinases in HIV-1 Tat and gp120-induced apoptosis and neurite degeneration in striatal neurons. *Neuroscience, 135*, 781–790.

Smith, D. G., Guillemin, G. J., Pemberton, L., Kerr, S., Nath, A., Smythe, G. A., et al. (2001). Quinolinic acid is produced by macrophages stimulated by platelet activating factor, Nef and Tat. *Journal of Neurovirology, 7*, 56–60.

Smurzynski, M., Wu, K., Letendre, S., Robertson, K., Bosch, R. J., Clifford, D. B., et al. (2011). Effects of central nervous system antiretroviral penetration on cognitive functioning in the ALLRT cohort. *AIDS, 25*, 357–365.

Sodhi, A., Montaner, S., & Gutkind, J. S. (2004). Viral hijacking of G-protein-coupled-receptor signalling networks. *Nature Reviews Molecular Cell Biology, 5*, 998–1012.

Stevenson, M. (2003). HIV-1 pathogenesis. *Nature Medicine, 9*, 853–860.

Sui, Z., Fan, S., Sniderhan, L., Reisinger, E., Litzburg, A., Schifitto, G., et al. (2006). Inhibition of mixed lineage kinase 3 prevents HIV-1 Tat-mediated neurotoxicity and monocyte activation. *Journal of Immunology, 177*, 702–711.

Sui, Z., Sniderhan, L. F., Schifitto, G., Phipps, R. P., Gelbard, H. A., Dewhurst, S., et al. (2007). Functional synergy between CD40 ligand and HIV-1 Tat contributes to inflammation: Implications in HIV type 1 dementia. *Journal of Immunology, 178*, 3226–3236.

Tachibana, K., Hirota, S., Iizasa, H., Yoshida, H., Kawabata, K., Kataoka, Y., et al. (1998). The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature, 393*, 591–594.

Tenneti, L., D'Emilia, D. M., Troy, C. M., & Lipton, S. A. (1998). Role of caspases in N-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. *Journal of Neurochemistry, 71*, 946–959.

Toggas, S. M., Masliah, E., & Mucke, L. (1996). Prevention of HIV-1 gp120-induced neuronal damage in the central nervous system of transgenic mice by the NMDA receptor antagonist memantine. *Brain Research, 706*, 303–307.

Toggas, S. M., Masliah, E., Rockenstein, E. M., Rall, G. F., Abraham, C. R., & Mucke, L. (1994). Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. *Nature, 367*, 188–193.

Toggas, S. M., & Mucke, L. (1996). Transgenic models in the study of AIDS dementia complex. *Current Topics in Microbiology and Immunology, 206*, 223–241.

Tran, P. B., & Miller, R. J. (2003). Chemokine receptors: Signposts to brain development and disease. *Nature Reviews Neuroscience, 4*, 444–455.

Trillo-Pazos, G., Farlane-Abdulla, E., Campbell, I. C., Pilkington, G. J., & Everall, I. P. (2000). Recombinant nef HIV-IIIB protein is toxic to human neurons in culture. *Brain Research, 864*, 315–326.

Tun, C., Guo, W., Nguyen, H., Yun, B., Libby, R. T., Morrison, R. S., et al. (2007). Activation of the extrinsic caspase pathway in cultured cortical neurons requires p53-mediated down-regulation of the X-linked inhibitor of apoptosis protein to induce apoptosis. *Journal of Neurochemistry, 102*, 1206–1219.

Turchan, J., Pocernich, C. B., Gairola, C., Chauhan, A., Schifitto, G., Butterfield, D. A., et al. (2003). Oxidative stress in HIV demented patients and protection ex vivo with novel antioxidants. *Neurology, 60*, 307–314.

Turrin, N. P., & Rivest, S. (2004). Unraveling the molecular details involved in the intimate link between the immune and neuroendocrine systems. *Experimental Biology and Medicine (Maywood, N.J.), 229*, 996–1006.

Tyor, W. R., Power, C., Gendelman, H. E., & Markham, R. B. (1993). A model of human immunodeficiency virus encephalitis in scid mice. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 8658–8662.

Urbina, A., & Jones, K. (2004). Crystal methamphetamine, its analogues, and HIV infection: Medical and psychiatric aspects of a new epidemic. *Clinical Infectious Diseases, 38*, 890–894.

van Marle, G., Henry, S., Todoruk, T., Sullivan, A., Silva, C., Rourke, S. B., et al. (2004). Human immunodeficiency virus type 1 Nef protein mediates neural cell death: A neurotoxic role for IP-10. *Virology, 329*, 302–318.

Verani, A., & Lusso, P. (2002). Chemokines as natural HIV antagonists. *Current Molecular Medicine, 2*, 691–702.

Wahl, L. M., Corcoran, M. L., Pyle, S. W., Arthur, L. O., Harel-Bellan, A., & Farrar, W. L. (1989). Human immunodeficiency virus glycoprotein (gp120) induction of monocyte arachidonic acid metabolites and interleukin 1. *Proceedings of the National Academy of Sciences of the United States of America, 86*, 621–625.

Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S., & Alizon, M. (1985). Nucleotide sequence of the AIDS virus, LAV. *Cell, 40*, 9–17.

Walmsley, S. L., Winn, L. M., Harrison, M. L., Uetrecht, J. P., & Wells, P. G. (1997). Oxidative stress and thiol depletion in plasma and peripheral blood lymphocytes from HIV-infected patients: Toxicological and pathological implications. *AIDS, 11*, 1689–1697.

Walsh, K. A., Megyesi, J. F., Wilson, J. X., Crukley, J., Laubach, V. E., & Hammond, R. R. (2004). Antioxidant protection from HIV-1 gp120-induced neuroglial toxicity. *Journal of Neuroinflammation, 1*, 8.

Wang, Y., White, M. G., Akay, C., Chodroff, R. A., Robinson, J., Lindl, K. A., et al. (2007). Activation of cyclin-dependent kinase 5 by calpains contributes to human immunodeficiency virus-induced neurotoxicity. *Journal of Neurochemistry, 103*, 439–455.

Welch, K., & Morse, A. (2002). The clinical profile of end-stage AIDS in the era of highly active antiretroviral therapy. *AIDS Patient Care and STDs, 16*, 75–81.

Wesselingh, S. L., Takahashi, K., Glass, J. D., McArthur, J. C., Griffin, J. W., & Griffin, D. E. (1997). Cellular localization of tumor necrosis factor mRNA in neurological tissue from HIV-infected patients by combined reverse transcriptase/polymerase chain reaction in situ hybridization and immunohistochemistry. *Journal of Neuroimmunology, 74*, 1–8.

Wiley, C. A., Masliah, E., & Achim, C. L. (1994). Measurement of CNS HIV burden and its association with neurologic damage. *Advances in Neuroimmunology, 4*, 319–325.

Winston, A., Duncombe, C., Li, P. C., Gill, J. M., Kerr, S. J., Puls, R., et al. (2010). Does choice of combination antiretroviral therapy (cART) alter changes in cerebral function testing after 48 weeks in treatment-naive, HIV-1-infected individuals commencing cART? A randomized, controlled study. *Clinical Infectious Diseases, 50*, 920–929.

Xiao, H., Neuveut, C., Tiffany, H. L., Benkirane, M., Rich, E. A., Murphy, P. M., et al. (2000). Selective CXCR4 antagonism by Tat: Implications for in vivo expansion of coreceptor use by HIV-1. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 11466–11471.

Xie, Z., Smith, C. J., & Van Eldik, L. J. (2004). Activated glia induce neuron death via MAP kinase signaling pathways involving JNK and p38. *Glia, 45*, 170–179.

Yeh, M. W., Kaul, M., Zheng, J., Nottet, H. S., Thylin, M., Gendelman, H. E., et al. (2000). Cytokine-stimulated, but not HIV-infected, human monocyte-derived macrophages produce neurotoxic levels of L -cysteine. *Journal of Immunology, 164*, 4265–4270.

Yi, Y., Chen, W., Frank, I., Cutilli, J., Singh, A., Starr-Spires, L., et al. (2003). An unusual syncytia-inducing human immunodeficiency virus type 1 primary isolate from the central nervous system that is restricted to CXCR4, replicates efficiently in macrophages, and induces neuronal apoptosis. *Journal of Neurovirology, 9*, 432–441.

Yi, Y., Rana, S., Turner, J. D., Gaddis, N., & Collman, R. G. (1998). CXCR-4 is expressed by primary macrophages and supports CCR5-independent infection by dual-tropic but not T-tropic isolates of human immunodeficiency virus type 1. *Journal of Virology, 72*, 772–777.

Zhang, Y., Wang, M., Li, H., Zhang, H., Shi, Y., Wei, F., et al. (2012). Accumulation of nuclear and mitochondrial DNA damage in the frontal cortex cells of patients with HIV-associated neurocognitive disorders. *Brain Research, 1458*, 1–11.

Zheng, J., Thylin, M. R., Ghorpade, A., Xiong, H., Persidsky, Y., Cotter, R., et al. (1999). Intracellular CXCR4 signaling, neuronal apoptosis and neuropathogenic mechanisms of HIV-1-associated dementia. *Journal of Neuroimmunology, 98*, 185–200.

Zhou, D., Masliah, E., & Spector, S. A. (2011). Autophagy is increased in postmortem brains of persons with HIV-1-associated encephalitis. *Journal of Infectious Diseases, 203*, 1647–1657.

Zou, Y. R., Kottmann, A. H., Kuroda, M., Taniuchi, I., & Littman, D. R. (1998). Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature, 393*, 595–599.

Cancer-Mediated Neurotoxicity

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Contents

Abstract

The central nervous system is particularly susceptible to cancer, and its treatment side effects result in significant neurologic morbidity and mortality. Conversely, recent improvements in treatment protocols have extended average life expectancies in various subgroups of cancer patients. As a result of the extended survival, quality of survival is becoming much more important, and attention is increasingly being paid to the disease-free period between treatment and death. The majority of the research, however, focuses on potential side effects of various therapeutic modalities. The following contribution is devoted to

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exploration of a relatively new concept of tumor-related neurotoxicity with special emphasis on the role of glutamate and glutamate-dependent pathways in tumor development and progression as well as in glutamate-dependent excitotoxicity and its consequences.

Keywords
Cancer • Glutamate • Neurotoxicity • Receptors

List of Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4 isoxazolepropionate
EAAT	Excitatory amino acid transporter
ECM	Extracellular matrix
FAK	Focal adhesion kinase
GDH	Glutamate dehydrogenase
Glu	Glutamate
HAA	Hydroxyanthranilic acid
HDI	Histone deacetylase inhibitor
IDO	Indoleamine 2,3-dioxygenase
iGluR	Inotropic glutamate receptor
KA	Kainate
KYNA	Kynurenic acid
LTD	Long-term depression
LTP	Long-term potentiation
MAGUK	Membrane-associated guanylate kinase
mGluR	Metabotropic glutamate receptor
NMDA	*N*-methyl-D-aspartate
NOS	Nitric oxide synthase
PIC	Picolinic acid
QA	Quinolinic acid
TMZ	Temozolomide
TSC	Tumor stem cell

1 Introduction

According to GLOBOCAN database of the International Agency for Research on Cancer, almost 12.7 million new cases of cancer were reported worldwide in 2008, resulting in more than 7.6 million deaths (Ferlay et al. 2010). A significant percentage of those patients are expected to develop complications in sites distant to the original tumor location. The nervous system is particularly susceptible to such complications that result in significant neurologic morbidity and mortality. These effects may be direct, with direct cancer involvement of the brain, spine, or peripheral nervous system (PNS) or indirect as in paraneoplastic neurologic syndromes (Giglio and Gilbert 2010) with symptoms ranging from delirium, amnesia,

aphasia, psychotic disturbance, sensory disturbance, ataxia, and autonomic dysfunction to eye movement disorders (Bataller and Dalmau 2004). Still, the impairments of cognitive functions such as language, memory, visuospatial perception, time–space orientation, and attention or executive functions are the most prevalent (Reid-Arndt et al. 2010). From this perspective a distinct subpopulation of cancer patients is patients with brain tumors, both primary and metastatic ones who, in fact, show signs of cognitive decline in almost all of the cases (Hottinger et al. 2009). As a matter of fact, a cognitive dysfunction often is present at diagnosis in many of those patients, more frequently in rapid-growing tumors such as glioblastoma than in slow-growing ones such as low-grade gliomas (Correa 2010). On the other hand, improvements in treatments have extended average life expectancies in various subgroups of patients with brain tumors, although in most instances the patient cannot be cured and will eventually die from the disease (DeAngelis 2001). As a result of the extended survival, quality of survival is becoming much more important, and attention is increasingly being paid to the disease-free period between treatment and death (Weitzner and Meyers 1997).

The exact pathophysiology of cognitive deficits in patients with brain tumors is not entirely understood. Studies have shown that tumor itself, its progression, tumor-related neurological complications, and epilepsy can cause them (Klein et al. 2002; Aoyama et al. 2007). Diaschisis, in which there is dysfunction of brain areas connected to the original lesion, owing to altered metabolism and blood flow, might also contribute (Taphoorn and Klein 2004). On top of that, disturbances in connectivity between functional networks in the brain have also been reported (Bartolomei et al. 2006). The question remains, however: what is the underlying mechanism responsible for these disturbances?

A recent discovery of glutamate as a neurotrophic factor for gliomas, acting in autocrine or paracrine fashion, not only revealed a potential signaling pathway important for glioma invasion and established a new tool for cancer treatment but also opened a new avenue of research into the mechanisms responsible for neuronal network impairments found in brain tumor patients. Glial tumors, especially malignant gliomas, release significant amounts of Glu into extracellular space, which acts as a growth factor for malignant cells. In the last decade evidence has emerged implicating a role for glutamate as a signal mediator that stimulates cancer cell proliferation and motility via activation of glutamate receptors (GluRs) (de Groot and Sontheimer 2011). Moreover, excess of glutamate causes neurodegeneration of the peritumoral tissue, enabling tumor progression (de Groot and Sontheimer 2011). On the other side, elevated glutamate concentrations in the brain exert neurotoxicity, resulting in neuronal excitotoxicity and cell death, enabling brain tumors to expand, and cause seizures in the tumor-bearing patients (Sontheimer 2008; Buckingham et al. 2011). Importantly, glutamate is considered as one of the major excitatory mediators in the central nervous system (CNS). It affects several physiological processes such us learning, memory, or behavior (Peng et al. 2011). Excessive activation of glutametergic system (high levels of Glu and activation of respective receptors) results in neural cells death called excitotoxicity that is involved in

pathophysiology of various neurodegenerative diseases and other CNS pathologies (epilepsy, hypoxia, stroke) (Lau and Tymianski 2010). Thereby, glioma-derived glutamate seems to be important endogenous neurotoxin.

Glutamate activates two principal classes of receptors: ionotropic (iGluRs) and metabotropic (mGluRs) glutamate receptors. The mGluR receptor family comprises of eight subtypes (mGluR1-8) that are subdivided into three groups based on the sequence similarities and G-protein coupling to specific signal transduction mechanisms. mGluR1 and mGluR5, which are coupled to the phospholipase C, belong to group I. Group II consists of mGluR2 and mGluR3, whereas group III contains mGluR4, mGluR6, mGluR7, and mGluR8, which are all negatively coupled to adenylate cyclase. mGluRs function as dimers, with two glutamate molecules being required for full receptor activation (Shin et al. 2008; Kew and Kemp 2005). In neurons mGluR receptors modulate synaptic transmission and are involved in activity-dependent modification of synaptic transmission such as long-term potentiation and long-term depression, whereas in glial cells they exert a variety of effects that are crucial for glial function and glial–neuronal interaction under physiologic and pathologic conditions (D'Antoni et al. 2008). iGluRs, on the other hand, are subdivided into three groups according to structural similarities and synthetic agonists that activate them: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (kainate, KA) receptors.

N-methyl-D-aspartate (NMDA) tetrameric receptors consist of two obligatory NR1 subunits and two of four types of regulatory subunits: NR2A, B, C, and D. The NR1 subunit is necessary for calcium conductivity of the channel, while the NR2 and NR3 subunits determine electrophysiological and pharmacological properties of the receptor. Activation of NMDA receptor requires binding of two agonists: glutamate and glycine. Concurrent membrane depolarization abrogates the magnesium block, which allows calcium and sodium cations to enter the cell. NR3 subunits may assemble with NR1 subunits in order to create a functional glycine receptor that is not affected by glutamate. NMDA receptors are implicated in synaptic transmission and neuronal plasticity phenomena such as long-term potentiation (LTP) and long-term depression (LTD) (Kew and Kemp 2005; Traynelis et al. 2010).

AMPA receptors are homo- or heterotetramers composed of subunits known as GluR1–GluR4. They mediate fast excitatory synaptic signaling in the brain and are involved in activity-dependent modulation of synaptic plasticity. AMPA receptors gate Na+/Ca^{2+} in response to ligand binding. Conductance and kinetic properties of the channel depend upon subunit composition. Influx of ions causes a fast excitatory postsynaptic response in neurons, and Ca^{2+} can also activate second messenger pathways. The GluR2 subunit plays a critical role in the determination of the permeability of AMPARs. The genomic DNA of the GluR2 subunit contains a glutamine (Q) residue at amino acid 607, but during the process of nuclear RNA editing, glutamine is replaced by arginine (R) in the vast majority of neuronal cells, which results in a very low calcium permeability for receptors containing the

GluR2 subunit (Madden 2002; Kew and Kemp 2005). The kinetics and amplitude of the excitatory synaptic response are determined by the biophysical properties of the receptor subunit combination (Traynelis et al. 2010).

KA receptors are composed of tetrameric assemblies of GluR5–7 and KA1/2 subunits. GluR5–7 subunits can form homomeric functional receptors as well as combine with KA1 and KA2 to form heteromeric receptors with distinct pharmacological properties. KA1 and KA2 subunits do not form homomeric functional receptors. Similar to GluR2, GluR5 and GluR6 undergo glutamine/arginine editing (Contractor et al. 2011).

The glutamate cellular system includes also a number of proteins transporting glutamate, namely, high-affinity sodium and potassium-coupled excitatory amino acid transporters (EAAT1/GLAST, EAAT2/GLT-1, EAAT3/EAAC1, EAAT4, EAAT5). The major function of these transporters is to maintain the extracellular glutamate concentration at a low, nontoxic level in order to prevent overstimulation of glutamate receptors. Glu uptake occurs primarily through EAAT1 and EAAT2 transporters present on membranes of nonmalignant astrocytes (de Groot and Sontheimer 2011).

2 Developments in the Field

Neurotoxic effects of glutamate and its relevance for the CNS as a potential neurotransmitter were recognized for the first time over 60 years ago. In 1952, severe convulsions were observed following glutamate administration into the central nervous system (CNS) of dogs and monkeys (Hayashi 1952), and in 1957 the neurotoxic effects of sodium-L-glutamate on the inner layers of the retina were described (Lucas and Newhouse 1957). In 1959, direct evidence that Glu depolarized individual neurons of the spinal cord was provided (Curtis et al. 1959). With the identification of natural or synthetic substances (NMDA, AMPA, KA) capable to discriminate and activate different glutamate receptors, followed by receptor antagonist/agonist development and mapping of the various iGluR subtypes to distinct CNS regions by radioligand binding techniques, and finally with molecular receptor characterization based on structure-function analysis, significant progress was made in elucidating the role of GluRs in physiology and CNS disorders (Bowie 2008).

Various studies have proven glutamate to be the major excitatory neurotransmitter in the brain that plays a key role in learning and memory, thanks to its role in the induction and maintenance of long-term potentiation (LTP) and long-term depression (LTD). For that, its signaling pathways have been extensively studied in the CNS. Accordingly, aside from the physiological functions of Glu, much effort has been made in order to resolve its mechanism of action causing neurotoxicity, termed excitotoxicity (Lau and Tymianski 2010).

Excessive glutamate levels cause neuronal dysfunction and degeneration by the complex excitotoxic mechanism, which involves hyperactivation of glutamate receptors, massive influx of Ca^{2+} cations resulting in “overactivation” of selected

signaling pathways. Different levels of magnitude and heterogeneity of neurodegeneration were observed following glutamate application in cultured neurons, however. Depending on the intensity of glutamatergic insult, apoptotic or necrotic types of cell death can occur. Intense glutamatergic stimulation causes necrosis, mediated by acute mitochondrial dysfunction, whereas milder glutamate influence results in the activation of several intracellular pathways, associated with other cell death types (Lau and Tymianski 2010).

The crucial player in glutamate-mediated excitotoxicity is a calcium influx into the cells, mainly through NMDA receptor channels (Lau and Tymianski 2010). NMDA receptors (dependent on subunit-specific differences) are approximately 3–4 times more permeable to Ca^{2+} than unedited Ca^{2+}-permeable AMPA, and Ca^{2+}-permeable AMPA receptors still show a slightly greater Ca^{2+} permeability than KA receptors (Traynelis et al. 2010). After three-phase time-dependent rise of calcium that includes initial 5–10-min-lasting acute phase, subsequent 2-h latent phase with normal Ca^{2+} concentration followed by calcium sequestration in mitochondria in third sustained phase, a metabolic acidosis and free radical generation develop that result in mitochondrial damage. Glutamate-mediated calcium-dependent neurodegeneration has also been linked to the generation of nitric oxide (NO for a nitric oxide synthase (NOS) knockout mice demonstrated attenuated NMDA-mediated excitotoxicity). More detailed studies revealed a structural scaffold – a postsynaptic density protein of molecular weight 95 kDa (PSD-95) connecting NR2B subunit of NMDA receptor and NOS. It is of particular importance for NO is directly neurotoxic, nitrosylating GADPH (glyceraldehyde 3-phosphate dehydrogenase, an ubiquitous glycolysis enzyme), which together with ubiquitin ligase (Siah1) enhances p300/CBP-associated acetylation of nuclear proteins (Lau and Tymianski 2010), including histones, thereby potentially influencing gene expression. Produced NO, together with free superoxide radicals, also causes protein modifications, lipid peroxidation, and DNA damage, resulting in subsequent cell death. Caspases, classical apoptotic cysteine proteases, activated downstream of calcium influx, and mitochondrial dysfunction play a significant role in this process as well. Other cellular cysteine proteases, calpains, might also be involved in glutamatergic excitoxicity by the cleavage and release of apoptosis-inducing factor (AIF) from the mitochondria, which after nuclear translocation causes cell death, preceded by chromatin condensation and DNA fragmentation. Moreover, AIF release is linked to the activation of PARP-1 (poly(ADP)-ribose polymerase), enzyme involved in DNA repair in an alternative excitotoxic mechanism (Lau and Tymianski 2010).

The normal nontoxic Glu concentration between 1 and 3 μM in the brain is maintained by excitatory amino acid transporters, mainly EAAT1 and EAAT2, predominantly expressed in nonmalignant astrocytes. However, as demonstrated in vitro, Glu concentration exceeding 500 μM is present in cultured glioma cells. Likewise Glu concentrations in the range of 100 μM were reported at the tumor margin of the glioblastoma-bearing patients (de Groot and Sontheimer 2011).

In contrast to neurons and glial cells, glioma cells release Glu mainly by employing x_c^- cysteine–Glu exchanger system, which requires extracellular

cysteine to be incorporated to the cell and intracellular Glu to be released outside the cell. It has been suggested that Glu may be generated from glutamine via a glutaminase reaction and/or by glutamate dehydrogenase (GDH). These notions are supported by the observation that high levels of liver type glutaminase and GDH have been demonstrated in gliomas. Their expression might be promoted by the oncogene *c-myc*, which stimulates glutamine metabolism and is overexpressed in treatment-resistant glioma stem cells. Additionally, EAAT1/2 transporters are often dysfunctional in glioma cells, and their expression (especially EAAT2) seems to be inversely correlated with the degree of malignancy with almost no expression in GBM cells (de Groot and Sontheimer 2011). In consequence glioma cells release high amounts of glutamate while having impaired glutamate uptake system, which results in excessive amounts of Glu in peritumoral area.

On the other hand, iGluRs and mGluRs are ubiquitously present on neurons and glial cells (Bowie 2008). Glu released from gliomas may then exert peritumoral excitotoxicity directed to neurons and oligodendrocytes. Astrocytes are usually resistant, even to high glutamate concentration (millimolar range). Still, peritumoral astrocytes probably lose their glutamate uptake potential and/or ability to synthesize glutamine from Glu. Thereby, high-grade gliomas probably also kill astrocytes, which is supported by observations that the space surrounding expansive tumors is devoid of normal brain cells (de Groot and Sontheimer 2011).

Another NMDA receptor agonist quinolinic acid (QA) might be considered as an additional endogenous neurotoxin involved in cancer-mediated neurotoxicity. QA is produced in kynurenine pathway, metabolizing an essential amino acid tryptophan (TRP). To achieve the final product, nicotinamide adenosine dinucleotide (NAD), yet another potential neurotoxin, 3-hydroxyanthranilic acid (HAA), and two endogenous neuroactive intermediates – the NMDA antagonist, kynurenic acid (KYNA), and the neuroprotectant, picolinic acid (PIC) – are generated along the pathway. The kynurenine pathway has been shown to be present in neurons, astrocytes, and microglial cells, as well as in brain tumor cells (Chen and Guillemin 2009). The differential pattern of kynurenine pathway expression was also demonstrated in neuroblastoma cells when compared to neurons. Neuroprotective PIC was produced primarily by immature and mature neurons, but not by the tumor cells. In contrast, neuroblastoma cells were able to produce the excitotoxin QA (Guillemin et al. 2007). QA is a heterocyclic amino acid that selectively activates the neuronal NMDA receptors and has been demonstrated to have immunomodulatory and neurotoxic properties, causing neuronal cell death after direct intracerebral administration (Chen and Guillemin 2009). It has been shown that QA can directly increase the proliferation rate of brain tumor cells in vitro (Guillemin et al. 2007) while being directly toxic to the surrounding neurons and thus mirroring the effects mediated by high amounts of glutamate. Interestingly, this effect can be abrogated by endogenous neuroprotectants, synthesized in the same pathway. KYNA and PIC may effectively block QA-induced neurotoxicity without affecting its excitatory effect. Kynurenic acid acts on the glycine modulatory site at low concentrations while at higher ones on the glutamate site of the NMDA receptors. KYNA was also shown to antagonize AMPA receptors and the alpha 7 nicotinic acetylcholine receptors.

Accordingly, KYNA was reported to exert sedative and anticonvulsant effects; it was also proved to be protective against brain ischemia. PIC is less potent and appears to act via a different mechanism attenuating calcium-dependent glutamate release and/or chelating endogenous zinc (Chen and Guillemin 2009). Interestingly, neurons seem to synthesize it, while neuroblastoma cells do not (Guillemin et al. 2007).

Furthermore, quinolinic acid and 3-hydroxyanthranilic acid can effectively suppress T cell proliferation (Chen and Guillemin 2009) thus potentially attenuating the immune defense system. This could be mediated by overexpressed indoleamine 2,3-dioxygenase (IDO) enzyme, a component of the kynurenic pathway, involved in the conversion of tryptophan to *N*-formylkynurenine. IDO is being expressed by a plethora of cells, including glial tumor cells, dendritic cells, and monocytes in the tumor's vicinity, which in turn has implicated this molecule as one of the key mediators of the tumor immune escape. Beyond the tryptophan depletion, accumulation of IDO metabolites, 3-hydroxyanthranilic acid, KYNA, and QA, within the tumor environment and in the blood seems to increase the suppression of antitumor immune responses being quite detrimental in particular for the T cell, inducing both their anergy and death, as well as to impair NK cellular cytotoxicity by particularly targeting the expression of key NK receptors involved in target recognition (Zamanakou et al. 2007).

A commonly voiced concern about the possible role of QA in neuronal damage is that its concentrations in the blood and cerebrospinal fluid (CSF) are usually lower than these required to induce neuronal damage. However, the QA levels may be many times greater in the extracellular space around activated glial cells (Stone et al. 2012), or brain tumors, and there may be subsets of neurons which are particularly sensitive to quinolinic acid toxicity like neurons within the dentate gyrus of the hippocampus.

Notably, an enhanced degradation of TRP is observed in patients with various types of cancer. On the other hand patients with decreased TRP serum/plasma levels and increased concentrations of tryptophan catabolites have been reported to be involved in the development of mood disorders, especially depression. IDO activation, described above, leads to the accumulation of neurotoxic metabolites, which are supposed to induce depressive-like behavior. Resulting imbalance between neuroprotective (KYNA) and neurotoxic (QA) metabolites has been proposed to be critical for the development of depressive symptoms in patients with CNS tumors. On the other hand, decreased tryptophan availability might also affect neurosignaling by impaired synthesis of neurotransmitters serotonin and NAD, resulting in the development of depression (Kurz et al. 2011).

Taken all together, aberrant activation of the kynurenine pathway may contribute to the persistence and development of brain tumors and might be, at least in part, responsible for neurotoxicity associated with them.

3 Important Scientific Research

Direct evidence for neurotoxic function of glutamate released by glioma cells was provided for the first time in 1999. It was demonstrated then that established glioma

cell lines and primary cultures grown from the patient tissues harvested during biopsy release large amounts of glutamate, resulting in elevations of Glu that exceed 100 μM within hours sufficient to activate glutamate receptors on hippocampal neurons in coculture experiments and to induce delayed Ca^{2+}-dependent cell death (Ye and Sontheimer 1999).

Crucial data, which confirmed that glutamate excitotoxicity contributes to tumor expansion in vivo, were obtained by implanting different clones of rat glioma C6 cells in striata of adult rats. Clones that actively released glutamate developed aggressive tumors that expanded faster than tumors composed of wt C6 cells or derived from clones that were deficient in glutamate. Tumor size correlated with glutamate secretion by these clones and was not affected by proliferation rate and mitotic activity; as expected it also correlated with survival time of tumor-bearing animals. Consistent with the coculture experiments, tumors from clones releasing high amounts of Glu generated high degree of degenerated neurons, accompanied by the inflammatory response in surrounding brain areas (Takano et al. 2001). Based on this data it was concluded that glutamate acts as an important autocrine/paracrine signal that promotes glioma cell invasion and the formation of tumor metastasis (Lyons et al. 2007).

Subsequent studies proved that GluR antagonists negatively affect proliferation of different types of cancer cells in vitro (Rzeski et al. 2001) and growth of implanted tumors in vivo (Stepulak et al. 2005; Takano et al. 2001). Accordingly, the involvement of glutamate receptors in the proliferation and migration of cancer cells was also suggested. Follow-up study by Ishiuchi et al. provided evidence that gliomas express functional AMPA receptors (Ishiuchi et al. 2002). NMDA and kainate receptor subunits were also demonstrated in brain tumor-derived cell lines (Stepulak et al. 2009). Still, their functional significance was not unequivocally proved. AMPA receptors seem to be crucial for promoting tumor cell proliferation and invasion. The presence of GluR2 subunit of the AMPAR is critical to the receptor function. In its edited form GluR2 makes AMPAR impermeable for calcium entry into the cell, resulting in attenuated ability of glioma cells to invade in vitro and in vivo, as demonstrated by overexpression of GluR2 subunit (Ishiuchi et al. 2002). Ca^{2+} imaging experiments showed also that the released glutamate activates AMPAR and induced intracellular Ca^{2+} oscillations that are essential for cell migration (Lyons et al. 2007). Interestingly, brain-derived tumor cells expressed also unedited form of GluR2 subunit (Stepulak et al. 2009), thus enabling Ca^{2+} influx through AMPAR functioning ion channel. A similar situation might take place in brain tumors in vivo; direct proof requires further studies, though.

The significance of AMPAR expression in brain tumors was also demonstrated in the clinic. Lack of GluR2 expression has been shown to be an independent prognostic factor for a poor prognosis in patients with high-grade glioma (Colman et al. 2010). GluR2 was absent in fast-growing GBM-derived tumor stem cells and high-grade glioma specimens but was expressed in slow-growing stem cells and low-grade glioma specimens (Beretta et al. 2009). Increased GluR1 expression was observed in glioblastoma samples when compared to anaplastic astrocytomas and low-grade gliomas. AMPA-mediated signaling in glioma cell lines was attributed to

MAP kinase (ERK1/2) activation, and gene silencing of the GluR1 AMPA receptor subunit results in abrogation of AMPA-mediated signaling and tumor growth (de Groot et al. 2008). It has been also shown that GluR1 overexpression in glioma cells with subsequent activation of focal adhesion kinase (FAK) is responsible for increased cell adhesion to the extracellular matrix (ECM) in vitro (de Groot and Sontheimer 2011).

GluR2 overexpression in glioma cells inhibits proliferation and induces apoptosis by inactivating extracellular signal-regulated kinase (ERK)1⁄2 via Src kinase (Beretta et al. 2009). MAP kinase seems to be an intracellular mediator connecting mGluR3 receptor with bone morphogenetic proteins (BMP)-Smads transcription factors signaling. Activation of mGluR3 results in sustained undifferentiated state of glioma-initiating cells, whereas pharmacological blockade of mGluR3 stimulates differentiation of these cells and limits the growth of implanted tumors (Ciceroni et al. 2008). Akt kinase pathway, crucial for proliferation of various cancer cells types, seems to be another important downstream effector of AMPAR-mediated signaling in glioma cells. A dominant-negative form of Akt inhibits cell proliferation and migration accelerated by overexpression of Ca^{2+}-permeable AMPA receptor. In contrast, introduction of a constitutively active form of Akt rescues tumor cells from apoptosis induced by the conversion of Ca^{2+}-permeable AMPA receptor to Ca^{2+}-impermeable receptors by the delivery of GluR2 cDNA (Ishiuchi et al. 2007) proving the importance of this type of signaling for tumor cell homeostasis. Since glutamate receptors appear to be vital for glioma invasion and metastasis, inhibition of these receptors and/or GluR-dependent signaling pathways opens a new field for therapeutic intervention that should result in an inhibition of tumor invasion, along with reduction of neurotoxic side effects mediated by the tumor.

Expansion of the tumor may explain a frequent occurrence of seizures initiated by the tumor and the surrounding brain. Over 80 % of glioma patients suffer seizures during the course of their diseases (de Groot and Sontheimer 2011). However, the mechanism responsible for these seizures has not been unequivocally proved and may be related to the expression and activation of GluRs in different types of brain tumors and glial cells. Subunits from each receptor class, including ionotropic NR1, NR2A/B (NMDA) GluR1, GluR2 (AMPA), and GluR5-7 (kainate) and metabotropic mGluR1, mGluR2/3, mGluR5, and mGluR7a subtypes, were demonstrated in glioneuronal tumors from patients with intractable epilepsy. Group I mGluRs (mGluR1 and mGluR5) were highly represented, whereas low expression of mGluR2/3 (group II) and mGluR7 (group III) was observed in the neuronal component of the tumors, supporting the central role of glutamatergic transmission in the mechanisms underlying the intrinsic and high epileptogenicity of these lesions. Increased expression of mGluR2/3, mGluR5, and GluR5-7 proteins in peritumoral reactive astrocytes suggests its possible contribution to the generation and propagation of seizure activity (Aronica et al. 2001). In astrocytes iGluRs, especially AMPARs, are able to form functional ion channels, and their activation can alter astrocytes morphology and function, whereas mGluRs appear to regulate the expression of Glu transporters (de Groot and Sontheimer 2011).

Astrocytes predominantly express mGluR3 and mGluR5, and their activation enhances glutamate uptake, thus protecting neurons from glutamate-mediated toxicity. These mechanisms are often disrupted under pathologic conditions. The mGluR5-mediated upregulation of glutamate transport is lost in chemically activated astrocytes in rat ALS model, and an enhanced release of glutamate induced by the activation of glial mGluR5 receptors contributing to glutamate-mediated neuronal death was observed after status epilepticus (D'Antoni et al. 2008). Thus, tumor-released glutamate may potentiate excitotoxicity mediated by astrocytes by activating mGluR5 receptors. In microglia, activation of mGluR2 induces mitochondrial depolarization and apoptosis or, alternatively, induces an activated phenotype, which underlies microglia-mediated neurotoxicity, mediated probably by the secretion of TNFa and Fas ligand from activated microglia (D'Antoni et al. 2008).

Recently, the involvement of KARs activated by the endogenous agonist glutamate in the generation and propagation of epileptiform seizures was discussed (Vincent and Mulle 2009). Nonsynaptic source of glutamate derived from the activity of astrocytes increases the activation of KARs in neighboring interneurons. On the other hand, apoptotic and necrotic death of neurons is associated with dose-dependent kainate (KA)-induced excitotoxicity in vivo (Vincent and Mulle 2009; Wu et al. 2005) and involvement of KA receptors in epileptiform seizure induction was demonstrated by injection of KA in rodents (Vincent and Mulle 2009). Thereby, it is possible that activation of KAR by glioma-released glutamate could contribute to the development of the seizures observed in glioblastoma patients. This hypothesis is supported by the observation that NMDA receptor antagonists given before or after the induction of KA-mediated seizures prevent most of the excitotoxic damage, suggesting pivotal role of NMDA receptors in KA-induced excitotoxicity (Vincent and Mulle 2009). Seizures might be also activated by quinolinic acid (QA) acting on NMDA receptors and producing neuronal damage (Stone et al. 2012), which have been shown to be produced by neuroblastoma cells (Guillemin et al. 2007).

4 Recent Findings

Despite recent improvements in treatment strategies, the results of therapy in patients with glioblastoma are unsatisfactory. Effective treatment is limited due to biological properties of the tumor and the clinical advancement of the disease upon diagnosis. Because of the low efficacy of the traditional chemotherapy, new therapeutic tools acting selectively on specific metabolic pathways pathologically deregulated in glioma cells are still needed. On the other hand, development of new tools attenuating tumor- and glutamate-induced neurotoxicity is of great importance.

Since, new types of the targeted therapy, including blockade of growth factors, like EGFR, GFs-mediated downstream signaling (Ras–ERK1/2, Akt–mTOR, PKC), proteasome, and microtubule inhibitors, proved to be of limited value in the clinic (Adamson et al. 2009), glutamatergic signaling system has attracted great

attention. As described above, glutamate receptor signaling has been implicated in tumor biology, and receptor antagonists have been shown to be sufficient to suppress cancer growth in experimental studies (Arcella et al. 2005). Unfortunately, the majority of tested compounds, although promising in animal experiments, failed in clinical trials (Lau and Tymianski 2010). It is of interest, however, that in contrast to other NMDA receptor antagonists, memantine has been approved for the treatment of Alzheimer disease and has shown some antitumor effects in vitro (Rzeski et al. 2001; Takano et al. 2001). It is worth mentioning that doses, which inhibit tumor cells proliferation, are much higher than those applicable for Alzheimer disease. The other iGluR antagonists show similar properties, even though it has been shown that some of them exert their activities at relatively low, clinically relevant doses both in vitro (Rzeski et al. 2001) and in vivo (Stepulak et al. 2005). Unfortunately, potential side effects, psychotic in particular, are limiting factors of iGluR antagonists' therapy (Lau and Tymianski 2010).

On the other hand, very promising results of treatment of newly diagnosed glioblastoma patients with talampanel combined with temozolomide (TMZ) and radiation therapy (RT) were reported. Talampanel, an oral, noncompetitive AMPAR antagonist with excellent brain penetration, combined with RT and TMZ, significantly prolonged median survival time, without any additional toxicity, as compared to RT–TMZ-treated patients (Grossman et al. 2009). This provides a solid base for future studies with similar, newly synthesized inhibitors.

Recently, another group of potential therapeutic agents that inhibit glutamate release has draw attention. Glioma cells in the presence of deficient glutamate uptake systems upregulate the glutamate exchanger X_c- cysteine–glutamate antiporter that leads to progressive Glu accumulation in peritumoral tissue (de Groot and Sontheimer 2011). A disruption of this system using shRNA attenuated Glu release in vivo, prolonged survival of tumor-bearing animals, and diminished glioma-induced neurodegeneration and brain edema (Savaskan et al. 2008). It has been shown that an inhibition of the X_c- transporter by sulfasalazine results in tumor volume reduction by 60–80 % in animal model (Sontheimer 2008). Despite several limitations such us poor systemic bioavailability because of its rapid cleavage by colonic bacteria and a half-life of ~80 min in circulation, clinical trials with sulfasalazine in the treatment of newly diagnosed low-grade gliomas are ongoing (de Groot and Sontheimer 2011).

Another drug aimed on inhibition of glutamate release and attenuation of its neurotoxicity is riluzole (2-amino-6-trifluoromethoxybenzothiazole). Riluzole is an anticonvulsant and neuroprotective agent approved by FDA for different indications (treatment of amyotrophic lateral sclerosis – ALS). Its main mechanisms of action involve inhibition of Na^+-gated channels, postsynaptic blockade of ionotropic GluRs without direct receptor interaction, and potentiation of glutamate uptake by activation of glutamate transporters (Bellingham 2011).

Riluzole has been shown to inhibit proliferation and glutamate release in melanoma, both in vitro and in vivo (Namkoong et al. 2007); however, it has not been tested in glioma treatment, yet. Nevertheless, inhibition of cellular systems of glutamate release seems to be an interesting target for future therapies of brain tumors aimed on reduction of tumor-mediated neurotoxicity.

On top of tumor-mediated neurotoxicity, one should remember that present therapeutic strategies result in significant toxicity directed to brain cells (Rinne et al. 2012). Thus, the development of new neuroprotective agents has become a necessity. One of the most promising approaches involves application of a novel class of drugs aimed at disruption of protein complexes between GluRs and scaffolding proteins. It is of particular value that these specific peptides disrupt interactions between NMDAR and PSD95 or KAR and PSD95 without influencing synaptic activity or calcium influx, which in turn inhibits downstream neurotoxic signaling without interfering with neurotransmission (Vincent and Mulle 2009). Another group of peptides, named membrane-associated guanylate kinase (MAGUK) inhibitors, dissociates spatial relationship between NMDAR and NOS in a competitive manner, inhibiting NOS-mediated neurotoxicity. One of these peptides – NA1 – is currently in a phase II clinical trial (Lau and Tymianski 2010).

For years, agents such as psychostimulants and acetylcholinesterase inhibitors have been used to treat cognitive dysfunction in patients with brain tumors. Recently, however an improvement in cognitive functions in high-grade glioma patients treated with methylphenidate and modafinil, known for their neuroprotective capabilities (van Vliet et al. 2008; Ludolph et al. 2006), was reported. Donepezil, another compound with proven neuroprotective activity against glutamate-induced excitotoxicity (Shen et al. 2010), has also been shown to improve cognitive performance in patients with brain tumors.

Currently clinical trials are being conducted in order to determine the potential neuroprotective effects of memantine and lithium in patients with brain metastases (Correa 2010). Finally, there is some evidence that GluR5-containing KARs represent a novel target for AED (antiepileptic drug) development, since GluR5 antagonists prevent and interrupt limbic seizures without overt side effects in the experimental models (Vincent and Mulle 2009), thereby are of potential interest also for glioma-mediated seizures.

5 Conclusion

Brain tumors, especially glioblastoma, are still a therapeutic conundrum in the context of short survival times and low quality of life of the patients that arise from the neurotoxicity mediated by both tumor and side effects of applied therapies. An improvement of anticancer therapy on one side and attenuation of neurotoxicity on the other define future challenges on the field. Thereby, additional therapeutic approaches are needed to supplement existing strategies (Candolfi et al. 2009). A deregulated glutamatergic system in glioma is definitely one of the plausible therapeutic targets. Still, a number of questions regarding the role of ionotropic glutamate receptors in carcinogenesis remain unanswered. Even though initial reports confirmed the role of glutamate and its receptors in oncogenesis and their antagonists proved to exhibit a therapeutic potential, a practical implementation of those compounds remains elusive. The main obstacle relates to their serious side effects, in particular related to the CNS. Moreover, relevant information on the role

of the aforementioned receptors in peripheral tissues is also missing. The receptors themselves exist in various configurations of subunits that affect their pharmacological proprieties; on top of that receptor subunits undergo posttranslational and epigenetic modifications, which additionally increase their variability. iGluR antagonists are usually receptor or even subunit specific; accordingly a prospective therapy will probably require prior analysis of receptors in a given tumor. Nonetheless, existing data, in particular the results of clinical trials with talampanel in gliomas, are encouraging and support the search for novel antagonists of ionotropic glutamate receptors with superior pharmacologic proprieties to be implemented into antitumor and neuroprotective therapeutic protocols. On the other hand, strategies aimed on the inhibition of glutamate release with subsequent disruption of autocrine glutamate signaling loop, or strategies involving intracellular targets, where different signaling pathways converge, including those implementing histone deacetylase inhibitors (HDI) together with other standard or targeted types of therapy, are also of interest.

Another important advance that has recently changed our way of thinking about glial tumors biology relates to the concept of the so-called tumor stem or tumor-initiating cells (TSCs). It has been recently proven that within a total population of glioblastoma cells, there appears to be a small subpopulation of cells that are highly tumorigenic, with capacity for self-renewal (Singh et al. 2004). Because they share many properties with neural stem cells, it has been suggested that they originate from neuronal stem cells (Alcantara Llaguno et al. 2009). These cells are functionally defined with self-renewal and cell differentiation in vitro and their capability for tumor propagation in vivo (Li et al. 2009). The most important aspect of their presence within glial tumors is that this population appears to be particularly resistant to conventional radiation (Bao et al. 2006) and chemotherapy (Liu et al. 2006). What is of particular interest, however, is the fact that only recently this specific cellular subpopulation has been proven to express high concentrations of functional calcium-permeable AMPA receptors when compared to the differentiated tumor cultures consisting of non-stem cells. This raise the possibility that glutamate secretion in the glioblastoma microenvironment may stimulate brain tumor-derived cancer stem cells (Oh et al. 2012). This finding suggests that therapeutic strategies aimed at glutamate-dependent excitotoxic pathway disruption might also influence TSCs, possibly initiating their differentiation thus rendering them more susceptible to conventional therapeutic modalities. This hypothesis requires further substantiation, though.

A possible strategy to overcome these obstacles involves more personalized diagnosis and treatment, including analysis of genetic abnormalities of particular tumor cell populations, as well as assessment of potential treatment efficacies of arrays of drugs tested on primary cell cultures, derived from samples of tumors harvested during surgery.

In the context of neurotoxicity approaches that render physiological glutamatergic transmission intact and simultaneously disrupt excitotoxic pathways are of particular interest. Another potential therapeutic strategy for neuroprotection involves mimicry of the glutamate blocking activity of endogenous neuroprotective agents, such as kynurenic acid or melatonin. Nevertheless, a lack of significant

success with therapeutic protocols that interfere with NMDA–AMPA-dependent mechanisms of excitotoxicity implies that some other potential therapeutic targets have to be explored.

References

Adamson, C., Kanu, O. O., Mehta, A. I., Di, C., Lin, N., Mattox, A. K., & Bigner, D. D. (2009). Glioblastoma multiforme: A review of where we have been and where we are going. *Expert Opinion on Investigational Drugs, 18*, 1061–1083.

Alcantara Llaguno, S., Chen, J., Kwon, C. H., Jackson, E. L., Li, Y., Burns, D. K., Alvarez-Buylla, A., & Parada, L. F. (2009). Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer Cell, 15*, 45–56.

Aoyama, H., Tago, M., Kato, N., Toyoda, T., Kenjyo, M., Hirota, S., Shioura, H., Inomata, T., Kunieda, E., Hayakawa, K., Nakagawa, K., Kobashi, G., & Shirato, H. (2007). Neurocognitive function of patients with brain metastasis who received either whole brain radiotherapy plus stereotactic radiosurgery or radiosurgery alone. *International Journal of Radiation Oncology, Biology, and Physics, 68*, 1388–1395.

Arcella, A., Carpinelli, G., Battaglia, G., D'Onofrio, M., Santoro, F., Ngomba, R. T., Bruno, V., Casolini, P., Giangaspero, F., & Nicoletti, F. (2005). Pharmacological blockade of group II metabotropic glutamate receptors reduces the growth of glioma cells in vivo. *Neuro-Oncology, 7*, 236–245.

Aronica, E., Yankaya, B., Jansen, G. H., Leenstra, S., van Veelen, C. W., Gorter, J. A., & Troost, D. (2001). Ionotropic and metabotropic glutamate receptor protein expression in glioneuronal tumours from patients with intractable epilepsy. *Neuropathology and Applied Neurobiology, 27*, 223–237.

Bao, S., Wu, Q., McLendon, R. E., Hao, Y., Shi, Q., Hjelmeland, A. B., Dewhirst, M. W., Bigner, D. D., & Rich, J. N. (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature, 444*, 756–760.

Bartolomei, F., Bosma, I., Klein, M., Baayen, J. C., Reijneveld, J. C., Postma, T. J., Heimans, J. J., van Dijk, B. W., de Munck, J. C., de Jongh, A., Cover, K. S., & Stam, C. J. (2006). Disturbed functional connectivity in brain tumour patients: Evaluation by graph analysis of synchronization matrices. *Clinical Neurophysiology, 117*, 2039–2049.

Bataller, L., & Dalmau, J. O. (2004). Paraneoplastic disorders of the central nervous system: Update on diagnostic criteria and treatment. *Seminars in Neurology, 24*, 461–471.

Bellingham, M. C. (2011). A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis: what have we learned in the last decade? *CNS Neuroscience and Therapeutics, 17*, 4–31.

Beretta, F., Bassani, S., Binda, E., Verpelli, C., Bello, L., Galli, R., & Passafaro, M. (2009). The GluR2 subunit inhibits proliferation by inactivating Src-MAPK signalling and induces apoptosis by means of caspase 3/6-dependent activation in glioma cells. *The European Journal of Neuroscience, 30*, 25–34.

Bowie, D. (2008). Ionotropic glutamate receptors & CNS disorders. *CNS & Neurological Disorders Drug Targets, 7*, 129–143.

Buckingham, S. C., Campbell, S. L., Haas, B. R., Montana, V., Robel, S., Ogunrinu, T., & Sontheimer, H. (2011). Glutamate release by primary brain tumors induces epileptic activity. *Nature Medicine, 17*, 1269–1274.

Candolfi, M., Kroeger, K. M., Muhammad, A. K., Yagiz, K., Farrokhi, C., Pechnick, R. N., Lowenstein, P. R., & Castro, M. G. (2009). Gene therapy for brain cancer: Combination therapies provide enhanced efficacy and safety. *Current Gene Therapy, 9*, 409–421.

Chen, Y., & Guillemin, G. J. (2009). Kynurenine pathway metabolites in humans: Disease and healthy States. *International Journal of Tryptophan Research, 2*, 1–19.

Ciceroni, C., Arcella, A., Mosillo, P., Battaglia, G., Mastrantoni, E., Oliva, M. A., Carpinelli, G., Santoro, F., Sale, P., Ricci-Vitiani, L., De Maria, R., Pallini, R., Giangaspero, F., Nicoletti, F., & Melchiorri, D. (2008). Type-3 metabotropic glutamate receptors negatively modulate bone morphogenetic protein receptor signaling and support the tumourigenic potential of glioma-initiating cells. *Neuropharmacology, 55*, 568–576.

Colman, H., Zhang, L., Sulman, E. P., McDonald, J. M., Shooshtari, N. L., Rivera, A., Popoff, S., Nutt, C. L., Louis, D. N., Cairncross, J. G., Gilbert, M. R., Phillips, H. S., Mehta, M. P., Chakravarti, A., Pelloski, C. E., Bhat, K., Feuerstein, B. G., Jenkins, R. B., & Aldape, K. (2010). A multigene predictor of outcome in glioblastoma. *Neuro-Oncology, 12*, 49–57.

Contractor, A., Mulle, C., & Swanson, G. T. (2011). Kainate receptors coming of age: Milestones of two decades of research. *Trends in Neurosciences, 34*, 154–163.

Correa, D. D. (2010). Neurocognitive function in brain tumors. *Current Neurology and Neuroscience Reports, 10*, 232–239.

Curtis, D. R., Phillis, J. W., & Watkins, J. C. (1959). Chemical excitation of spinal neurones. *Nature, 183*, 611–612.

D'Antoni, S., Berretta, A., Bonaccorso, C. M., Bruno, V., Aronica, E., Nicoletti, F., & Catania, M. V. (2008). Metabotropic glutamate receptors in glial cells. *Neurochemical Research, 33*, 2436–2443.

de Groot, J., & Sontheimer, H. (2011). Glutamate and the biology of gliomas. *Glia, 59*, 1181–1189.

de Groot, J. F., Piao, Y., Lu, L., Fuller, G. N., & Yung, W. K. (2008). Knockdown of GluR1 expression by RNA interference inhibits glioma proliferation. *Journal of Neuro-Oncology, 88*, 121–133.

DeAngelis, L. M. (2001). Brain tumors. *The New England Journal of Medicine, 344*, 114–123.

Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., & Parkin, D. M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer, 127*, 2893–2917.

Giglio, P., & Gilbert, M. R. (2010). Neurologic complications of cancer and its treatment. *Current Oncology Reports, 12*, 50–59.

Grossman, S. A., Ye, X., Chamberlain, M., Mikkelsen, T., Batchelor, T., Desideri, S., Piantadosi, S., Fisher, J., & Fine, H. A. (2009). Talampanel with standard radiation and temozolomide in patients with newly diagnosed glioblastoma: A multicenter phase II trial. *Journal of Clinical Oncology, 27*, 4155–4161.

Guillemin, G. J., Cullen, K. M., Lim, C. K., Smythe, G. A., Garner, B., Kapoor, V., Takikawa, O., & Brew, B. J. (2007). Characterization of the kynurenine pathway in human neurons. *The Journal of Neuroscience, 27*, 12884–12892.

Hayashi, T. (1952). A physiological study of epileptic seizures following cortical stimulation in animals and its application to human clinics. *The Japanese Journal of Physiology, 3*, 46–64.

Hottinger, A. F., Yoon, H., DeAngelis, L. M., & Abrey, L. E. (2009). Neurological outcome of long-term glioblastoma survivors. *Journal of Neuro-Oncology, 95*, 301–305.

Ishiuchi, S., Tsuzuki, K., Yoshida, Y., Yamada, N., Hagimura, N., Okado, H., Miwa, A., Kurihara, H., Nakazato, Y., Tamura, M., Sasaki, T., & Ozawa, S. (2002). Blockage of Ca(2+)-permeable AMPA receptors suppresses migration and induces apoptosis in human glioblastoma cells. *Nature Medicine, 8*, 971–978.

Ishiuchi, S., Yoshida, Y., Sugawara, K., Aihara, M., Ohtani, T., Watanabe, T., Saito, N., Tsuzuki, K., Okado, H., Miwa, A., Nakazato, Y., & Ozawa, S. (2007). Ca^{2+} −permeable AMPA receptors regulate growth of human glioblastoma via Akt activation. *The Journal of Neuroscience, 27*, 7987–8001.

Kew, J. N., & Kemp, J. A. (2005). Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology, 179*, 4–29.

Klein, M., Heimans, J. J., Aaronson, N. K., van der Ploeg, H. M., Grit, J., Muller, M., Postma, T. J., Mooij, J. J., Boerman, R. H., Beute, G. N., Ossenkoppele, G. J., van Imhoff, G. W., Dekker, A. W., Jolles, J., Slotman, B. J., Struikmans, H., & Taphoorn, M. J. (2002). Effect of radiotherapy and other treatment-related factors on mid-term to long-term cognitive sequelae in low-grade gliomas: A comparative study. *Lancet, 360*, 1361–1368.

Kurz, K., Schroecksnadel, S., Weiss, G., & Fuchs, D. (2011). Association between increased tryptophan degradation and depression in cancer patients. *Current Opinion in Clinical Nutrition and Metabolic Care, 14*, 49–56.

Lau, A., & Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflügers Archiv, 460*, 525–542.

Li, Z., Bao, S., Wu, Q., Wang, H., Eyler, C., Sathornsumetee, S., Shi, Q., Cao, Y., Lathia, J., McLendon, R. E., Hjelmeland, A. B., & Rich, J. N. (2009). Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell, 15*, 501–513.

Liu, G., Yuan, X., Zeng, Z., Tunici, P., Ng, H., Abdulkadir, I. R., Lu, L., Irvin, D., Black, K. L., & Yu, J. S. (2006). Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Molecular Cancer, 5*, 67.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Archives of Ophthalmology, 58*, 193–201.

Ludolph, A. G., Schaz, U., Storch, A., Liebau, S., Fegert, J. M., & Boeckers, T. M. (2006). Methylphenidate exerts no neurotoxic, but neuroprotective effects in vitro. *Journal of Neural Transmission, 113*, 1927–1934.

Lyons, S. A., Chung, W. J., Weaver, A. K., Ogunrinu, T., & Sontheimer, H. (2007). Autocrine glutamate signaling promotes glioma cell invasion. *Cancer Research, 67*, 9463–9471.

Madden, D. R. (2002). The structure and function of glutamate receptor ion channels. *Nature Reviews Neuroscience, 3*, 91–101.

Namkoong, J., Shin, S. S., Lee, H. J., Marin, Y. E., Wall, B. A., Goydos, J. S., & Chen, S. (2007). Metabotropic glutamate receptor 1 and glutamate signaling in human melanoma. *Cancer Research, 67*, 2298–2305.

Oh, M. C., Kim, J. M., Safaee, M., Kaur, G., Sun, M. Z., Kaur, R., Celli, A., Mauro, T. M., & Parsa, A. T. (2012). Overexpression of calcium-permeable glutamate receptors in glioblastoma derived brain tumor initiating cells. *PloS One, 7*, e47846.

Peng, S., Zhang, Y., Zhang, J., Wang, H., & Ren, B. (2011). Glutamate receptors and signal transduction in learning and memory. *Molecular Biology Reports, 38*, 453–460.

Reid-Arndt, S. A., Hsieh, C., & Perry, M. C. (2010). Neuropsychological functioning and quality of life during the first year after completing chemotherapy for breast cancer. *Psycho-Oncology, 19*, 535–544.

Rinne, M. L., Lee, E. Q., & Wen, P. Y. (2012). Central nervous system complications of cancer therapy. *The Journal of Supportive Oncology, 10*, 133–141.

Rzeski, W., Turski, L., & Ikonomidou, C. (2001). Glutamate antagonists limit tumor growth. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 6372–6377.

Savaskan, N. E., Heckel, A., Hahnen, E., Engelhorn, T., Doerfler, A., Ganslandt, O., Nimsky, C., Buchfelder, M., & Eyupoglu, I. Y. (2008). Small interfering RNA-mediated xCT silencing in gliomas inhibits neurodegeneration and alleviates brain edema. *Nature Medicine, 14*, 629–632.

Shen, H., Kihara, T., Hongo, H., Wu, X., Kem, W. R., Shimohama, S., Akaike, A., Niidome, T., & Sugimoto, H. (2010). Neuroprotection by donepezil against glutamate excitotoxicity involves stimulation of alpha7 nicotinic receptors and internalization of NMDA receptors. *British Journal of Pharmacology, 161*, 127–139.

Shin, S. S., Martino, J. J., & Chen, S. (2008). Metabotropic glutamate receptors (mGlus) and cellular transformation. *Neuropharmacology, 55*, 396–402.

Singh, S. K., Hawkins, C., Clarke, I. D., Squire, J. A., Bayani, J., Hide, T., Henkelman, R. M., Cusimano, M. D., & Dirks, P. B. (2004). Identification of human brain tumour initiating cells. *Nature, 432*, 396–401.

Sontheimer, H. (2008). A role for glutamate in growth and invasion of primary brain tumors. *Journal of Neurochemistry, 105*, 287–295.

Stepulak, A., Sifringer, M., Rzeski, W., Endesfelder, S., Gratopp, A., Pohl, E. E., Bittigau, P., Felderhoff-Mueser, U., Kaindl, A. M., Buhrer, C., Hansen, H. H., Stryjecka-Zimmer, M.,

Turski, L., & Ikonomidou, C. (2005). NMDA antagonist inhibits the extracellular signal-regulated kinase pathway and suppresses cancer growth. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 15605–15610.

Stepulak, A., Luksch, H., Gebhardt, C., Uckermann, O., Marzahn, J., Sifringer, M., Rzeski, W., Staufner, C., Brocke, K. S., Turski, L., & Ikonomidou, C. (2009). Expression of glutamate receptor subunits in human cancers. *Histochemistry and Cell Biology, 132*, 435–445.

Stone, T. W., Forrest, C. M., & Darlington, L. G. (2012). Kynurenine pathway inhibition as a therapeutic strategy for neuroprotection. *The FEBS Journal, 279*, 1386–1397.

Takano, T., Lin, J. H., Arcuino, G., Gao, Q., Yang, J., & Nedergaard, M. (2001). Glutamate release promotes growth of malignant gliomas. *Nature Medicine, 7*, 1010–1015.

Taphoorn, M. J., & Klein, M. (2004). Cognitive deficits in adult patients with brain tumours. *Lancet Neurology, 3*, 159–168.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., & Dingledine, R. (2010). Glutamate receptor ion channels: Structure, regulation, and function. *Pharmacological Reviews, 62*, 405–496.

van Vliet, S. A., Blezer, E. L., Jongsma, M. J., Vanwersch, R. A., Olivier, B., & Philippens, I. H. (2008). Exploring the neuroprotective effects of modafinil in a marmoset Parkinson model with immunohistochemistry, magnetic resonance imaging and spectroscopy. *Brain Research, 1189*, 219–228.

Vincent, P., & Mulle, C. (2009). Kainate receptors in epilepsy and excitotoxicity. *Neuroscience, 158*, 309–323.

Weitzner, M. A., & Meyers, C. A. (1997). Cognitive functioning and quality of life in malignant glioma patients: A review of the literature. *Psycho-Oncology, 6*, 169–177.

Wu, G., Lu, Z. H., Wang, J., Wang, Y., Xie, X., Meyenhofer, M. F., & Ledeen, R. W. (2005). Enhanced susceptibility to kainate-induced seizures, neuronal apoptosis, and death in mice lacking gangliotetraose gangliosides: protection with LIGA 20, a membrane-permeant analog of GM1. *The Journal of Neuroscience, 25*, 11014–11022.

Ye, Z. C., & Sontheimer, H. (1999). Glioma cells release excitotoxic concentrations of glutamate. *Cancer Research, 59*, 4383–4391.

Zamanakou, M., Germenis, A. E., & Karanikas, V. (2007). Tumor immune escape mediated by indoleamine 2,3-dioxygenase. *Immunology Letters, 111*, 69–75.

Drug Treatments for Alzheimer's Disease: Hopes and Challenges

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Abstract

At present, no effective cure is available to slow down or prevent progressive neuronal loss in the pathogenesis of Alzheimer's disease (AD). All currently approved therapeutic agents provide symptomatic relief only. However, current agents under development exhibit potential protective effects on disease course. In this chapter, we consider currently marketed drugs for AD, including acetylcholinesterase inhibitors (AChEI) and antagonists of *N*-methyl-D-aspartate (NMDA) receptors. This work also discusses potential cognitive enhancers under clinical trial. We also review new therapies that are likely to slow down disease progression by mechanisms unrelated to amyloid or tau.

Keywords

ACE inhibitors • Alzheimer's disease • Amyloid • Antioxidants • Tau

1 Introduction

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder characterized by impaired learning and memory loss. One of the major pathological hallmarks of AD is the presence of a high density of extracellular senile plaques containing amyloid beta (Aβ) in the hippocampus and cerebral cortex (Shen et al. 2008). Irregular processing and accumulation of Aβ can impact negatively on important metabolic pathways within neurons and glial cells leading to neuronal and synaptic dysfunction and neurodegeneration. Aβ generation is regulated by the sequential proteolytic activity of β-secretase (BACE) and γ-secretase proteases (De Strooper et al. 2010; Hashimoto et al. 2010; Marks and Berg 2010; Neugroschl and Sano 2010; Tabaton et al. 2010). When the α-secretase pathway is used for the processing of amyloid precursor protein, no Aβ is generated (Nord et al. 2010). Presenilins 1 and 2 are the core components for the catalytic activity of γ-secretases, which are necessary for Aβ formation and progression of AD (Rocher-Ros et al. 2010).

Intracellular neurofibrillary tangles (NFTs) containing hyperphosphorylated tau protein are the second hallmark for AD (Attems et al. 2012; Wei et al. 2012). In the healthy neuronal population, tau protein helps to stabilize microtubules in the cytoskeleton (Kon et al. 2012). Although the mechanism for tau hyperphosphorylation in AD remains unclear, this hyperphosphorylation also occurs in numerous dementias and in the pathogenesis of other neurodegenerative disorders including Parkinson's disease (PD) (Lasagna-Reeves et al. 2012).

Prodromal mild cognitive impairment precedes AD, with more than half of these individuals progressing to AD within 4 years of symptomatic onset. Most individuals with mild cognitive impairment show early signs of AD pathology in their brain, including enhanced Aβ deposition and NFTs (Petersen et al. 1999). Owing to the postmitotic nature of neuronal cells, therapeutic agents that can slow down and/or reverse the pathogenic AD cascade prior to irreversible neuronal damage may alter the disease course in AD (Olazaran et al. 2010).

AD is the most common cause of dementia among elderly people over 65 years of age. For every 5-year age group beyond 65, the percentage of people with AD doubles. Scientists estimate that around 4.5 million people worldwide suffer from AD (Olazaran et al. 2010). Despite the alarming increase in the prevalence of AD, there are no disease-modifying drugs available. Currently approved treatments include the acetylcholinesterase inhibitors (AChEI) donepezil, galantamine, and rivastigmine and memantine, an antagonist of *N*-methyl-D-aspartate (NMDA) receptors. Other proposed therapies include β- and γ-secretase inhibitors, glucagon-like peptide-1 (GLP-1), bapineuzumab, solanezumab, statins, nonsteroidal anti-inflammatory drugs (NSAIDs), Cerebrolysin, psychotropic agents, and dietary supplements, vitamins, and nutraceuticals.

2 Acetylcholinesterase Inhibitors

The progressive degeneration of cholinergic neurons in the basal forebrain and reduced cholinergic neurotransmission in the cerebral cortex and other areas contribute significantly to impaired learning and memory observed in patients with AD. This neurochemical abnormality has provided the rationale for the development of first-line drugs for the treatment of AD which induce the cholinergic system in which the neurotransmitter acetylcholine is lacking (Pepeu and Giovannini 2009). Acetylcholinesterase inhibitors (AChEIs) are the first class of agents approved by the US Food and Drug Administration (FDA) for the treatment of AD. Tacrine (Cognex) was first approved in 1993 but is now rarely used due to elevations in hepatic enzymes and will not be discussed here. In 1996, donepezil was approved under the trade names Aricept, Adonep, Yasnal, and others. It was followed by rivastigmine (Exelon, Prometax, Ristidic, Vastigmex, or Evertas) in 2000 and galantamine (Reminyl, Razadyne, or Nivalin) (Pohanka 2011).

Inhibitors of acetylcholinesterase (AChE) share similar mechanisms of action while exhibiting additional pharmacological properties. The response to treatment with AChEIs is similar, and no individual AChEI has been shown to display superiority to others in efficacy studies. AChEIs bind to the synaptic cleft, thus stimulating the release of acetylcholine from the presynaptic cholinergic terminal and increasing the potential for interaction with the postsynaptic cholinergic receptor, therefore promoting the function of the cholinergic receptor (Pohanka 2011). Donepezil is a selective reversible AChEI with an affinity to AChE which is 1,000-fold higher than towards butyrylcholinesterase (BCHE) (Perdomo et al. 2003).

Table 1 Pharmacodynamics of AChEIs

	AChEIs		
Characteristics	Donepezil	Rivastigmine	Galantamine
Indications	Mild to moderate and severe AD	Mild to moderate AD; Parkinson's disease dementia	Mild to moderate AD
Half-life (hrs)	70	1.5	7
Administration regime	q.d.	b.i.d. for capsule; q.d. for the patch	b.i.d. for the non-ER form; q.d. for the ER form
Metabolism by hepatic CYP enzymes	2D6, 3A4	No	2D6, 3A4
Protein binding (%)	96	40	18
Bioavailability	100	40	90
Absorption delayed by food (hrs)	No	No	Yes (1–2.5)
Time to reach peak serum concentration (hrs)	3–4	1	1 (2.5 with food); 4.5 with ER form
Titration schedule	Initial dose of 5 mg then advance to 10 mg after 1 month	Oral form: 1.5 mg b.i.d. for 4 weeks then 3 mg for 4 weeks then to 6 mg b.i.d. if tolerated	Non-ER form: 4 mg b.i.d. then 8 mg b.i.d. after 1 month to 12 mg b.i.d. after another month
		Patch form: initial patch of 5 cm^2 for 1 month then the 10 cm^2 patch	ER form: 8 mg q.d. after 1 month then to 16 mg for 1 month then 24 mg q.d. after another month
Adverse effects	Gastrointestinal, nausea, vomiting	Gastrointestinal, nausea, vomiting	Gastrointestinal, nausea, vomiting
	Reduced heart rate, sleep disturbances, muscle cramps	Reduced heart rate	Reduced heart rate

Abbreviations: q.d. every day; b.i.d. twice a day; ER extended release

Rivastigmine is a nonselective mixed inhibitor of both AChE and BCHE (Naik et al. 2009). Galantamine is an allosteric modulator of AChE with limited affinity towards BCHE. Apart from interaction with AChE, galantamine neuroprotective effects can be further attributed to its allosteric modulation of nicotinic acetylcholine receptor (nAChR) (Feng et al. 2010). The basic pharmacology and pharmacodynamics of these drugs are summarized in Table 1.

It remains unclear as to whether AChEIs have any disease-modifying properties in AD. It has been suggested that enhanced cholinergic transmission can slow down Aβ production (Kimura et al. 2005). Several imaging studies have shown a marked reduction in brain atrophy in patients treated with AChEIs (Krishnan et al. 2003). One study

has shown reduced decline in cognitive function in patients treated with AChEIs or in combination with memantine compared to placebo (Lopez et al. 2009). However, no disease-modifying effects have been reported in another study (Jack et al. 2008).

Huperzine, a metabolite in the Chinese lycopod *Huperzia serrata* (Huperziaceae), is another drug that has been shown to have a neuroprotective effect suitable for the treatment of AD. It exists in two forms: A and B. However, the A variant is most likely pharmacologically relevant. It is a noncompetitive inhibitor of AChE, with no effect on BCHE. As well, it is a potent antagonist of the NMDA receptor (Gao et al. 2009). It can also attenuate oxidative stress and upregulate endogenous antioxidant activity in the CNS (Coleman et al. 2008). Huperzine has become target for extensive pharmacological research and is available for the treatment of neurodegenerative diseases in China. However, it is yet to be approved in the USA, Europe, and Australia. Favorable outcomes from a current clinical trial will allow the drug to be approved for therapy purposes in those countries (Ma et al. 2007).

3 Memantine

Memantine antagonizes the NMDA receptor by replacing the potassium in the NMDA ion channel to reduce the efflux of intracellular calcium into neurons and prevent excitotoxicity and subsequent apoptosis. Prior to its approval for the treatment of AD, memantine was used for the treatment of Parkinson's disease in Germany (Winblad et al. 1999). In 2004, memantine (Namenda, Ebixa, or Axura) was approved for the treatment of moderate to severe AD by the FDA in the USA (Ferris et al. 2003; Moebius et al. 2003a, b; Reisberg et al. 2003) (Table 2).

Whether the neuroprotective effects of memantine on neuronal cells observed in vitro are responsible for the symptomatic relief observed in clinical trials remains to be examined. Reduced Aβ deposition and tau hyperphosphorylation have been reported in in vitro studies after treatment with memantine (Martinez-Coria et al. 2010). Memantine has also been shown to provide beneficial effects on long-term potentiation (LTP), which correlates with memory function (Frankiewicz and Parsons 1999). This observation provides a plausible explanation for the symptomatic relief reported in patients undergoing therapy with memantine.

Memantine may be administered alone or in combination with an AChEI. No adverse effects have been reported with patients receiving both drugs. Pharmacotherapy with an AChEI and memantine continues until the patient either succumbs to AD or the quality of life of the patient is significantly compromised that the combination therapy is vain. Some long-term open-labeled studies have shown that combination therapy with an AChEI and memantine may attenuate the progression of AD, although other studies show conflicting results (Lopez et al. 2009; Schneider et al. 2009; Schneider and Sano 2009).

Table 2 Pharmacodynamics of memantine

Indications	Moderate to severe AD
Half-life (hrs)	60–80
Metabolism by hepatic CYP enzymes	No
Protein binding (%)	50
Absorption delayed by food	No
Time to reach peak serum concentration (hrs)	3–7
Titration schedule	Initially 5 mg q.d. for 1 week, 5 mg b.i.d. for the next week, 10 mg in the morning and 5 mg in the evening for 1 week, and 10 mg hereafter

Abbreviations: q.d. every day; b.i.d. twice a day

4 β-Secretase Inhibitors

β-Secretase cleaves APP into two peptide fragments, Aβ40 and Aβ42 (Zhou et al. 2012). Therefore, inhibition of β-secretase may reduce plaque formation and slow down the progression of AD. Preliminary studies using β-secretase knockout models showed only minor phenotypic changes, suggesting that β-secretase inhibitors may be an effective therapeutic strategy (Tang and Ghosh 2011). Novel β-secretase inhibitors have been developed as potential preventative and/or intervention therapy for AD.

Treatment with the β-secretase inhibitor (GRL-8234) has been shown to attenuate age-mediated decline in cognitive function in a transgenic mice model expressing APP after long-term inhibitor treatment (Chang et al. 2011). Moreover, the same study showed that GRL-8234 reduced brain Aβ plaque load in these mice, suggesting a potential treatment for patients with late-stage AD (Chang et al. 2011).

TAK-070, a non-peptidic compound, has also been demonstrated to be a potent β-secretase inhibitor (Fukumoto et al. 2010; Takahashi et al. 2010). This compound can reduce the levels of soluble Aβ, promote neurotrophic levels of APP, and inhibit the extracellular deposition of insoluble Aβ. A clinical trial using TAK-070 is currently under consideration. Additional efficacy studies in rodents are underway (Fukumoto et al. 2010; Takahashi et al. 2010).

5 γ-Secretase Inhibitors

Inhibition of γ-secretase may also represent an important process to prevent the proteolytic processing of APP and regulate Aβ formation (De Strooper and Annaert 2010). However, these inhibitors have been shown to cause adverse effects in preclinical animal models and human clinical trials. In one study, inhibition of γ-secretase induced embryonic toxicity, likely due to impaired activation of the notch, which is vital for cellular development and differentiation (Donoviel et al. 1999).

In a Phase II clinical trial, the γ-secretase inhibitor LY450139 leads to unwanted side effects including drug-related rashes and lightening of hair color with none

reported in the placebo group (Fleisher et al. 2008). Similarly, a Phase III trial for the γ-secretase inhibitor semagacestat was halted after the cognitive symptoms worsened in patients receiving the drug compared those who were given the placebo. Moreover, the incidence of skin cancers was increased in patients receiving semagacestat (Samson 2010). Therefore, the key question is whether undesirable side effects may result from the inhibition of cleavage of additional substrates for APP processing and whether a therapeutic window is necessary for the administration of these inhibitors to reduce Aβ deposition.

Several agents, including NSAIDs and other organic compounds, have been recently shown to regulate γ-secretase activity, without interfering with notch signalling (Imbimbo and Giardina 2011; Imbimbo et al. 2011). These γ-secretase modulators may be efficacious for the treatment of AD without the serious adverse effects.

6 Bapineuzumab

Active immunization with synthetic full-length Aβ peptide (AN1792) has been discontinued due to significant cognitive and neurological impairment occurring as a result of meningoencephalitis occurring in 6 % of patients (Orgogozo et al. 2003). The antibody-mediated response to AN1792 has been associated with the N-terminal residues (Lee et al. 2005). Subsequent studies using antibodies which target the N-terminus of Aβ which do not contain T-cell activating epitopes can be protective against neurotoxicity and inhibit fibrillogenesis (McLaurin et al. 2002).

Bapineuzumab (AAB-001) is a humanized monoclonal antibody directed against the N-terminal region of Aβ. Magnetic resonance imaging (MRI) abnormalities have been noted in a Phase I trial which generally resolve. There are also reports of vasogenic edema, likely due to an interaction of bapineuzumab with Aβ occurring in the blood vasculature. These cases are largely associated with microhemorrhage which is asymptomatic (Black et al. 2010).

Additionally, bapineuzumab can improve cognitive performance assessed using the mini-mental state examination at lower doses compared to the placebo, while no change is observed in higher doses (>5 mg/kg). In a Phase II study, CSF t-tau and p-tau levels were significantly reduced at the end of the study compared with baseline in bapineuzumab-treated patients, while no change was detected in the placebo group. Interestingly, no difference was observed in Aβ levels in either group (Blennow et al. 2012; Winblad et al. 2012). These studies highlight the downstream effects of bapineuzumab on the neurodegenerative process in AD, which may prove useful as a disease-modifying therapy in the early stages of AD.

7 Solanezumab

Solanezumab (LY2062430) is a humanized monoclonal antibody that targets the central region of Aβ (Lachno et al. 2011; Reichert 2011; Samadi and Sultzer 2011).

Preclinical studies have shown that acute and subchronic treatment with solanezumab can ameliorate or reverse memory deficits in transgenic mice (Siemers et al. 2010; Panza et al. 2012; Uenaka et al. 2012). Unlike other disease-modifying immunotherapies, no effects on incidence or severity of cerebral amyloid angiopathy-associated microhemorrhages, a serious adverse effect associated with bapineuzumab, have been reported. Solanezumab displayed a good safety profile in a Phase II clinical study for AD and can significantly reduce cerebrospinal and plasma levels of Aβ and tau (Carlson et al. 2011; Farlow et al. 2012; Imbimbo et al. 2012).

In a Phase III trial, primary endpoints, both cognitive and functional, were not met in patients with mild to moderate AD. However, a statistically significant slowing of cognitive decline was shown in a prespecified secondary analysis of pooled data in patients with mild AD. Although solanezumab may be the first passive vaccine for the prevention of AD, immense scepticism still exists regarding the ability of this drug to slow down the progressive degeneration in patients with AD (Prodouz 2012). The next steps for solanezumab will be determined after discussions with pharmaceutical regulators.

8 Statins

Conflicting reports are available regarding the role of statins in improving brain function in AD. Epidemiological studies have shown a reduction in the risk of developing AD in patients using statins compared to those that have not been treated with cholesterol-lowering drugs. However, there are also numerous reports indicating significant cognitive decline following treatment with statins (Haag et al. 2009). Therefore, the usefulness of these drugs for the treatment of AD remains uncertain.

The neuroprotective effects of statins may be due to several mechanisms. Statins have demonstrated beneficial effects in the AD brain by enhancing degradation of extracellular Aβ by inducing the release of insulin-degrading enzymes from the resident microglial cells. Increase levels of soluble Aβ have been reported in microglia-depleted mice (Tamboli et al. 2010). However, since increased cholesterol levels is a risk factor for AD, it is difficult to determine whether the beneficial effects of statins in AD are related to the action of the statin or the improved lipid profile.

Numerous studies have been performed to determine the role of lipid-lowering agents for the treatment of AD. The Lipitor's Effect in Alzheimer's Dementia study found no significant difference in cognition or global function in patients with mild to moderate AD receiving Atorvastatin 80 mg/day for 72 weeks (Feldman et al. 2010). The Cholesterol Lowering Agent to Slow Progression of AD is a Phase III study currently underway to examine the safety and effectiveness of simvastatin to slow the progression of AD (McGuinness et al. 2010). Additional studies are needed to clarify the risks and benefits of statin therapy for the treatment of AD.

9 NSAIDs

A retrospective human epidemiological study showed that long-term use of NSAIDs, particularly ibuprofen, can protect against AD (Vlad et al. 2008). Similarly, experiments using rodent models for AD have predicted the effects of NSAIDs in humans, although little is known regarding the effect of NSAIDs on AD risk and pathogenesis. Prophylactic treatment of young 3xTg-AD mice with ibuprofen reduced intraneuronal Aβ oligomers and hyperphosphorylated tau and enhanced cognitive function (McKee et al. 2008). Ibuprofen has also been shown to reduce reactive oxygen species production by inhibiting the activity of nicotinamide adenine dinucleotide phosphate oxidase (Wilkinson et al. 2012). NSAIDs represent a promising therapeutic target also due to their modulatory effect on γ-secretase activity, without inhibiting important γ-secretase-dependent signalling pathways (Pettersson et al. 2011).

10 Glucagon-Like Peptide

Type 2 diabetes is a major risk factor for AD. Impaired insulin signalling appears to be a converging biochemical phenomena present in both diseases (Gotz et al. 2009; Ke et al. 2009). Apart from the pathological hallmarks in the AD brain, insulin receptors are reported to be desensitized in the AD brain, and this is referred to as type 3 diabetes (Steen et al. 2005). Therefore, effective treatment regime against type 2 diabetes may be of value in AD.

Activation of the glucagon-like peptide-1 (GLP-1) receptor has been recently shown to reduce Aβ deposition and downstream toxicity in both cellular and rodent models for AD (Li et al. 2010). Moreover, GLP-1 has been shown to reduce neuronal APP levels in vitro (Perry et al. 2003). Taken together, these studies provide further support for the association between diabetes and AD.

11 Cerebrolysin

Cerebrolysin is a parenterally administered digested peptide preparation present in the pig brain and mimics the action of endogenous neurotrophic factors. This compound has demonstrated neuroprotective effects. Positive effects of Cerebrolysin on Aβ- and tau-related pathologies, neuroinflammation, neurotrophic factors, oxidative stress, excitotoxicity, neurotransmission, brain metabolism, neuroplasticity, neuronal apoptosis and degeneration, neurogenesis, and cognition were demonstrated in experimental conditions (Allegri and Guekht 2012; Heiss et al. 2012; Masliah and Diez-Tejedor 2012; Menon et al. 2012; Sharma et al. 2012a, b; Thome and Doppler 2012; Vazquez-Roque et al. 2012; Xiao et al. 2012). As well, numerous studies have demonstrated symptomatic benefits in AD (Okamura et al. 2008). As a result, this drug is widely used in several countries,

although more definitive trials are needed to ensure the effectiveness of Cerebrolysin as a monotherapy or in conjunction with other treatment regimens.

12 Psychotropic Agents

Behavioral disturbances are common in AD and include depression, agitation, irritability, abnormal motor behaviors, and psychosis (Cummings 2003; Porter et al. 2003). At present, no drug is currently available to treat these behavioral disturbances. The use of conventional and atypical antipsychotics can increase the risk of mortality from ~ 2.6 % to ~ 4.5 % in patients with AD (Kales et al. 2007).

Several 1–12-week-long clinical trials have shown that risperidone is efficacious in reducing psychosis in nursing home patients with AD at low doses (Ballard and Howard 2006; Ballard and Waite 2006; Fossey et al. 2006). Similarly, there is no evidence for the use of antidepressants in AD (Rosenberg et al. 2010; Weintraub et al. 2010). Several studies have shown that valproate has no benefit for the treatment of agitation compared to placebo and induces severe toxicity (Herrmann and Lanctot 2007; Herrmann et al. 2007). Anxiolytics should be avoided in patients with AD as they induce confusion. Short-term benzodiazepines such as lorazepam or clonazepam may be useful in patients with frequent episodes of agitation (Tariot et al. 1998). Given the current evidence for benefit and harm for the indication of antipsychotics in AD, these drugs should only be prescribed with caution to patients where non-pharmacological therapeutics have failed and their behavioral change is extreme, administering the treatment only when needed and informing the patient and responsible carer of the associated risks.

13 Dietary Supplements, Vitamins, and Nutraceuticals

Dietary supplements and vitamins have been used in clinical trials for AD. Nutraceuticals describe nontraditional compounds that display pharmaceutical effects but cannot be advertised for a health disease claim. Formulations of these substances vary, and no regulatory standards are available to verify the potency of these compounds except that these products contain the active substance advertised.

13.1 Docosahexaenoic Acid

Docosahexaenoic acid (DHA) is the main constituent of the cellular membrane in the CNS. The levels of DHA are reduced with age but can be replenished to normal levels following dietary supplementation. DHA has also been shown to reduce Aβ deposition in mice and improve cognitive performance when administered as a dietary supplement (Geleijnse et al. 2012; Morris 2012; Rosales-Corral et al. 2012; Stough et al. 2012). One trial reported improvements in episodic

memory after post hoc analyses in older individuals who have cognitive test scores below the normal threshold for young adults (Okamura et al. 2008). However, DHA supplementation did not alter cognitive function in another study (Quinn et al. 2010).

13.2 B Vitamins

Increased level of homocysteine in the blood is a major risk factor for cardiovascular disease, AD, and other dementia-related disorders and can potentiate the neurotoxic effect of Aβ peptides in vitro. Homocysteine levels can be reduced by at least 30 % using regimens of B vitamins (B12 1 mg/day, B6 25 mg/day, and folic acid 5 mg/day). One trial showed a substantial reduction in homocysteine levels with these vitamins but did not reduce the rate of cognitive deterioration in AD patients compared to the placebo (Aisen et al. 2008).

13.3 Vitamin E

The antioxidant effects of vitamin E might be useful for the treatment of AD. In one study, vitamin E (1,000 IU b.i.d.) combined with selegiline (5 mg b.i.d.) delayed the time until patients with moderate AD require nursing home placement or succumb to the disorder, although no effect on cognition was reported (Sano et al. 1997). Another study reported no significant delay in conversion to AD or dementia when vitamin E was administered alone or with donepezil in patients with mild cognitive impairment (Petersen et al. 2005).

13.4 Homotaurine

Homotaurine (Vivimind) is an inhibitor of Aβ aggregation that is naturally found in certain seaweeds. It is marketed in Canada as a nutraceutical with no specific health claims. Although a Phase II safety trial reported reduced CSF Aβ levels, two follow-up 18-month Phase III trials did not show any efficacy (Aisen et al. 2006, 2011).

13.5 *Ginkgo biloba*

Ginkgo biloba leaves and extracts are currently used as over-the-counter preparations for the treatment of numerous medical conditions. The rationale for the use of *Ginkgo biloba* for the treatment of AD stems from its free radical scavenging activity or the inhibition of pro-inflammatory pathways or the potentiation of the cell stress response (Uluoglu and Guney 2010; Walesiuk and Braszko 2010; Serby et al. 2011). However, clinical studies are yet to validate the beneficial effects of *Ginkgo biloba* in improving cognitive function in AD patients.

13.6 Resveratrol

Resveratrol is a naturally occurring phytochemical that is found in highest levels in red wine and the skin of red grapes. A recent epidemiological study showed that monthly and weekly consumption of red wine is associated with a lower risk of dementia (Daffner 2010). Several studies have shown that caloric restriction enhance healthy living by activating a new class of histone deacetylase enzymes known as sirtuins (Ramesh et al. 2010; Smith et al. 2010; Wang et al. 2010). Resveratrol is a substance found in some plants which may activate sirtuins either directly or by its action on upstream signalling pathways to stimulate caloric restriction and slow down brain atrophy (Albani et al. 2010; Braidy et al. 2012; Massudi et al. 2012). Despite its poor penetration into the brain, some studies have shown that resveratrol can reduce brain Aβ aggregation (Chandrashekaran et al. 2010; Ladiwala et al. 2010; Sun et al. 2010). An NIA-funded clinical trial examining the importance of resveratrol for the treatment of AD is currently underway.

14 Conclusion

AChEIs and the NMDA receptor antagonist memantine are currently approved for the treatment of AD. Despite this, numerous agents have been identified that exert a plethora of neuroprotective effects that may be useful for the treatment of AD. The clinical impact of current agents that may slow down the progression of AD will be significant if these drugs are administered early during disease onset.

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References

Aisen, P. S., Saumier, D., et al. (2006). A phase II study targeting amyloid-beta with 3APS in mild-to-moderate Alzheimer disease. *Neurology, 67*(10), 1757–1763.

Aisen, P. S., Schneider, L. S., et al. (2008). High-dose B vitamin supplementation and cognitive decline in Alzheimer disease – a randomized controlled trial. *JAMA : The Journal of the American Medical Association, 300*(15), 1774–1783.

Aisen, P. S., Gauthier, S., et al. (2011). Tramiprosate in mild-to-moderate Alzheimer's disease - a randomized, double-blind, placebo-controlled, multi-centre study (the alphase study). *Archives of Medical Science, 7*(1), 102–111.

Albani, D., Polito, L., et al. (2010). Sirtuins as novel targets for Alzheimer's disease and other neurodegenerative disorders: Experimental and genetic evidence. *Journal of Alzheimer's Disease, 19*(1), 11–26.

Allegri, R. F., & Guekht, A. (2012). Cerebrolysin improves symptoms and delays progression in patients with Alzheimer's disease and vascular dementia. *Drugs of Today, 48*, 25–41.

Attems, J., Thal, D. R., et al. (2012). The relationship between subcortical tau pathology and Alzheimer's disease. *Biochemical Society Transactions, 40*, 711–715.

Ballard, C., & Howard, R. (2006). Neuroleptic drugs in dementia: Benefits and harm. *Nature Reviews Neuroscience, 7*(6), 492–500.

Ballard, C., & Waite, J. (2006). The effectiveness of atypical antipsychotics for the treatment of aggression and psychosis in Alzheimer's disease. *Cochrane Database of Systematic Reviews, 1*, CD003476.

Black, R. S., Sperling, R. A., et al. (2010). A single ascending dose study of bapineuzumab in patients with Alzheimer disease. *Alzheimer Disease and Associated Disorders, 24*(2), 198–203.

Blennow, K., Zetterberg, H., et al. (2012). Effect of immunotherapy with bapineuzumab on cerebrospinal fluid biomarker levels in patients with mild to moderate Alzheimer disease. *Archives of Neurology, 69*(8), 1002–1010.

Braidy, N., Jayasena, T., et al. (2012). Sirtuins in cognitive ageing and Alzheimer's disease. *Current Opinion in Psychiatry, 25*(3), 226–230.

Carlson, C., Estergard, W., et al. (2011). Prevalence of asymptomatic vasogenic edema in pretreatment Alzheimer's disease study cohorts from phase 3 trials of semagacestat and solanezumab. *Alzheimer's & Dementia, 7*(4), 396–401.

Chandrashekaran, I. R., Adda, C. G., et al. (2010). Inhibition by flavonoids of amyloid-like fibril formation by *Plasmodium falciparum* merozoite surface protein 2. *Biochemistry, 49*(28), 5899–5908.

Chang, W. P., Huang, X., et al. (2011). Beta-secretase inhibitor GRL-8234 rescues age-related cognitive decline in APP transgenic mice. *The FASEB Journal, 25*(2), 775–784.

Coleman, B. R., Ratcliffe, R. H., et al. (2008). [+]-Huperzine a treatment protects against N-methyl-D-aspartate-induced seizure/status epilepticus in rats. *Chemico-Biological Interactions, 175*(1–3), 387–395.

Cummings, J. L. (2003). Toward a molecular neuropsychiatry of neurodegenerative diseases. *Annals of Neurology, 54*(2), 147–154.

Daffner, K. R. (2010). Promoting successful cognitive aging: A comprehensive review. *Journal of Alzheimers Disease, 19*(4), 1101–1122.

De Strooper, B., & Annaert, W. (2010). Novel research horizons for presenilins and gamma-secretases in cell biology and disease. *Annual Review of Cell and Developmental Biology, 26*(26), 235–260.

De Strooper, B., Vassar, R., et al. (2010). The secretases: Enzymes with therapeutic potential in Alzheimer disease. *Nature Reviews. Neurology, 6*(2), 99–107.

Donoviel, D. B., Hadjantonakis, A. K., et al. (1999). Mice lacking both presenilin genes exhibit early embryonic patterning defects. *Genes & Development, 13*(21), 2801–2810.

Farlow, M., Amold, S. E., et al. (2012). Safety and biomarker effects of solanezumab in patients with Alzheimer's disease. *Alzheimer's & Dementia, 8*(4), 261–271.

Feldman, H. H., Doody, R. S., et al. (2010). Randomized controlled trial of atorvastatin in mild to moderate Alzheimer disease: LEADe. *Neurology, 74*(12), 956–964.

Feng, J., Huang, R., et al. (2010). A rapid review of economic evaluations of donepezil, rivastigmine, galantamine and memantine for Alzheimer's disease (Ad). *Value in Health, 13*(7), A560–A560.

Ferris, S. H., Schmitt, F. A., et al. (2003). Long-term treatment with the NMDA antagonist, memantine: Results of a 24-week, open-label extension study in moderate to severe Alzheimer's disease. *International Psychogeriatrics, 15*, 258–259.

Fleisher, A. S., Raman, R., et al. (2008). Phase 2 safety trial targeting amyloid beta production with a gamma-secretase inhibitor in Alzheimer disease. *Archives of Neurology, 65*(8), 1031–1038.

Fossey, J., Ballard, C., et al. (2006). Effect of enhanced psychosocial care on antipsychotic use in nursing home residents with severe dementia: Cluster randomised trial. *British Medical Journal, 332*(7544), 756–758A.

Frankiewicz, T., & Parsons, C. G. (1999). Memantine restores long term potentiation impaired by tonic N-methyl-D-aspartate (NMDA) receptor activation following reduction of Mg^{2+} in hippocampal slices. *Neuropharmacology, 38*(9), 1253–1259.

Fukumoto, H., Takahashi, H., et al. (2010). A noncompetitive BACE1 inhibitor TAK-070 ameliorates Abeta pathology and behavioral deficits in a mouse model of Alzheimer's disease. *The Journal of Neuroscience, 30*(33), 11157–11166.

Gao, X., Zheng, C. Y., et al. (2009). Huperzine a protects isolated rat brain mitochondria against beta-amyloid peptide. *Free Radical Biology & Medicine, 46*(11), 1454–1462.

Geleijnse, J. M., Giltay, E. J., et al. (2012). Effects of n-3 fatty acids on cognitive decline: A randomized, double-blind, placebo-controlled trial in stable myocardial infarction patients. *Alzheimer's & Dementia, 8*(4), 278–287.

Gotz, J., Ittner, L. M., et al. (2009). Common features between diabetes mellitus and Alzheimer's disease. *Cellular and Molecular Life Sciences, 66*(8), 1321–1325.

Haag, M. D., Hofman, A., et al. (2009). Statins are associated with a reduced risk of Alzheimer disease regardless of lipophilicity. The Rotterdam study. *Journal of Neurology, Neurosurgery, and Psychiatry, 80*(1), 13–17.

Hashimoto, T., Ogino, K., et al. (2010). Age-dependent increase in lysosome-associated membrane protein 1 and early-onset behavioral deficits in APPSL transgenic mouse model of Alzheimer's disease. *Neuroscience Letters, 469*(2), 273–277.

Heiss, W. D., Brainin, M., et al. (2012). Cerebrolysin in patients with acute ischemic stroke in Asia results of a double-blind, placebo-controlled randomized trial. *Stroke, 43*(3), 630–636.

Herrmann, N., & Lanctot, K. L. (2007). Pharmacologic management of neuropsychiatric symptoms of Alzheimer disease. *Canadian Journal of Psychiatry-Revue Canadienne De Psychiatrie, 52*(10), 630–646.

Herrmann, N., Lanctot, K. L., et al. (2007). A placebo-controlled trial of valproate for agitation and aggression in Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders, 23*(2), 116–119.

Imbimbo, B. P., & Giardina, G. A. (2011). Gamma-secretase inhibitors and modulators for the treatment of Alzheimer's disease: Disappointments and hopes. *Current Topics in Medicinal Chemistry, 11*(12), 1555–1570.

Imbimbo, B. P., Panza, F., et al. (2011). Therapeutic intervention for Alzheimer's disease with gamma-secretase inhibitors: Still a viable option? *Expert Opinion on Investigational Drugs, 20*(3), 325–341.

Imbimbo, B. P., Ottonello, S., et al. (2012). Solanezumab for the treatment of mild-to-moderate Alzheimer's disease. *Expert Review of Clinical Immunology, 8*(2), 135–149.

Jack, C. R., Petersen, R. C., et al. (2008). Longitudinal MRI findings from the vitamin E and donepezil treatment study for MCI. *Neurobiology of Aging, 29*(9), 1285–1295.

Kales, H. C., Valenstein, M., et al. (2007). Mortality risk in patients with dementia treated with antipsychotics versus other psychiatric medications. *The American Journal of Psychiatry, 164*(10), 1568–1576.

Ke, Y. D., Delerue, F., et al. (2009). Experimental diabetes mellitus exacerbates tau pathology in a transgenic mouse model of Alzheimer's disease. *PloS One, 4*(11), e7917.

Kimura, M., Akasofu, S., et al. (2005). Protective effect of donepezil against Abeta (1–40) neurotoxicity in rat septal neurons. *Brain Research, 1047*(1), 72–84.

Kon, T., Mori, F., et al. (2012). Abnormal tau deposition in neurons, but not in glial cells in the cerebral tissue surrounding arteriovenous malformation. *Neuropathology, 32*(3), 267–271.

Krishnan, K. R. R., Charles, H. C., et al. (2003). Randomized, placebo-controlled trial of the effects of donepezil on neuronal markers and hippocampal volumes in Alzheimer's disease. *The American Journal of Psychiatry, 160*(11), 2003–2011.

Lachno, D. R., Romeo, M. J., et al. (2011). Validation of ELISA methods for quantification of total Tau and phosporylated-Tau(181) in human cerebrospinal fluid with measurement in specimens from Two Alzheimer's disease studies. *Journal of Alzheimer's Disease, 26*(3), 531–541.

Ladiwala, A. R. A., Lin, J. C., et al. (2010). Resveratrol selectively remodels soluble oligomers and fibrils of amyloid Abeta into off-pathway conformers. *The Journal of Biological Chemistry, 285*(31), 24228–24237.

Lasagna-Reeves, C. A., Castillo-Carranza, D. L., et al. (2012). Identification of oligomers at early stages of tau aggregation in Alzheimer's disease. *The FASEB Journal, 26*(5), 1946–1959.

Lee, M., Bard, F., et al. (2005). Abeta42 Immunization in Alzheimer's disease generates Abeta N-terminal antibodies. *Annals of Neurology, 58*(3), 430–435.

Li, Y., Duffy, K. B., et al. (2010). GLP-1 receptor stimulation reduces amyloid-beta peptide accumulation and cytotoxicity in cellular and animal models of Alzheimer's disease. *Journal of Alzheimers Disease, 19*(4), 1205–1219.

Lopez, O. L., Baker, J. T., et al. (2009). Long-term effects of the concomitant use of memantine with cholinesterase inhibition in Alzheimer disease (vol 80, pg 600, 2009). *Journal of Neurology, Neurosurgery, and Psychiatry, 80*(9), 1056–1056.

Ma, X. Q., Tan, C. H., et al. (2007). Huperzine a from Huperzia species - an ethnopharmacological review. *Journal of Ethnopharmacology, 113*(1), 15–34.

Marks, N., & Berg, M. J. (2010). BACE and gamma-secretase characterization and their sorting as therapeutic targets to reduce amyloidogenesis. *Neurochemical Research, 35*(2), 181–210.

Martinez-Coria, H., Green, K. N., et al. (2010). Memantine improves cognition and reduces Alzheimer's-like neuropathology in transgenic mice. *The American Journal of Pathology, 176*(2), 870–880.

Masliah, E., & Diez-Tejedor, E. (2012). The pharmacology of neurotrophic treatment with cerebrolysin: Brain protection and repair to counteract pathologies of acute and chronic neurological disorders. *Drugs of Today, 48*, 3–24.

Massudi, H., Grant, R., et al. (2012). NAD(+) metabolism and oxidative stress: The golden nucleotide on a crown of thorns. *Redox Report, 17*(1), 28–46.

McGuinness, B., O'Hare, J., et al. (2010). Statins for the treatment of dementia. *Cochrane Database of Systematic Reviews, 8*, CD007514.

McKee, A. C., Carreras, I., et al. (2008). Ibuprofen reduces Abeta, hyperphosphorylated tau and memory deficits in Alzheimer mice. *Brain Research, 1207*, 225–236.

McLaurin, J., Cecal, R., et al. (2002). Therapeutically effective antibodies against amyloid-beta peptide target amyloid-beta residues 4–10 and inhibit cytotoxicity and fibrillogenesis. *Nature Medicine, 8*(11), 1263–1269.

Menon, P. K., Muresanu, D. F., et al. (2012). Cerebrolysin, a mixture of neurotrophic factors induces marked neuroprotection in spinal cord injury following intoxication of engineered nanoparticles from metals. *CNS & Neurological Disorders Drug Targets, 11*(1), 40–49.

Moebius, H. J., Galasko, D., et al. (2003a). Functional improvement from treatment with the NMDA antagonist memantine: Results of a 28-week, randomized, placebo-controlled study in advanced Alzheimer's disease. *European Neuropsychopharmacology, 13*, S388–S388.

Moebius, H. J., Reisberg, B., et al. (2003b). Long-term efficacy and safety benefits from treatment with the NMDA antagonist memantine: results of a 24-week, open-label extension study in moderate to severe Alzheimer's disease. *European Neuropsychopharmacology, 13*, S388–S389.

Morris, M. C. (2012). Symposium 1: Vitamins and cognitive development and performance nutritional determinants of cognitive aging and dementia. *The Proceedings of the Nutrition Society, 71*(1), 1–13.

Naik, R. S., Hartmann, J., et al. (2009). Effects of rivastigmine and donepezil on brain acetylcholine levels in acetylcholinesterase-deficient mice. *Journal of Pharmacy and Pharmaceutical Sciences, 12*(1), 79–85.

Neugroschl, J., & Sano, M. (2010). Current treatment and recent clinical research in Alzheimer's disease. *The Mount Sinai Journal of Medicine, 77*(1), 3–16.

Nord, L. C., Sundqvist, J., et al. (2010). Analysis of oestrogen regulation of alpha-, beta- and gamma-secretase gene and protein expression in cultured human neuronal and glial cells. *Neurodegenerative Diseases, 7*(6), 349–364.

Okamura, N., Funaki, Y., et al. (2008). In vivo visualization of donepezil binding in the brain of patients with Alzheimer's disease. *British Journal of Clinical Pharmacology, 65*(4), 472–479.

Olazaran, J., Reisberg, B., et al. (2010). Nonpharmacological therapies in Alzheimer's disease: A systematic review of efficacy. *Dementia and Geriatric Cognitive Disorders, 30*(2), 161–178.

Orgogozo, J. M., Gilman, S., et al. (2003). Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology, 61*(1), 46–54.

Panza, F., Frisardi, V., et al. (2012). Immunotherapy for Alzheimer's disease: From anti-beta-amyloid to tau-based immunization strategies. *Immunotherapy, 4*(2), 213–238.
Pepeu, G., & Giovannini, M. G. (2009). Cholinesterase inhibitors and beyond. *Current Alzheimer Research, 6*(2), 86–96.
Perdomo, C., Wilcock, G. K., et al. (2003). Donepezil: A meta-analysis of individual patient data from randomized controlled trials in the treatment of patients with mild to moderate Alzheimer's disease. *Journal of General Internal Medicine, 18*, 197–197.
Perry, T., Lahiri, D. K., et al. (2003). Glucagon-like peptide-1 decreases endogenous amyloid-beta peptide (Abeta) levels and protects hippocampal neurons from death induced by Abeta and iron. *Journal of Neuroscience Research, 72*(5), 603–612.
Petersen, R. C., Smith, G. E., et al. (1999). Mild cognitive impairment - clinical characterization and outcome. *Archives of Neurology, 56*(3), 303–308.
Petersen, R. C., Thal, L. J., et al. (2005). Donepezil and vitamin E in mild cognitive impairment. *Journal of the American Geriatrics Society, 53*(4), S14–S14.
Pettersson, M., Kauffman, G. W., et al. (2011). Novel gamma-secretase modulators: A review of patents from 2008 to 2010. *Expert Opinion on Therapeutic Patents, 21*(2), 205–226.
Pohanka, M. (2011). Alzheimer's disease and related neurodegenerative disorders: Implication and counteracting of melatonin. *Journal of Applied Biomedicine, 9*(4), 185–196.
Porter, V. R., Buxton, W. G., et al. (2003). Frequency and characteristics of anxiety among patients with Alzheimer's disease and related dementias. *The Journal of Neuropsychiatry and Clinical Neurosciences, 15*(2), 180–186.
Prodouz, S. (2012). LILLY (ELI) & COMPANY - LLY's Results of Phase 3 Solanezumab EXPEDITION Studies.
Quinn, J. F., Raman, R., et al. (2010). Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease a randomized trial. *JAMA : The Journal of the American Medical Association, 304*(17), 1903–1911.
Ramesh, B. N., Rao, T. S. S., et al. (2010). Neuronutrition and Alzheimer's disease. *Journal of Alzheimer's Disease, 19*(4), 1123–1139.
Reichert, J. M. (2011). Antibody-based therapeutics to watch in 2011. *MAbs, 3*(1), 76–99.
Reisberg, B., Doody, R., et al. (2003). Memantine in moderate-to-severe Alzheimer's disease. *The New England Journal of Medicine, 348*(14), 1333–1341.
Rocher-Ros, V., Marco, S., et al. (2010). Presenilin modulates EGFR signaling and cell transformation by regulating the ubiquitin ligase Fbw7. *Oncogene, 29*(20), 2950–2961.
Rosales-Corral, S. A., Acuna-Castroviejo, D., et al. (2012). Alzheimer's disease: Pathological mechanisms and the beneficial role of melatonin. *Journal of Pineal Research, 52*(2), 167–202.
Rosenberg, P. B., Drye, L. T., et al. (2010). Sertraline for the treatment of depression in Alzheimer disease. *The American Journal of Geriatric Psychiatry, 18*(2), 136–145.
Samadi, H., & Sultzer, D. (2011). Solanezumab for Alzheimer's disease. *Expert Opinion on Biological Therapy, 11*(6), 787–798.
Samson, K. (2010). NerveCenter: Phase III Alzheimer trial halted: Search for therapeutic biomarkers continues. *Annals of Neurology, 68*(4), A9–A12.
Sano, M., Ernesto, C., et al. (1997). A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. *The New England Journal of Medicine, 336*(17), 1216–1222.
Schneider, L. S., & Sano, M. (2009). Current Alzheimer's disease clinical trials: Methods and placebo outcomes. *Alzheimer's & Dementia, 5*(5), 388–397.
Schneider, B. M., Dodman, N. H., et al. (2009). Use of memantine in treatment of canine compulsive disorders. *Journal of Veterinary Behavior-Clinical Applications and Research, 4*(3), 118–126.
Serby, M. J., Burns, S. J., et al. (2011). Treatment of memory loss with herbal remedies. *Current Treatment Options in Neurology, 13*(5), 520–528.
Sharma, A., Muresanu, D. F., et al. (2012a). Superior neuroprotective effects of cerebrolysin in nanoparticle-induced exacerbation of hyperthermia-induced brain pathology. *CNS & Neurological Disorders Drug Targets, 11*(1), 7–25.

Sharma, H. S., Sharma, A., et al. (2012b). Neuroprotective effects of cerebrolysin, a combination of different active fragments of neurotrophic factors and peptides on the whole body hyperthermia-induced neurotoxicity: Modulatory roles of Co-morbidity factors and nanoparticle intoxication. *New Perspectives of Central Nervous System Injury and Neuroprotection, 102*, 249–276.

Shen, C. Y., Chen, Y. F., et al. (2008). Hydrogen peroxide promotes Abeta production through JNK-dependent activation of gamma-secretase. *The Journal of Biological Chemistry, 283*(25), 17721–17730.

Siemers, E. R., Friedrich, S., et al. (2010). Safety and changes in plasma and cerebrospinal fluid amyloid beta after a single administration of an amyloid beta monoclonal antibody in subjects with Alzheimer disease. *Clinical Neuropharmacology, 33*(2), 67–73.

Smith, P. J., Blumenthal, J. A., et al. (2010). Effects of the dietary approaches to stop hypertension diet, exercise, and caloric restriction on neurocognition in overweight adults with high blood pressure. *Hypertension, 55*(6), 1331–U1385.

Steen, E., Terry, B. M., et al. (2005). Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? *Journal of Alzheimer's Disease, 7*(1), 63–80.

Stough, C., Downey, L., et al. (2012). The effects of 90-day supplementation with the Omega-3 essential fatty acid docosahexaenoic acid (DHA) on cognitive function and visual acuity in a healthy aging population. *Neurobiology of Aging, 33*(4), 824.e1–824.e3.

Sun, A. Y., Wang, Q., et al. (2010). Resveratrol as a therapeutic agent for neurodegenerative diseases. *Molecular Neurobiology, 41*(2–3), 375–383.

Tabaton, M., Zhu, X. W., et al. (2010). Signaling effect of amyloid-beta(42) on the processing Abeta PP. *Experimental Neurology, 221*(1), 18–25.

Takahashi, H., Fukumoto, H., et al. (2010). Ameliorative effects of a non-competitive BACE1 inhibitor TAK-070 on Abeta peptide levels and impaired learning behavior in aged rats. *Brain Research, 1361*, 146–156.

Tamboli, I. Y., Barth, E., et al. (2010). Statins promote the degradation of extracellular amyloid {beta}-peptide by microglia via stimulation of exosome-associated insulin-degrading enzyme (IDE) secretion. *The Journal of Biological Chemistry, 285*(48), 37405–37414.

Tang, J., & Ghosh, A. (2011). Treating transgenic Alzheimer mice with a beta-secretase inhibitor, what have we learned? *Aging-Us, 3*(1), 14–16.

Tariot, P. N., Erb, R., et al. (1998). Efficacy and tolerability of carbamazepine for agitation and aggression in dementia. *The American Journal of Psychiatry, 155*(1), 54–61.

Thome, J., & Doppler, E. (2012). Safety profile of cerebrolysin: Clinical experience from dementia and stroke trials. *Drugs of Today, 48*, 63–69.

Uenaka, K., Nakano, M., et al. (2012). Comparison of pharmacokinetics, pharmacodynamics, safety, and tolerability of the amyloid beta monoclonal antibody solanezumab in Japanese and white patients with mild to moderate Alzheimer disease. *Clinical Neuropharmacology, 35*(1), 25–29.

Uluoglu, C., & Guney, H. Z. (2010). Drug research in the elderly people with dementia. *Turkish Journal of Geriatrics-Turk Geriatri Dergisi, 13*, 61–69.

Vazquez-Roque, R. A., Ramos, B., et al. (2012). Chronic administration of the neurotrophic agent cerebrolysin ameliorates the behavioral and morphological changes induced by neonatal ventral hippocampus lesion in a rat model of schizophrenia. *Journal of Neuroscience Research, 90*(1), 288–306.

Vlad, S. C., Miller, D. R., et al. (2008). Protective effects of NSAIDs on the development of Alzheimer disease. *Neurology, 70*(19), 1672–1677.

Walesiuk, A., & Braszko, J. J. (2010). Gingkoselect alleviates chronic corticosterone-induced spatial memory deficits in rats. *Fitoterapia, 81*(1), 25–29.

Wang, J., Fivecoat, H., et al. (2010). The role of Sirt1: At the crossroad between promotion of longevity and protection against Alzheimer's disease neuropathology. *Biochimica Et Biophysica Acta-Proteins and Proteomics, 1804*(8), 1690–1694.

Wei, Y., Miao, J. Y., et al. (2012). Endogenous and exogenous factors in hyperphosphorylation of Tau in Alzheimer's disease. *Progress in Biochemistry and Biophysics, 39*(8), 778–784.

Weintraub, D., Rosenberg, P. B., et al. (2010). Sertraline for the treatment of depression in Alzheimer disease: Week-24 outcomes. *The American Journal of Geriatric Psychiatry, 18*(4), 332–340.

Wilkinson, B. L., Cramer, P. E., et al. (2012). Ibuprofen attenuates oxidative damage through NOX2 inhibition in Alzheimer's disease. *Neurobiology of Aging, 33*(1), 197 e121–132.

Winblad, B., Poritis, N. (1999). Memantine in severe dementia: results of the 9M-Best Study (Benefit and efficacy in severely demented patients during treatment with memantine). *Int J Geriatr Psychiatry, 14*, 135–146.

Winblad, B., Andreasen, N., et al. (2012). Safety, tolerability, and antibody response of active Abeta immunotherapy with CAD106 in patients with Alzheimer's disease: Randomised, double-blind, placebo-controlled, first-in-human study. *Lancet Neurology, 11*(7), 597–604.

Xiao, S. F., Xue, H. B., et al. (2012). Therapeutic effects of cerebrolysin added to risperidone in patients with schizophrenia dominated by negative symptoms. *The Australian and New Zealand Journal of Psychiatry, 46*(2), 153–160.

Zhou, J. W., Cheng, X. R., et al. (2012). The activity and mRNA expression of beta-secretase, cathepsin D, and cathepsin B in the brain of senescence-accelerated mouse. *Journal of Alzheimer's Disease, 28*(2), 471–480.

Epilepsy

Stjepana Kovac and Matthew C. Walker

Contents

Abstract

Epilepsy is a chronic disorder defined by recurrent and unprovoked seizures and is one of the commonest disorders of the brain. Despite the burgeoning of new antiepileptic drugs, the burden of medically intractable epilepsy has not reduced significantly. One-third of patients with epilepsy continue to have unprovoked seizures despite advances in treatment. Cell death in seizures has been observed in in vitro and in vivo models of epilepsy and is the hallmark of hippocampal sclerosis, the commonest pathology associated with drug-resistant epilepsy.

Neuroprotection in epilepsy has a long tradition and with the discovery of new pathways involved in seizure induced cell death, strategies have been refined accordingly. In this chapter we will provide a comprehensive overview on the proposed mechanisms underlying cell death in epilepsy and how these can be targeted by exogenous and endogenous neuroprotectants. We will focus on the NMDA receptor and its downstream signalling cascades and their role in seizure induced cell death and potential drug targets arising from this central position within the cascade of cell death. We will summarize evidence

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supporting the involvement of AMPA receptors in seizure induced cell death. In the last part the focus will be on mitochondria and ROS as pivotal players in seizure induced cell death and how these are linked to NMDA receptor dysfunction. We will outline strategies targeting these downstream signalling cascades.

1 Introduction

Epilepsy is a chronic disorder defined by recurrent and unprovoked seizures and is one of the commonest disorders of the brain. Despite the burgeoning of new antiepileptic drugs in the twentieth and twenty-first century, the burden of medically intractable epilepsy has not reduced significantly. One-third of patients with epilepsy continue to have unprovoked seizures despite advances in treatment (Duncan et al. 2006). Cell death in seizures has been observed in in vitro and in vivo models of epilepsy and is the hallmark of hippocampal sclerosis, the commonest pathology associated with drug-resistant epilepsy (Swanson 1995; Deshpande et al. 2008). This neuronal death is critically dependent upon excessive neuronal activity and the duration of such activity (Pitkänen et al. 2002). Seizures are usually self-limiting; generalized tonic-clonic seizures typically last less than 90 s (Theodore et al. 1994). However, seizures can be prolonged, and status epilepticus (defined as continuous seizure activity lasting longer than 30 min), the maximal expression of epilepsy, can occur in people with or without a prior history of epilepsy (often as the result of acute brain pathology, e.g., stroke, infection or head injury). This condition is a medical emergency, as it can lead to substantial neuronal cell death, resulting in cognitive and neurological deficits. Even single prolonged seizures in humans can lead to permanent damage in the hippocampus (Pinto et al. 2011). Animal studies support this. The first studies, showing that electrographic seizure discharges alone rather than secondary systemic complications during generalized seizures can lead to cell death, were published by Meldrum and colleagues (1973).

Neuroprotection in epilepsy has a long tradition and was mainly aimed at preventing seizure-induced cell death. However, there is growing evidence that prolonged seizures result in not only neuronal cell death but also neuronal and network dysfunction, which also contribute to the associated morbidities. Therefore, a definition of neuroprotection in epilepsy should include preventing not just neuronal cell death but also network dysfunction (Sutula et al. 2003; Walker 2007). This is difficult to capture in an experimental setting, especially in experimental animal work. In contrast, robust assays are available to measure neuronal cell death in animals. Much research into neuroprotection in epilepsy has therefore focussed on seizure-induced cell death.

This chapter will provide a comprehensive overview on the proposed mechanisms underlying cell death in epilepsy and how these can be targeted by exogenous and endogenous neuroprotectants. The first part will focus on the NMDA receptor and its downstream signalling cascades and their role in seizure-induced cell death and potential drug targets arising from this central position within the cascade of cell death. Evidence supporting the involvement of AMPA

receptors in seizure-induced cell death will be summarized. The final part will focus on mitochondria and ROS as pivotal players in seizure-induced cell death and how these are linked to NMDA receptor dysfunction. Strategies targeting these downstream signalling cascades will be outlined.

2 Neuronal Cell Death in Epilepsy

For over 100 years, it has been recognized that status epilepticus can result in damage and injury to the hippocampus (Pfleger 1880). Prolonged convulsive seizures can lead to hypotension, ventilatory dysfunction, and metabolic acidosis due to excessive muscle activity that can lead to neuronal damage (Walton 1993). It was thought that this physiological compromise was the main mechanism underlying neuronal death, which would therefore be restricted to convulsive status epilepticus. Pioneering experiments by Meldrum and colleagues in baboons in the 1970s challenged the prevailing orthodoxy; they showed that brain seizure activity can cause neuronal damage even in the absence of a systemic convulsion; seizure-induced brain damage is therefore not just a secondary phenomenon (Meldrum et al. 1973). That seizure activity itself can cause neuronal injury is now widely accepted and it is appreciated that even non-convulsive status epilepticus, a condition encountered in certain clinical settings, such as in comatose patients in the intensive care unit, can result in neuronal damage (Schmutzhard and Pfausler 2011). A more vexed question is whether even single, brief seizures can cause neuronal damage. Longitudinal neuroimaging studies in humans and histopathological studies in animals indicate that repeated seizures over time can result in damage, in particular to the hippocampus (Kälviäinen and Salmenperä 2002; Kotloski et al. 2002).

3 NMDA Receptors and Seizure-Induced Cell Death

The NMDA receptor has been linked to epilepsy and is an important neuroprotective target in epilepsy. NMDA receptor dysfunction as in NMDA receptor encephalitis, where autoantibodies are directed against the NMDA receptor, can cause seizures (Dalmau et al. 2008). Anti-NMDA receptor antibody mediate reversible decreases in NMDAR surface density and synaptic localization (Hughes et al. 2010), which could explain the associated cognitive deficits seen with this condition but does not easily explain the common occurrence of seizures. NMDAR involvement is seen not only in rare cases of autoantibody-mediated epilepsy but also in more common epilepsies. Epileptogenic lesions such as focal cortical dysplasia have an altered pattern of NMDA receptor expression with an abundance of the NR2B subunit (Ying et al. 2004; Möddel et al. 2005). NMDA receptor activation during prolonged seizures may also regulate the expression and function of other channels. The current first-line treatments for status epilepticus target the benzodiazepine site of the GABA(A) receptor. However, prolonged seizure activity leads to NMDA receptor activation and internalization of GABA(A)

receptors (Muir et al. 2010) and therefore after ~30 min of seizure activity benzodiazepines lose their efficacy (Naylor et al. 2005). The reverse holds true for the NMDA receptor. NMDA receptor subunits are mobilized to the synaptic membrane after continuous seizure activity (Chen and Wasterlain 2006).

NMDA receptors have also been identified as the crucial mediator of glutamate-induced neurotoxicity, and it was later discovered that this is mainly mediated by Ca^{2+} influx (Lucas and Newhouse 1957; Curtis et al. 1959). NMDA receptor antagonists given early after status epilepticus in animal models have been shown to be neuroprotective (Schauwecker 2010; Loss et al. 2012). There is evidence that NMDA receptor excitotoxicity depends on the type of subunit expressed and on the location relative to the synapse with many proposing that the predominantly extrasynaptic NR2B subunit mediates detrimental effects on neurons (Monyer et al. 1994; Tovar and Westbrook 1999; Hardingham et al. 2002; Liu et al. 2007). In keeping with this, ifenprodil, which predominantly targets the NR2B-containing NMDA receptors, has been shown to be neuroprotective if given after status epilepticus (Frasca et al. 2011). However, the NMDA receptor not only has negative effects on neurons, as it is pivotal for long-term potentiation, a fundamental mechanism underlying memory consolidation. Moreover, the NMDA receptor has protective effects (Hardingham and Bading 2010). Such protective effects are mediated by CREB (cyclic-AMP response element-binding protein), a transcription factor which is activated by nuclear Ca^{2+}. CREB activation enhances pro-survival gene expression and therefore provides neuroprotection against insults (Chrivia et al. 1993; Hardingham et al. 2001; Papadia et al. 2005). CREB is regulated by the ERK 1/2 pathway and CaM kinase (Wu et al. 2001). Other important pathways that have been implicated in NMDA receptor-mediated neuroprotection include suppression of FOXO and boosting of antioxidant defense genes (Papadia et al. 2008). Interestingly, these pathways are affected by synaptic and extrasynaptic NMDAR activation in opposing fashions, emphasizing the dichotomy between protective synaptic and neurotoxic extrasynaptic NMDAR activation (Hardingham and Bading 2010; Fig. 1). More recently it has been discovered that synaptic NMDARs are gated by different endogenous co-agonists from those that gate non-synaptic NMDARs with the former being gated by *d*-serine and the latter by glycine (Papouin et al. 2012). This differential gating provides novel more specific targets for either the synaptic or extrasynaptic NMDARs. Interestingly, the same group found that NMDA-induced neurotoxicity relies solely on synaptic NMDAR activation, thereby challenging previous reports, which identified the extrasynaptic NMDAR as the major culprit for NMDAR-induced neurotoxicity.

One endogenous substance that acts at the glycine site of the NMDA receptor is kynurenic acid. The kynurenine pathway is the main route of amino acid tryptophan metabolism from dietary intake that is not used for protein metabolism. The two main members along this pathway are quinolinic acid and kynurenic acid. Both are linked to NMDA receptor function. Quinolinic acid acts as an agonist, whereas kynurenic acid acts as an antagonist at NMDA receptors (Stone and Perkins 1981; Vamos et al. 2009). Kynurenic acid acts at both the glutamate and at the allosteric glycine site of the NMDA receptor and at higher concentrations inhibits AMPA receptors

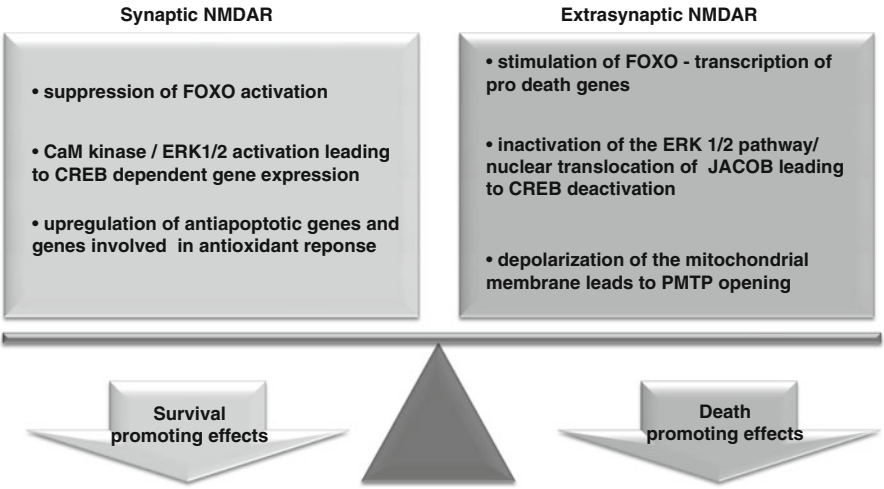

Fig. 1 Opposing effects of synaptic versus extrasynaptic NMDA receptors. *FOXO* forkhead box O class of transcription factors, *CREB* cyclic-AMP response element-binding protein, *CaM* nuclear Ca^{2+}/calmodulin-dependent protein. A more detailed summary of the opposing effects of synaptic versus extrasynaptic NMDA receptors is available in Hardingham and Bading (2010)

(Ganong and Cotman 1986; Watkins et al. 1990). Both receptors have been linked to epilepsy and epileptogenesis; kynurenic acid (KYNA) could therefore be an ideal neuroprotectant in epilepsy. There is evidence that the kynurenine pathway is involved in epilepsy and epileptogenesis. Depletion of KYNA within the hippocampus has been shown in pentylenetetrazole-induced seizures (Maciejak et al. 2009), and endogenously produced KYNA showed strong inhibitory effect on epileptiform activity vitro epilepsy model (Scharfman et al. 1999).

4 AMPA Receptors and Seizure-Induced Cell Death

The AMPA receptor is a tetrameric ionotropic transmembrane receptor for glutamate with fast synaptic transmission properties. It is the most abundant ionotropic glutamate receptor in the central nervous system. AMPA receptors are upregulated in neocortical tissue from patients suffering from pharmacoresistant epilepsy (Zilles et al. 1999; Palomero-Gallagher et al. 2012). Ion permeability of the receptor is determined by its subunit composition; GluR2-lacking receptors are permeable to Ca^{2+}, Na^{+}, and K^{+} and are inwardly rectifying, while channels containing the GluR2 subunit are selective for Na^{+} and K^{+} only (Isaac et al. 2007). Such differences in permeability are important, since in refractory status epilepticus, GluR2-subunits are internalized and the GluR2-lacking AMPARs are preferentially expressed on the surface of the cell. Furthermore, Rajasekaran and colleagues have shown that these receptor changes in the hippocampi of rats subjected to status epilepticus translate into changes in the functional properties of AMPAR-mediated currents, which show

increased inward rectification and the activation of which results in intracellular accumulation of Ca^{2+} (Rajasekaran et al. 2012). Drugs that target AMPA receptors have consequently also been shown to be neuroprotective in status epilepticus (Fritsch et al. 2010). AMPA receptor changes have not only been found in acquired epilepsy models but also in genetic models of epilepsy. The stargazer mice (lacking stargazing) have a trafficking defect in synaptic AMPARs in reticular thalamic nucleus cells and enhanced synaptic NMDARs activity leading to increases in thalamic excitability (Lacey et al. 2012). Targeting AMPA receptor trafficking therefore may reduce seizure-induced cell death.

5 Seizure-Induced Cell Death, Epilepsy, and Mitochondria

The common feature of both NMDA and GluR2-lacking AMPAR activation is an intracellular increase in Ca^{2+}. Ca^{2+} homeostasis is closely linked to the mitochondria and mitochondrial dysfunction leads to epilepsy in humans. It is well established that mutations in the mitochondrial genome or mutations in the nuclear genes coding for mitochondrial proteins lead to epileptic phenotypes (DiMauro 2004; Schapira 2006). Furthermore, a tight link between mitochondrial ATP production and seizures can be postulated, since seizures are a prominent feature in Leigh syndrome patients showing mutations in ATPase 6 gene (Canafoglia et al. 2001). Classical examples of mutations in the mitochondrial genome leading to seizures are MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) and MERRF (myoclonic epilepsy with ragged red fibres) syndrome (Rahman 2012). As implied in the names of mitochondrial disorders, multiple systems are affected; cerebral involvement represents only one facet of these multiorgan conditions. Epilepsies due to mutations in mitochondrial genes or nuclear genes coding for mitochondrial function are rare, when compared to the vast majority of epilepsies in general neurological practice. However, there is growing evidence that mitochondria and mitochondrial dysfunction may play an important role in many other epilepsies (Kann and Kovács 2007; Kann et al. 2011). Ongoing seizure activity induces ionic shifts and thereby increasing the demand for energy substrates to maintain transmembrane ion homeostasis (Lux et al. 1986). Therefore, it seems reasonable to link seizure activity to mitochondria, which are the main organelles responsible for the maintenance of transmembrane ion homeostasis. That mitochondria may play a prominent role in seizure generation is supported by the observation that mitochondrial membrane potential and Ca^{2+} concentrations change dramatically during epileptiform activity in vitro (Kovacs et al. 2005; Kovac et al. 2012). Mitochondrial complex I deficiency has been found in tissue resected from patient undergoing epilepsy surgery thus linking mitochondrial respiration to chronic seizure activity (Kunz et al. 2000). Another link comes from the effective treatment of refractory seizures with the ketogenic diet, a high-fat, low-carbohydrate diet (Bough et al. 2006; Bough 2008). Further analysis of the changes in gene expression secondary to the ketogenic diet showed mainly

upregulation of genes involved in oxidative phosphorylation and TCA cycle function (Bough et al. 2006). Finally, the pathology underlying one of the most common forms of epilepsy is hippocampal sclerosis which is characterized by apoptosis and necrosis, features that are tightly linked to mitochondria (Sloviter 1996).

But how do mitochondria contribute to seizure-induced cell death? There are two recognized forms of cell death: apoptosis and necrosis, and the mitochondria are important players in both. Apoptosis is defined as programmed cell death. A cascade of events leads to characteristic morphological cell changes with shrinkage of the cell with an intact cell membrane, fragmentation of DNA, and finally phagocytosis leading to cell death. In contrast necrotic cell death is described as a premature uncontrolled neuronal death that involves activation of hydrolytic enzymes and a rupture of the cell membrane followed by an inflammatory response.

Apoptosis involves intrinsic (activation within the cell or mitochondrion) and extrinsic (activation through plasma membrane receptors) pathways that converge to activate the executioner caspases, caspase 3 and caspase 7 (Tait and Green 2010). Inhibition of caspase 3 has been shown to be neuroprotective even if treatment is delayed for after the status epilepticus (Narkilahti et al. 2003b). Both pathways rely on mitochondrial outer membrane permeabilization (MOMP), which indicates a key point of no return. MOMP leads to release of cytochrome C. Cytochrome C is involved in electron transport during oxidative phosphorylation. However, it also leads to caspase activation after permeabilization of the outer mitochondrial membrane. Cells lacking cytochrome C fail to activate caspases and exhibit resistance to intrinsic apoptosis (Li et al. 2000).

Mitochondria act as a gatekeeper tuning Ca^{2+} concentrations in the cell and in cytoplasmic domains by buffering the Ca^{2+} during Ca^{2+} signalling. However, this buffering can be a starting point for apoptotic and necrotic cell death, if the matrix Ca^{2+} exceeds mitochondrial Ca^{2+} buffering capacity. This process leads to permeability transition, which is a phenomenon induced by high levels of matrix Ca^{2+}. It is characterized by the opening of the permeability transition pore (MPTP). The characteristic feature of MPTP opening is a permeability increase of the inner mitochondrial membrane to substances with small molecular masses (up to 1,500 Da) (Bernardi 1999; Crompton et al. 1999; Bernardi et al. 2006). Factors that have been indentified to contribute to mitochondrial permeability transition are Ca^{2+} accumulation and mitochondrial membrane potential depolarization, whereas a low pH, Mg^{2+}, and ADP prevent MPTP opening. The exact structure of the MPTP still needs to be characterized; however, it is most likely a multimeric complex, which may be assembled following activation (Zorov et al. 2009). Cyclophilin D (CypD) is a component of the MPTP, which is used pharmacologically, as the CypD inhibitors, such as cyclosporin A, are potent inhibitors of MPTP opening (Elrod et al. 2010). Strong evidence that MPTP could be a target for drug development comes from recent studies in ischemic heart disease. Inhibition of MPTP opening has been hypothesized to underlie the protective effect of ischemic preconditioning and post conditioning of myocytes (Hausenloy et al. 2009).

These strategies have been translated to the brain and MPTP opening has become a target in stroke and traumatic brain injury (Sullivan et al. 2005; Osman et al. 2011). Cyclosporin A prevents seizure-induced mitochondrial membrane depolarization, an event that leads to seizure-induced cell death (Kovac et al. 2012). Persistent MPTP opening leads to a depolarization of the mitochondrial membrane potential with a subsequent decrease in ATP production and matrix swelling induces breaks in the outer mitochondrial membrane. Release of cytochrome C which contributes to the formation of the apoptosome finally activates the executor caspases. Besides permanent opening, the MPTP can undergo transient "flickering" states which may prevent permanent opening through decreasing intra-matrix Ca^{2+} concentrations (Petronilli et al. 1999). Whether the cell will undergo apoptotic or necrotic cell death depends on the proportion of mitochondria undergoing MPTP opening. Mitochondria undergoing MPTP opening are not capable of ATP generation due to a dissipation of the mitochondrial membrane potential. However, ATP is required to aggregate the apoptosome which is necessary to induce programmed cell death via caspases. If not enough ATP is available for apoptosome formation, the rise in cytosolic Ca^{2+} will activate hydrolytic enzymes leading to necrosis (Gramaglia et al. 2004). It is important to note that increases in reactive oxygen species (ROS) contribute to MPTP opening by increasing Ca^{2+} load via stimulation of IP_3/ryanodine receptors and via inhibition of SERCA pumps and plasma membrane Ca^{2+} channels (PMCA) (Camello-Almaraz et al. 2006).

Inhibition of cell death pathways would seem a rational approach to prevent seizure-induced cell death. However, studies using this strategy have varying and inconsistent results. Inhibiting caspase 3 activation, for example, has been shown to be neuroprotective after status epilepticus in one animal model, whereas another study showed that this pathway is not involved in seizure-induced neuronal necrosis (Fujikawa et al. 2002; Narkilahti et al. 2003a, b; Walker 2007). Possible explanations for this variation are differences in the epilepsy models used in these studies and the time point at which the intervention takes place. Indeed, there may be more than one downstream pathway involved in mediating neuronal death in epilepsy and the relative importance of these pathways may depend upon a host of factors, highlighting the significant challenges facing the search for neuroprotective targets in epilepsy.

Given that energy failure and starvation play a pivotal role in seizure-induced cell death, anaplerosis seems to be an appealing strategy to reduce seizure-induced cell death. Anaplerosis is the replenishment of energetic substrates and is a strategy that has been used to prevent seizure-induced cell death and seizures themselves (for a comprehensive review see (Kovac et al. 2012)). Anaplerotic substrates, such as pyruvate or alpha-ketoglutarate, for example, reduce seizure-induced cell death both in vitro and in vivo (Yamamoto 1990; Yamamoto and Mohanan 2003; Kim et al. 2007; Carvalho et al. 2011; Kovac et al. 2012).

Factors that limit the use of anaplerotic substrates for the treatment of seizures and seizure-induced cell death are pharmacokinetic properties of these compounds and their bioavailability. Some of the compounds of the tricarboxylic acid cycle are not

membrane permeable (e.g., succinyl-CoA) or lack adequate transporters in the neuron, (e.g., citrate), preventing their use as an anaplerotic substrate (Gnoni et al. 2009).

Pyruvate seems to be the most promising anaplerotic substrate given its pharmacokinetic properties and evidence from in vivo and in vitro animal and human data. In addition pyruvate exhibits antioxidant properties as it acts as a free radical scavenger (Andrae et al. 1985; Bassenge et al. 2000; Varma and Hegde 2007).

6 Seizure-Induced Cell Death and Reactive Oxygen Species (ROS)

ROS can contribute to neuronal damage and therefore to seizure-induced cell death through a myriad of pathways. ROS, especially of mitochondrial origin, contribute to MPTP opening by increasing Ca^{2+} load via stimulating IP_3/ryanodine receptors and inhibiting SERCA pumps and plasma membrane Ca^{2+} channels (PMCA) (Camello-Almaraz et al. 2006). More recently ROS generated as a direct consequence of NMDA receptor activation have been described (Brennan et al. 2009).

Due to their reactive nature, ROS can contribute to cell death in a more direct manner. These are lipid peroxidation, protein denaturation inducing inactivation of enzymes, and nucleic acid damage. It needs to be highlighted that all these events are consequences of a very high intracellular ROS burden, exceeding the cell's capacity for antioxidant defense.

With regard to the brain, lipid peroxidation is probably the most important detrimental ROS effect, due to the abundance of lipids within the brain. Lipid peroxidation leads to the production of conjugated dienic hydroperoxides that decompose into aldehydes, dienals, or alkanes. These are toxic to the neuron and can lead to apoptosis (McCracken et al. 2000). Nuclear damage to DNA can lead to activation of repair enzymes such as poly (ADP-ribose) polymerase (PARP). This enzyme is known to lead to rapid ATP depletion which can stimulate cascades leading to cell death (Szabó 1998; Heeres and Hergenrother 2007). On the other hand, ROS induce release of cytochrome C from the mitochondria which activates the apoptosome inducing the caspase cascade, which leads to caspase 3-induced cleavage of nuclear DNA repair enzyme (Kim et al. 2000).

Oxidative stress has been implicated in the pathology associated with acute seizures, status epilepticus, and epilepsy. Acute increases in reactive oxygen species leading to damage of proteins, lipids, and ultimately cell death were shown after repeated seizures (Bruce and Baudry 1995; Liang et al. 2000; Tejada et al. 2007). It has been shown that antioxidant compounds such as vitamin C prevent seizure-induced neuronal death (Santos et al. 2008, 2009). Moreover, the lack of cellular antioxidants, as in superoxide dismutase mutant mice, leads to seizures in vivo (Lynn et al. 2005). In humans, magnetic resonance spectroscopy has shown decreased brain levels of glutathione, indicative of oxidative stress, in the brain of patients with epilepsy when compared to age-matched healthy controls (Mueller et al. 2001). Reactive oxygen species can damage lipids in cell membranes.

ROS degrade polyunsaturated lipids, forming malondialdehyde (MDA), which therefore is a marker of oxidative damage within a cell. MDA has been shown to increase following chronic epilepsy implying that seizure-induced ROS production leads to oxidative damage of lipids and cell membranes (Bruce and Baudry 1995; Frantseva et al. 2000; Tejada et al. 2007). Animal studies have shown decreased levels of glutathione in rats after prolonged seizures (Sleven et al. 2006), and the ketogenic diet, an effective treatment for pharmacoresistant seizures, increased glutathione levels in rat hippocampi (Jarrett et al. 2008).

Two mechanistically different therapeutic approaches can reduce the oxidative burden within the cell. One is blocking the source of free radical generation, whereas the other aims at scavenging the free radicals that have already been produced within the cell. The first approach requires identifying the source of free radical generation within the cell.

Several enzymes generate ROS within the cell. The candidate enzymes for ROS generation during seizures are the mitochondria, NADPH oxidase, and xanthine oxidase. Mitochondria, through reactions in the electron transport chain at complex I and III, are the main producers of ROS within the cell. That the NADPH oxidase may play an important role in seizure-induced ROS generation is suggested by the finding that NMDA receptors play a pivotal role in seizure-induced neuronal damage (Schauwecker 2010) and that recent evidence indicates that direct pharmacological activation of NMDA receptors can activate NADPH oxidase, increasing free radical production and consequently neuronal death (Abramov et al. 2007; Brennan et al. 2009; Girouard et al. 2009). Moreover, xanthine oxidase (XO) is also a major potential source of ROS during periods of increased metabolism such as occur during seizure activity.

Excessive NMDA receptor activation is a crucial event in acute neurological disease with detrimental downstream effects on neurons which include free radical generation (Lipton 1999) and there is a strong link between epilepsy and NMDA receptor dysfunction (Ying et al. 1999; Schauwecker 2010). Recent data suggest that NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation (Brennan et al. 2009), and NADPH oxidase has been identified as a pivotal source of ROS generation in acute neurological disease such as stroke (Abramov et al. 2007).

In keeping with these findings, increases in NADPH oxidase activity have been linked to epilepsy models such as the pilocarpine and kainite model of epilepsy (Patel et al. 2005; Di Maio et al. 2011), and inhibition of NADPH oxidase was effective in reducing cell death in the in vivo pilocarpine model of temporal lobe epilepsy (Pestana et al. 2010).

ATP depletion results in adenine formation and consequently an increase in hypoxanthine and xanthine, substrates for xanthine oxidase (Xia and Zweier 1995; Kinugasa et al. 2003). Previous reports have suggested that there may be a beneficial effect of xanthine oxidase inhibition on seizures (Kramer et al. 1990; Tada et al. 1991; Zagnoni et al. 1994), although such an effect is not universally observed.

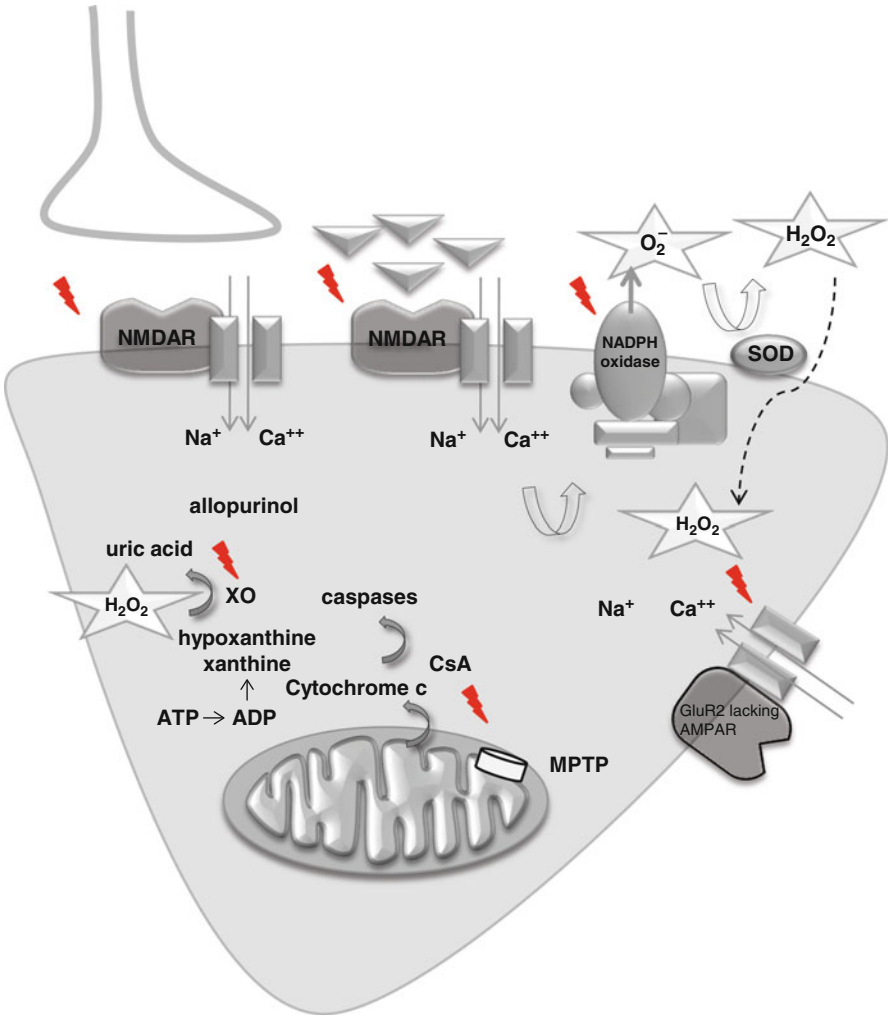

Fig. 2 Possible targets for neuroprotection in epilepsy. The figure illustrates potential targets of neuroprotection in epilepsy which include the ionotropic NMDA and AMPA glutamate receptor, the mitochondria and downstream mechanisms of ROS generation as the NADPH or xanthine oxidase. Abbreviations: *NADPH OX* NADPH oxidase, *MPTP* mitochondrial permeability transition pore, *CsA* cyclosporin A, *NMDAR* NMDA receptor, *AMPAR* AMPA receptor, *XO* xanthine oxidase, *SOD* superoxide dismutase

This chapter has outlined several mechanisms involved in seizure-induced cell death. The evidence for targeting NMDA and AMPA receptor, mitochondrial function, and ROS including several downstream targets of those receptors has been discussed. Neuroprotective targets in epilepsy are summarized in Fig. 2.

7 Conclusion

There are several interacting yet distinct mechanisms that can contribute to neuronal death in epilepsy, making common neuroprotective strategies challenging. The relative roles of each of the above mechanisms also show time dependency in status epilepticus with critical early steps likely to be the activation of ionotropic receptors and calcium influx, while later steps involve the activation of signalling and metabolic pathways. Directing treatments at the early steps has the advantage that there may be fewer targets but this strategy may be temporally less practical. Treating the later stages has a temporal advantage but the large number of pathways possibly involved presents a different challenge.

The observation that repeated brief seizures over time can lead to neuronal damage suggests that long-term neuroprotective strategies could offer the possibility of modifying comorbid declines in memory and other associated conditions such as depression.

References

Abramov, A. Y., Scorziello, A., & Duchen, M. R. (2007). Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation. *Journal of Neuroscience, 27*(5), 1129–1138.

Andrae, U., Singh, J., & Ziegler-Skylakakis, K. (1985). Pyruvate and related alpha-ketoacids protect mammalian cells in culture against hydrogen peroxide-induced cytotoxicity. *Toxicology Letters, 28*(2–3), 93–98.

Bassenge, E., Sommer, O., Schwemmer, M., & Bünger, R. (2000). Antioxidant pyruvate inhibits cardiac formation of reactive oxygen species through changes in redox state. *American Journal of Physiology. Heart and Circulatory Physiology, 279*(5), H2431–H2438.

Bernardi, P. (1999). Mitochondrial transport of cations: Channels, exchangers, and permeability transition. *Physiological Reviews, 79*(4), 1127–1155.

Bernardi, P., Krauskopf, A., Basso, E., Petronilli, V., Blachly-Dyson, E., Blalchy-Dyson, E., et al. (2006). The mitochondrial permeability transition from in vitro artifact to disease target. *FEBS Journal, 273*(10), 2077–2099.

Bough, K. (2008). Energy metabolism as part of the anticonvulsant mechanism of the ketogenic diet. *Epilepsia, 49*(Suppl 8), 91–93.

Bough, K. J., Wetherington, J., Hassel, B., Pare, J. F., Gawryluk, J. W., Greene, J. G., et al. (2006). Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Annals of Neurology, 60*(2), 223–235.

Brennan, A. M., Suh, S. W., Won, S. J., Narasimhan, P., Kauppinen, T. M., Lee, H., et al. (2009). NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. *Nature Neuroscience, 12*(7), 857–863.

Bruce, A. J., & Baudry, M. (1995). Oxygen free radicals in rat limbic structures after kainate-induced seizures. *Free Radical Biology & Medicine, 18*(6), 993–1002.

Camello-Almaraz, C., Gomez-Pinilla, P. J., Pozo, M. J., & Camello, P. J. (2006). Mitochondrial reactive oxygen species and Ca^{2+} signaling. *American Journal of Physiology. Cell Physiology, 291*(5), C1082–C1088.

Canafoglia, L., Franceschetti, S., Antozzi, C., Carrara, F., Farina, L., Granata, T., et al. (2001). Epileptic phenotypes associated with mitochondrial disorders. *Neurology, 56*(10), 1340–1346.

Carvalho, A. S. R., Torres, L. B., Persike, D. S., Fernandes, M. J. S., Amado, D., Naffah-Mazzacoratti, M. da. G., et al. (2011). Neuroprotective effect of pyruvate and oxaloacetate during pilocarpine induced status epilepticus in rats. *Neurochemistry International, 58*(3), 385–390.

Chen, J. W. Y., & Wasterlain, C. G. (2006). Status epilepticus: Pathophysiology and management in adults. *Lancet Neurology, 5*(3), 246–256.

Chrivia, J. C., Kwok, R. P., Lamb, N., Hagiwara, M., Montminy, M. R., & Goodman, R. H. (1993). Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature, 365*(6449), 855–859.

Crompton, M., Virji, S., Doyle, V., Johnson, N., & Ward, J. M. (1999). The mitochondrial permeability transition pore. *Biochemical Society Symposium, 66*, 167–179.

Curtis, D. R., Phillis, J. W., & Watkins, J. C. (1959). Chemical excitation of spinal neurones. *Nature, 183*(4661), 611–612.

Dalmau, J., Gleichman, A. J., Hughes, E. G., Rossi, J. E., Peng, X., Lai, M., et al. (2008). Anti-NMDA-receptor encephalitis: Case series and analysis of the effects of antibodies. *Lancet Neurology, 7*(12), 1091–1098.

Deshpande, L. S., Lou, J. K., Mian, A., Blair, R. E., Sombati, S., Attkisson, E., et al. (2008). Time course and mechanism of hippocampal neuronal death in an in vitro model of status epilepticus: Role of NMDA receptor activation and NMDA dependent calcium entry. *European Journal of Pharmacology, 583*(1), 73–83.

Di Maio, R., Mastroberardino, P. G., Hu, X., Montero, L., & Greenamyre, J. T. (2011). Pilocapine alters NMDA receptor expression and function in hippocampal neurons: NADPH oxidase and ERK1/2 mechanisms. *Neurobiology of Disease, 42*(3), 482–495.

DiMauro, S. (2004). Mitochondrial diseases. *Biochimica et Biophysica Acta, 1658*(1–2), 80–88.

Duncan, J. S., Sander, J. W., Sisodiya, S. M., & Walker, M. C. (2006). Adult epilepsy. *Lancet, 367*(9516), 1087–1100.

Elrod, J. W., Wong, R., Mishra, S., Vagnozzi, R. J., Sakthievel, B., Goonasekera, S. A., et al. (2010). Cyclophilin D controls mitochondrial pore-dependent Ca(2+) exchange, metabolic flexibility, and propensity for heart failure in mice. *The Journal of Clinical Investigation, 120*(10), 3680–3687.

Frantseva, M. V., Perez Velazquez, J. L., Tsoraklidis, G., Mendonca, A. J., Adamchik, Y., Mills, L. R., et al. (2000). Oxidative stress is involved in seizure-induced neurodegeneration in the kindling model of epilepsy. *Neuroscience, 97*(3), 431–435.

Frasca, A., Aalbers, M., Frigerio, F., Fiordaliso, F., Salio, M., Gobbi, M., et al. (2011). Misplaced NMDA receptors in epileptogenesis contribute to excitotoxicity. *Neurobiology of Disease, 43*(2), 507–515.

Fritsch, B., Stott, J. J., Joelle Donofrio, J., & Rogawski, M. A. (2010). Treatment of early and late kainic acid-induced status epilepticus with the noncompetitive AMPA receptor antagonist GYKI 52466. *Epilepsia, 51*(1), 108–117.

Fujikawa, D. G., Ke, X., Trinidad, R. B., Shinmei, S. S., & Wu, A. (2002). Caspase-3 is not activated in seizure-induced neuronal necrosis with internucleosomal DNA cleavage. *Journal of Neurochemistry, 83*(1), 229–240.

Ganong, A. H., & Cotman, C. W. (1986). Kynurenic acid and quinolinic acid act at N-methyl-D-aspartate receptors in the rat hippocampus. *Journal of Pharmacology and Experimental Therapeutics, 236*(1), 293–299.

Girouard, H., Wang, G., Gallo, E. F., Anrather, J., Zhou, P., Pickel, V. M., et al. (2009). NMDA receptor activation increases free radical production through nitric oxide and NOX2. *Journal of Neuroscience, 29*(8), 2545–2552.

Gnoni, G. V., Priore, P., Geelen, M. J. H., & Siculella, L. (2009). The mitochondrial citrate carrier: Metabolic role and regulation of its activity and expression. *IUBMB Life, 61*(10), 987–994.

Gramaglia, D., Gentile, A., Battaglia, M., Ranzato, L., Petronilli, V., Fassetta, M., et al. (2004). Apoptosis to necrosis switching downstream of apoptosome formation requires inhibition of both glycolysis and oxidative phosphorylation in a BCL-X(L)- and PKB/AKT-independent fashion. *Cell Death and Differentiation, 11*(3), 342–353.

Hardingham, G. E., & Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: Implications for neurodegenerative disorders. *Nature Reviews Neuroscience, 11*(10), 682–696.

Hardingham, G. E., Arnold, F. J., & Bading, H. (2001). Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity. *Nature Neuroscience, 4*(3), 261–267.

Hardingham, G. E., Fukunaga, Y., & Bading, H. (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nature Neuroscience, 5*(5), 405–414.

Hausenloy, D. J., Ong, S.-B., & Yellon, D. M. (2009). The mitochondrial permeability transition pore as a target for preconditioning and postconditioning. *Basic Research in Cardiology, 104*(2), 189–202.

Heeres, J. T., & Hergenrother, P. J. (2007). Poly(ADP-ribose) makes a date with death. *Current Opinion in Chemical Biology, 11*(6), 644–653.

Hughes, E. G., Peng, X., Gleichman, A. J., Lai, M., Zhou, L., Tsou, R., et al. (2010). Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *Journal of Neuroscience, 30*(17), 5866–5875.

Isaac, J. T. R., Ashby, M. C., & McBain, C. J. (2007). The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. *Neuron, 54*(6), 859–871.

Jarrett, S. G., Milder, J. B., Liang, L.-P., & Patel, M. (2008). The ketogenic diet increases mitochondrial glutathione levels. *Journal of Neurochemistry, 106*(3), 1044–1051.

Kälviäinen, R., & Salmenperä, T. (2002). Do recurrent seizures cause neuronal damage? A series of studies with MRI volumetry in adults with partial epilepsy. *Progress in Brain Research, 135*, 279–295.

Kann, O., & Kovács, R. (2007). Mitochondria and neuronal activity. *American Journal of Physiology. Cell Physiology, 292*(2), C641–C657.

Kann, O., Huchzermeyer, C., Kovács, R., Wirtz, S., & Schuelke, M. (2011). Gamma oscillations in the hippocampus require high complex I gene expression and strong functional performance of mitochondria. *Brain, 134*(2), 345–358.

Kim, G. W., Sugawara, T., & Chan, P. H. (2000). Involvement of oxidative stress and caspase-3 in cortical infarction after photothrombotic ischemia in mice. *Journal of Cerebral Blood Flow and Metabolism, 20*(12), 1690–1701.

Kim, T.-Y., Yi, J.-S., Chung, S.-J., Kim, D.-K., Byun, H.-R., Lee, J.-Y., et al. (2007). Pyruvate protects against kainate-induced epileptic brain damage in rats. *Experimental Neurology, 208*(1), 159–167.

Kinugasa, Y., Ogino, K., Furuse, Y., Shiomi, T., Tsutsui, H., Yamamoto, T., et al. (2003). Allopurinol improves cardiac dysfunction after ischemia-reperfusion via reduction of oxidative stress in isolated perfused rat hearts. *Circulation Journal, 67*(9), 781–787.

Kotloski, R., Lynch, M., Lauersdorf, S., & Sutula, T. (2002). Repeated brief seizures induce progressive hippocampal neuron loss and memory deficits. *Progress in Brain Research, 135*, 95–110.

Kovac, S., Domijan, A.-M., Walker, M. C., & Abramov, A. Y. (2012). Prolonged seizure activity impairs mitochondrial bioenergetics and induces cell death. *Journal of Cell Science, 125*(Pt 7), 1796–1806.

Kovacs, R., Kardos, J., Heinemann, U., & Kann, O. (2005). Mitochondrial calcium ion and membrane potential transients follow the pattern of epileptiform discharges in hippocampal slice cultures. *Journal of Neuroscience, 25*(17), 4260–4269.

Kramer, L. D., Locke, G. E., Nelson, L. G., & Ogunyemi, A. O. (1990). Status epilepticus following withdrawal of allopurinol. *Annals of Neurology, 27*(6), 691.

Kunz, W. S., Kudin, A. P., Vielhaber, S., Blümcke, I., Zuschratter, W., Schramm, J., et al. (2000). Mitochondrial complex I deficiency in the epileptic focus of patients with temporal lobe epilepsy. *Annals of Neurology, 48*(5), 766–773.

Lacey, C. J., Bryant, A., Brill, J., & Huguenard, J. R. (2012). Enhanced NMDA receptor-dependent thalamic excitation and network oscillations in stargazer mice. *Journal of Neuroscience, 32*(32), 11067–11081.

Li, K., Li, Y., Shelton, J. M., Richardson, J. A., Spencer, E., Chen, Z. J., et al. (2000). Cytochrome c deficiency causes embryonic lethality and attenuates stress-induced apoptosis. *Cell, 101*(4), 389–399.

Liang, L. P., Ho, Y. S., & Patel, M. (2000). Mitochondrial superoxide production in kainate-induced hippocampal damage. *Neuroscience, 101*(3), 563–570.

Lipton, P. (1999). Ischemic cell death in brain neurons. *Physiological Reviews, 79*(4), 1431–1568.

Liu, F.-Y., Wang, X.-F., Li, M.-W., Li, J.-M., Xi, Z.-Q., Luan, G.-M., et al. (2007). Upregulated expression of postsynaptic density-93 and N-methyl-D-aspartate receptors subunits 2B mRNA in temporal lobe tissue of epilepsy. *Biochemical and Biophysical Research Communications, 358*(3), 825–830.

Loss, C. M., Córdova, S. D., & De Oliveira, D. L. (2012). Ketamine reduces neuronal degeneration and anxiety levels when administered during early life-induced status epilepticus in rats. *Brain Research, 1474*, 110–117.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Archives of Ophthalmology, 58*(2), 193–201.

Lux, H. D., Heinemann, U., & Dietzel, I. (1986). Ionic changes and alterations in the size of the extracellular space during epileptic activity. *Advances in Neurology, 44*, 619–639.

Lynn, S., Huang, E. J., Elchuri, S., Naeemuddin, M., Nishinaka, Y., Yodoi, J., et al. (2005). Selective neuronal vulnerability and inadequate stress response in superoxide dismutase mutant mice. *Free Radical Biology & Medicine, 38*(6), 817–828.

Maciejak, P., Szyndler, J., Turzyńska, D., Sobolewska, A., Taracha, E., Skórzewska, A., et al. (2009). Time course of changes in the concentration of kynurenic acid in the brain of pentylenetetrazol-kindled rats. *Brain Research Bulletin, 78*(6), 299–305.

McCracken, E., Valeriani, V., Simpson, C., Jover, T., McCulloch, J., & Dewar, D. (2000). The lipid peroxidation by-product 4-hydroxynonenal is toxic to axons and oligodendrocytes. *Journal of Cerebral Blood Flow and Metabolism, 20*(11), 1529–1536.

Meldrum, B. S., Vigouroux, R. A., & Brierley, J. B. (1973). Systemic factors and epileptic brain damage. Prolonged seizures in paralyzed, artificially ventilated baboons. *Archives of Neurology, 29*(2), 82–87.

Möddel, G., Jacobson, B., Ying, Z., Janigro, D., Bingaman, W., González-Martínez, J., et al. (2005). The NMDA receptor NR2B subunit contributes to epileptogenesis in human cortical dysplasia. *Brain Research, 1046*(1–2), 10–23.

Monyer, H., Burnashev, N., Laurie, D. J., Sakmann, B., & Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron, 12*(3), 529–540.

Mueller, S. G., Trabesinger, A. H., Boesiger, P., & Wieser, H. G. (2001). Brain glutathione levels in patients with epilepsy measured by in vivo (1)H-MRS. *Neurology, 57*(8), 1422–1427.

Muir, J., Arancibia-Carcamo, I. L., MacAskill, A. F., Smith, K. R., Griffin, L. D., & Kittler, J. T. (2010). NMDA receptors regulate GABAA receptor lateral mobility and clustering at inhibitory synapses through serine 327 on the γ2 subunit. *Proceedings of the National Academy of Sciences of the United States of America, 107*(38), 16679–16684.

Narkilahti, S., Nissinen, J., & Pitkänen, A. (2003a). Administration of caspase 3 inhibitor during and after status epilepticus in rat: Effect on neuronal damage and epileptogenesis. *Neuropharmacology, 44*(8), 1068–1088.

Narkilahti, S., Pirttilä, T. J., Lukasiuk, K., Tuunanen, J., & Pitkänen, A. (2003b). Expression and activation of caspase 3 following status epilepticus in the rat. *European Journal of Neuroscience, 18*(6), 1486–1496.

Naylor, D. E., Liu, H., & Wasterlain, C. G. (2005). Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *Journal of Neuroscience, 25*(34), 7724–7733.

Osman, M. M., Lulic, D., Glover, L., Stahl, C. E., Lau, T., Van Loveren, H., et al. (2011). Cyclosporine-A as a neuroprotective agent against stroke: Its translation from laboratory research to clinical application. *Neuropeptides, 45*(6), 359–368.

Palomero-Gallagher, N., Schleicher, A., Bidmon, H.-J., Pannek, H.-W., Hans, V., Gorji, A., et al. (2012). Multireceptor analysis in human neocortex reveals complex alterations of receptor ligand binding in focal epilepsies. *Epilepsia, 53*(11), 1987–1997.

Papadia, S., Stevenson, P., Hardingham, N. R., Bading, H., & Hardingham, G. E. (2005). Nuclear Ca^{2+} and the cAMP response element-binding protein family mediate a late phase of activity-dependent neuroprotection. *Journal of Neuroscience, 25*(17), 4279–4287.

Papadia, S., Soriano, F. X., Léveillé, F., Martel, M.-A., Dakin, K. A., Hansen, H. H., et al. (2008). Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nature Neuroscience, 11*(4), 476–487.

Papouin, T., Ladépêche, L., Ruel, J., Sacchi, S., Labasque, M., Hanini, M., et al. (2012). Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. *Cell, 150*(3), 633–646.

Patel, M., Li, Q.-Y., Chang, L.-Y., Crapo, J., & Liang, L.-P. (2005). Activation of NADPH oxidase and extracellular superoxide production in seizure-induced hippocampal damage. *Journal of Neurochemistry, 92*(1), 123–131.

Pestana, R. R. F., Kinjo, E. R., Hernandes, M. S., & Britto, L. R. G. (2010). Reactive oxygen species generated by NADPH oxidase are involved in neurodegeneration in the pilocarpine model of temporal lobe epilepsy. *Neuroscience Letters, 484*(3), 187–191.

Petronilli, V., Miotto, G., Canton, M., Brini, M., Colonna, R., Bernardi, P., et al. (1999). Transient and long-lasting openings of the mitochondrial permeability transition pore can be monitored directly in intact cells by changes in mitochondrial calcein fluorescence. *Biophysical Journal, 76*(2), 725–734.

Pfleger, L. (1880). Beobachtungen uber Schrumpfung und Sklerose des Ammonshorns bei Epilepsie. *Allgemeine Zeitschrift für Psychiatrie, 35*, 359–365.

Pinto, A., Miller-Horn, J., Guilhoto, L., Harini, C., Morrison, P., Prabhu, S. P., et al. (2011). Teaching neuroimages: Mesial temporal sclerosis after a prolonged unprovoked seizure in an infant. *Neurology, 77*(19), e112–e113.

Pitkänen, A., Nissinen, J., Nairismägi, J., Lukasiuk, K., Gröhn, O. H. J., Miettinen, R., et al. (2002). Progression of neuronal damage after status epilepticus and during spontaneous seizures in a rat model of temporal lobe epilepsy. *Progress in Brain Research, 135*, 67–83.

Rahman, S. (2012). Mitochondrial disease and epilepsy. *Developmental Medicine and Child Neurology, 54*(5), 397–406.

Rajasekaran, K., Todorovic, M., & Kapur, J. (2012). Calcium-permeable AMPA receptors are expressed in a rodent model of status epilepticus. *Annals of Neurology, 72*(1), 91–102.

Santos, L. F. L., Freitas, R. L. M., Xavier, S. M. L., Saldanha, G. B., & Freitas, R. M. (2008). Neuroprotective actions of vitamin C related to decreased lipid peroxidation and increased catalase activity in adult rats after pilocarpine-induced seizures. *Pharmacology Biochemistry and Behavior, 89*(1), 1–5.

Santos, I. M. S., Tomé, A. da. R., Saldanha, G. B., Ferreira, P. M. P., Militão, G. C. G., Freitas, R. M. de. (2009). Oxidative stress in the hippocampus during experimental seizures can be ameliorated with the antioxidant ascorbic acid. *Oxidative Medicine and Cellular Longevity, 2*(4), 214–221

Schapira, A. H. V. (2006). Mitochondrial disease. *Lancet, 368*(9529), 70–82.

Scharfman, H. E., Hodgkins, P. S., Lee, S. C., & Schwarcz, R. (1999). Quantitative differences in the effects of de novo produced and exogenous kynurenic acid in rat brain slices. *Neuroscience Letters, 274*(2), 111–114.

Schauwecker, P. E. (2010). Neuroprotection by glutamate receptor antagonists against seizure-induced excitotoxic cell death in the aging brain. *Experimental Neurology, 224*(1), 207–218.
Schmutzhard, E., & Pfausler, B. (2011). Complications of the management of status epilepticus in the intensive care unit. *Epilepsia, 52*(Suppl 8), 39–41.
Sleven, H., Gibbs, J. E., Heales, S., Thom, M., & Cock, H. R. (2006). Depletion of reduced glutathione precedes inactivation of mitochondrial enzymes following limbic status epilepticus in the rat hippocampus. *Neurochemistry International, 48*(2), 75–82.
Sloviter, R. S. (1996). Hippocampal pathology and pathophysiology in temporal lobe epilepsy. *Neurologia, 11*(Suppl 4), 29–32.
Stone, T. W., & Perkins, M. N. (1981). Quinolinic acid: A potent endogenous excitant at amino acid receptors in CNS. *European Journal of Pharmacology, 72*(4), 411–412.
Sullivan, P. G., Rabchevsky, A. G., Waldmeier, P. C., & Springer, J. E. (2005). Mitochondrial permeability transition in CNS trauma: Cause or effect of neuronal cell death? *Journal of Neuroscience Research, 79*(1–2), 231–239.
Sutula, T. P., Hagen, J., & Pitkänen, A. (2003). Do epileptic seizures damage the brain? *Current Opinion in Neurology, 16*(2), 189–195.
Swanson, T. H. (1995). The pathophysiology of human mesial temporal lobe epilepsy. *Journal of Clinical Neurophysiology, 12*(1), 2–22.
Szabó, C. (1998). Role of poly(ADP-ribose)synthetase in inflammation. *European Journal of Pharmacology, 350*(1), 1–19.
Tada, H., Morooka, K., Arimoto, K., & Matsuo, T. (1991). Clinical effects of allopurinol on intractable epilepsy. *Epilepsia, 32*(2), 279–283.
Tait, S. W. G., & Green, D. R. (2010). Mitochondria and cell death: Outer membrane permeabilization and beyond. *Nature Reviews Molecular Cell Biology, 11*(9), 621–632.
Tejada, S., Sureda, A., Roca, C., Gamundí, A., & Esteban, S. (2007). Antioxidant response and oxidative damage in brain cortex after high dose of pilocarpine. *Brain Research Bulletin, 71*(4), 372–375.
Theodore, W. H., Porter, R. J., Albert, P., Kelley, K., Bromfield, E., Devinsky, O., et al. (1994). The secondarily generalized tonic-clonic seizure A videotape analysis. *Neurology, 44*(8), 1403.
Tovar, K. R., & Westbrook, G. L. (1999). The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *Journal of Neuroscience, 19*(10), 4180–4188.
Vamos, E., Pardutz, A., Klivenyi, P., Toldi, J., & Vecsei, L. (2009). The role of kynurenines in disorders of the central nervous system: Possibilities for neuroprotection. *Journal of Neurological Sciences, 283*(1–2), 21–27.
Varma, S. D., & Hegde, K. R. (2007). Lens thiol depletion by peroxynitrite. Protective effect of pyruvate. *Molecular and Cellular Biochemistry, 298*(1–2), 199–204.
Walker, M. (2007). Neuroprotection in epilepsy. *Epilepsia, 48*(Suppl 8), 66–68.
Walton, N. Y. (1993). Systemic effects of generalized convulsive status epilepticus. *Epilepsia, 34*(Suppl 1), S54–S58.
Watkins, J. C., Krogsgaard-Larsen, P., & Honoré, T. (1990). Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends in Pharmacological Sciences, 11*(1), 25–33.
Wu, G. Y., Deisseroth, K., & Tsien, R. W. (2001). Activity-dependent CREB phosphorylation: Convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. *Proceedings of the National Academy of Sciences of the United States of America, 98*(5), 2808–2813.
Xia, Y., & Zweier, J. L. (1995). Substrate control of free radical generation from xanthine oxidase in the postischemic heart. *Journal of Biological Chemistry, 270*(32), 18797–18803.
Yamamoto, H. (1990). Protection against cyanide-induced convulsions with alpha-ketoglutarate. *Toxicology, 61*(3), 221–228.

Yamamoto, H., & Mohanan, P. V. (2003). Effect of alpha-ketoglutarate and oxaloacetate on brain mitochondrial DNA damage and seizures induced by kainic acid in mice. *Toxicology Letters, 143*(2), 115–122.

Ying, Z., Babb, T. L., Mikuni, N., Najm, I., Drazba, J., & Bingaman, W. (1999). Selective coexpression of NMDAR2A/B and NMDAR1 subunit proteins in dysplastic neurons of human epileptic cortex. *Experimental Neurology, 159*(2), 409–418.

Ying, Z., Bingaman, W., & Najm, I. M. (2004). Increased numbers of coassembled PSD-95 to NMDA-receptor subunits NR2B and NR1 in human epileptic cortical dysplasia. *Epilepsia, 45*(4), 314–321.

Zagnoni, P. G., Bianchi, A., Zolo, P., Canger, R., Cornaggia, C., D'Alessandro, P., et al. (1994). Allopurinol as add-on therapy in refractory epilepsy: A double-blind placebo-controlled randomized study. *Epilepsia, 35*(1), 107–112.

Zilles, K., Qü, M. S., Köhling, R., & Speckmann, E. J. (1999). Ionotropic glutamate and GABA receptors in human epileptic neocortical tissue: Quantitative in vitro receptor autoradiography. *Neuroscience, 94*(4), 1051–1061.

Zorov, D. B., Juhaszova, M., Yaniv, Y., Nuss, H. B., Wang, S., & Sollott, S. J. (2009). Regulation and pharmacology of the mitochondrial permeability transition pore. *Cardiovascular Research, 83*(2), 213–225.

Excitotoxicity and Amyotrophic Lateral Sclerosis

Kim A. Staats and Ludo Van Den Bosch

Contents

Abstract

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by the progressive and selective death of motor neurons in the motor cortex, brainstem, and spinal cord. The only therapy with proven efficacy for ALS patients is a treatment with riluzole that offers patients a moderate

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increase in survival. Riluzole interferes with excitotoxicity, which is neuronal death caused by overstimulation of glutamate transporters. The high sensitivity of motor neurons to excitotoxicity is further illustrated by the observation that a number of exogenous toxic substances (excitotoxins) that stimulate glutamate receptors result in selective motor neuron death. This selective vulnerability of motor neurons to excitotoxic insults is due to their poor calcium buffering capacity in combination with the expression of calcium-permeable AMPA type of glutamate receptors. The AMPA receptor subunit responsible for calcium impermeability, GluR2, is expressed at lower levels, and RNA editing of GluR2 required for calcium impermeability is diminished in motor neurons of ALS patients. Not only calcium permeability of AMPA receptors but also glutamate transport into neighboring astrocytes plays an important role in ALS. Decreased levels of glutamate transporters are present in ALS patients and ALS animal models, implying an increased vulnerability to glutamate. This chapter aims to provide an overview of the known players and their interactions in the field of excitotoxic motor neuron death and in the context of ALS.

Keywords
ALS • Excitotoxicity • Motor neuron loss • Motor neuron disease • Glutamate

1 Introduction of ALS

1.1 Basics

Amyotrophic lateral sclerosis (ALS) is a devastating fatal neurodegenerative disease, with progressive loss of motor neurons of the motor cortex, brainstem, and spinal cord and denervation of muscle fibers, resulting in increasing muscle weakness and paralysis. The disease has an incidence of 2.7 cases per 100,000 people in Europe (Logroscino et al. 2010) and is more prevalent in the later years of life. In lack of a medical cure, average life expectancy is between 2 and 5 years. Patients usually die due to respiratory insufficiency or may opt for euthanasia where legislature permits (Maessen et al. 2010). Although ALS is characterized by degeneration of central nervous system tissue, mental functions remain largely unaffected resulting in a locked-in state (Kotchoubey et al. 2003). Only one drug is currently available to treat the disease, riluzole, and slows disease progression moderately (Miller et al. 2007).

1.2 ALS Genetics

There are both familial and sporadic cases of the disease, and a common disease mechanism is expected due to the indistinguishable clinical phenotypes of these patients. Approximately 90 % of ALS cases are sporadic in which a gene-environment

interaction is hypothesized. The remaining 10 % of ALS cases are inherited by mutations in a number of genes including superoxide dismutase 1 (SOD1), vesicle-associated membrane protein-associated protein B (VAPB), TAR DNA binding protein (TDP43), fused in sarcoma/translocated in liposarcoma (FUS/TLS), optineurin (OPTN), valosin-containing protein (VCP), ubiquilin2 (UBQLN2), and p62 (SQSTM1) which have been identified as causes of familial ALS (Van Deerlin et al. 2008; Rutherford et al. 2008; Del Bo et al. 2009; Kwiatkowski et al. 2009; Vance et al. 2009; Maruyama et al. 2010; Fecto et al. 2011; Deng et al. 2011; Johnson et al. 2010). In addition, hexanucleotide repeat expansions in the noncoding part of the "chromosome 9 open reading frame 72" (C9ORF72) gene were discovered as a new cause of ALS (Dejesus-Hernandez et al. 2011; Renton et al. 2011).

1.3 ALS Models

Overexpression of mutant forms of human SOD1 causes the ALS phenotype of transgenic SOD1 mice, which have made a large contribution to ALS research (Gurney et al. 1994). Many hallmarks of the disease are shared by patients and this rodent model, including specific motor neuron loss, aggregate formation, astrogliosis, microgliosis, and progressive paralysis. SOD1 detoxifies potentially cell-damaging free radicals, but the genetic ablation of SOD1 does not produce an ALS-like phenotype in mice (Reaume et al. 1996; Shefner et al. 1999), implying that mutant SOD1 causes the disease by a toxic gain of function. This gain of function may exert itself by protein misfolding, aggregation, impaired proteasome functioning, impaired retrograde transport, excitotoxic cell death, and others (reviewed in Bruijn et al. (2004)). Unfortunately, the discovery of the other ALS genes described above has not yet progressed into useful ALS model organisms, forcing most work described below to have been conducted on mutant SOD1 cells, on rodents, or on patients.

1.4 Non-cell Autonomous

ALS is a non-cell autonomous disease (Boillee et al. 2006a), meaning that multiple cell types affect disease pathology. By addition or deletion of mutant SOD1 in specific cell types, a number of cell types have been identified that influence the disease, including astrocytes (Yamanaka et al. 2008), microglia (Boillee et al. 2006b), Schwann cells (Lobsiger et al. 2009), and motor neurons (Jaarsma et al. 2008). Despite the influence of multiple cell types, mutant SOD1 expressed solely in neurons is sufficient to initiate the disease, with a slowed disease progression (Jaarsma et al. 2008). The vulnerability of motor neurons is also portrayed by the selective loss of motor neurons in the motor cortex, brainstem, and spinal cord in patients. There are a number of hypotheses that explain this cell type selectivity, including the long axons of these cells and the poor buffering of intracellular calcium.

1.5 Excitotoxicity in ALS

Glutamate is the neurotransmitter used to excite motor neurons that is the initiator of excitotoxicity in ALS. This neurotransmitter is the most abundant excitatory neurotransmitter in the brain and binds to *N*-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate (ionotropic) receptors and metabotropic glutamate receptors (mGluR). The levels of glutamate are increased in ALS patients (Fiszman et al. 2010; Spreux-Varoquaux et al. 2002), implying glutamate's role in the disease. The importance of excitotoxicity in ALS has been demonstrated mainly by the beneficial effects obtained by treating patients with riluzole. Although the precise mechanisms of this drug are not yet known, it has been shown to block NMDA receptors, it enhances reuptake of glutamate from the synaptic cleft, and it inhibits glutamate release by blocking voltage-gated sodium channels (Siniscalchi et al. 1999), thus preventing motor neuron cell death. Riluzole treatment increases predicted life span by 12 % in ALS mice (Lacomblez et al. 1996; Bensimon et al. 1994) and increases the probability of 1-year survival in patients by 9 % (Miller et al. 2007).

1.6 Non-glutamate Excitotoxins

A small number of substances are excitotoxic and can cause motor neuropathies and/or neurodegeneration. Domoic acid causes a motor neuropathy in some patients after food poisoning due to consumption of mussels containing high levels of this compound (Debonnel et al. 1989). Domoic acid is also associated with epileptic seizures in humans (Teitelbaum et al. 1990), sea lions (Scholin et al. 2000), and rats (Muha and Ramsdell 2011) and lowers the threshold for seizures in adult rats when exposed to it at neonatal stages (Gill et al. 2010). Also β-*N*-oxalyl-amino-L-alanine (BOAA), also known as β-oxalyl-L-α,β-diaminopropionic acid (ODAP), causes excitotoxicity. BOAA is highly present in the chickling pea (*Lathyrus sativus*) of which consumption leads to lathyrism (Streifler and Cohn 1981; Spencer et al. 1986). This substance is an AMPA receptor agonist (Willis et al. 1994; Kunig et al. 1994) that causes motor neuron degeneration (Chase et al. 1985; Weiss et al. 1989) and alternative effects on the central nervous system (Willis et al. 1994; Kunig et al. 1994). Another excitotoxin is one discovered by a high prevalence of ALS on the island of Guam, also known as the Western Pacific amyotrophic lateral sclerosis-Parkinsonism-dementia or the amyotrophic lateral sclerosis-Parkinsonism-dementia complex (ALS/PDC). Consumption of cycad seed products (*Cycas circinalis*) is responsible for the neurodegeneration in ALS/PDC of which β-methylamino-alanine (BMAA) is identified as a damaging substance (Steele and Guzman 1987; Hudson 1991; Wilson et al. 2002). BMAA is also a glutamate receptor agonist that causes motor neuron syndrome (Murch et al. 2004; Chang et al. 1993; Purdie et al. 2009) and damages motor neurons (Rao et al. 2006), cholinergic neurons in vitro (Liu et al. 2010; Brownson et al. 2002), and hippocampal neurons in vivo (Buenz and Howe 2007).

1.7 Poor Calcium Buffering

An additional explanation for the selective motor neuron vulnerability to excitotoxicity is poor capability of this cell type to buffer intracellular calcium; ALS-vulnerable spinal cord and brainstem motor neurons in mice display low endogenous calcium buffering capacities (Palecek et al. 1999; Lips and Keller 1998). In addition, ALS-resistant oculomotor neurons contain a far larger calcium buffering capacity than ALS-vulnerable motor neurons (von Lewinski and Keller 2005). This difference in calcium buffering capacity is directly due to the differential expression levels of the calcium-binding proteins calbindin-D28k and parvalbumin (Obal et al. 2006). The beneficial effect of calcium buffering by calcium-binding proteins is confirmed in experiments of acute neurodegeneration in a transgenic mouse by overexpressing the calcium-binding protein parvalbumin. According to expectations, parvalbumin overexpressing cells are protected against neurodegeneration induced by axotomy in vivo (Paizs et al. 2010) and excitotoxic cell death in vitro (Van Den Bosch et al. 2002a). In addition, parvalbumin overexpression delays disease onset and extends survival in ALS mice (Beers et al. 2001).

2 AMPA Receptors in Excitotoxicity in ALS

2.1 Basic Excitotoxicity

After release from the presynaptic neuron into the synaptic cleft, glutamate binds to NMDA, AMPA, or metabotropic receptors. The AMPA receptor plays an important role in motor neuron death in ALS. The AMPA receptor is a tetramer combining four different subunits (glutamate receptor units 1–4 (GluR1–4)) (Shi et al. 1999). All subunits bind glutamate, and the channels open after occupation of at least 2 binding sites (Mayer 2005). The importance of this receptor in ALS is demonstrated by the ablation of glutamate-induced apoptosis in cortical neurons in vitro (Cid et al. 2003) and in vivo when administering an AMPA receptor antagonist (Van Damme et al. 2003). The detrimental role of glutamate in the disease is demonstrated by the pronounced cell death that occurs to primary cultured neurons in vitro when exposed to low levels of glutamate, even as low as physiologically detected in cerebrospinal fluid (CSF) (Cid et al. 2003). To further illustrate the detrimental role of glutamate in ALS, administration of compounds that block the formation of glutamate increases cell survival, both in vivo and in vitro (Cid et al. 2003). In addition, decreasing vesicular glutamate transporter 2 (VGLUT2) extends survival of motor neurons in ALS mice but does not extend life span of ALS mice (Wootz et al. 2010).

2.2 AMPA Receptor-Mediated Cell Death

As discussed previously, motor neurons are excited by glutamate by, among others, the AMPA receptors. Interestingly, the AMPA receptors on motor neurons are

mainly calcium permeable in vitro (Van Den Bosch et al. 2000; Carriedo et al. 1996; Van Den Bosch et al. 2002b), which may explain the selective vulnerability of motor neurons to excitotoxic cell death. In addition, extracellular calcium entry via these calcium-permeable AMPA receptors is responsible for selective motor neuron death, as motor neuron death is inhibited by selective blockers of calcium-permeable AMPA receptors (Van Den Bosch et al. 2000). AMPA receptors of motor neurons have a lower rectification index and a higher relative calcium permeability ratio than other neurons (Van Damme et al. 2002). Not only in vitro but also in vivo motor neurons express calcium-permeable AMPA receptors (Greig et al. 2000). Interestingly, infusing the spinal cord of rats with AMPA receptor agonists induces neurodegeneration of specifically motor neurons and paralysis, which was blocked by coadministration of a selective blocker of calcium-permeable AMPA receptors (Corona and Tapia 2007; Sun et al. 2006). This demonstrates a specific vulnerability of motor neurons to AMPA receptor-mediated cell death.

2.3 GluR2 Subunit of the AMPA Receptor

The AMPA receptor plays an imperative role in excitotoxicity by its calcium permeability that is determined by the incorporation of the GluR2 subunit in the receptor complex. In most conditions, the AMPA receptor complex contains at least one GluR2 subunit and this prevents the influx of extracellular calcium into the neuron (Seeburg et al. 2001). In contrast, receptors lacking the GluR2 subunit are highly calcium permeable (Seeburg et al. 2001). Murine motor neurons contain lower levels of GluR2 mRNA compared to other neurons (Van Damme et al. 2002). In addition, laser capture microscopy and quantitative PCR demonstrated that the expression level of the GluR2 subunit is lower in (human) spinal motor neurons compared to other neurons (Heath et al. 2002; Kawahara et al. 2003), implying the intrinsic vulnerability of motor neurons to excitotoxicity. A general decrease of GluR2 is found in ALS model mice, portraying an increased vulnerability of these mice to excitotoxic insults (Zhao et al. 2008; Tortarolo et al. 2006). The role of GluR2 in ALS is investigated by genetically ablating GluR2 in ALS mice, which decreases survival in vivo and decreases cell survival in vitro (Van Damme et al. 2005). Upregulation of GluR2 expression in motor neurons of ALS mice increases survival (Tateno et al. 2004). In addition, pharmacological inhibition of the AMPA receptor prolongs survival in ALS mice (Van Damme et al. 2003; Tortarolo et al. 2006; Canton et al. 2001). Interestingly, the surrounding astrocytes influence the expression level of the GluR2 subunit in motor neurons, as soluble factors released from astrocytes affect GluR2 gene expression and neuronal vulnerability to excitotoxic insults, both in vitro and in vivo (Van Damme et al. 2007). Intriguingly, the presence of mutant SOD1 interferes with the production and/or secretion of these factors (Van Damme et al. 2007). Although not all these influencing factors are known, vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and glial cell-derived neurotrophic factor (GDNF) are able to upregulate GluR2 expression (Bogaert et al. 2010; Brene et al. 2000). Although the

role of AMPA receptors is established, there is no genetic evidence that polymorphisms in the GluR2 gene increase susceptibility for ALS (Bogaert et al. 2012).

2.4 GluR2 Editing

The GluR2 subunit's special characteristic of preventing calcium influx through the AMPA receptor is due to RNA editing at the Q/R site, and this process is also associated to ALS. This editing in the GluR2 pre-mRNA introduces a positively charged arginine at the Q/R site of the GluR2 peptide instead of the genetically encoded neutral glutamine (Burnashev et al. 1992). Under normal conditions, GluR2 pre-mRNA editing is virtually complete, but under pathological conditions it could become less efficient resulting in more calcium-permeable AMPA receptors. Indeed, it is reported that editing of pre-mRNA of GluR2 is defective in the spinal motor neurons of sALS patients (Kawahara et al. 2004). In addition, GluR2 gene expression and GluR2 RNA editing are reduced in the spinal cord of ALS patients (Takuma et al. 1999). Furthermore, the enzyme responsible for the RNA editing, adenosine deaminase acting on RNA 2 (ADAR2), is only present in 50 % of the motor neurons from sporadic ALS patients (Aizawa et al. 2010). Reduced editing at the Q/R site of GluR2 in mice results in a lethal phenotype accompanied with seizures and neurodegeneration (Brusa et al. 1995; Higuchi et al. 2000). Transgenic mice carrying a minigene with the GluR2 gene encoding an asparagine (GluR2-N) at the Q/R site, which makes editing impossible, are viable and fertile (Kuner et al. 2005) and are a useful tool to investigate the effect of calcium-permeable AMPA receptors, as AMPA receptors incorporating GluR2-N are permeable to calcium (Burnashev et al. 1992). The combined expression of the GluR2-N transgene and endogenous GluR2 alleles results in a twofold increase in permeability for calcium (Feldmeyer et al. 1999). Interestingly, these transgenic mice develop motor neuron degeneration late in life (Feldmeyer et al. 1999), and GluR2-N overexpression induces a progressive decline in function, as well as a degeneration of spinal motor neurons (Kuner et al. 2005). In accordance, GluR2-N expression in ALS mice exacerbates disease progression and reduces survival confirming the role for edited GluR2 in ALS (Kuner et al. 2005). In addition, conditional knockdown of ADAR2 in murine motor neurons causes a late-onset neurodegenerative condition (Hideyama et al. 2010).

3 Glutamate Transporters in ALS

3.1 Glutamate Transporter Levels in ALS

After release from the presynaptic neuron, glutamate is recycled by the glial and endothelial cells, including astrocytes. Astrocytic glutamate reuptake occurs by the glutamate transporters excitatory amino acid transporter 1 (EAAT1) and excitatory amino acid transporter 2 (EAAT2; also known as glutamate aspartate transporter

(GLAST1) and glutamate transporter 1 (GLT-1), respectively). These transporters internalize glutamate, for conversion to glutamine that is returned to the presynaptic neuron (Laake et al. 1995). Reduced glutamate uptake and EAAT2 protein expression are common in both ALS patients and ALS model systems (Staats and Van Den Bosch 2009). In vitro transfection of astrocytes with either mutant SOD1 or wild-type human SOD1 downregulates EAAT2 posttranscriptionally (Tortarolo et al. 2004), though it is unknown whether the downregulation of EAAT2 affects neuronal survival. Mutant SOD1 transfection also decreases glutamate transport in cell lines (Sala et al. 2005). Interestingly, this downregulation is present in ALS model rats at presymptomatic stages through to end stage (Howland et al. 2002), at end stage only (Warita et al. 2002), in ALS model mice at end stage (Bendotti et al. 2001), and in *post mortem* patient spinal cords by staining for EAAT2 (Sasaki et al. 2001). Interestingly, a decrease of EAAT2 protein is present in mutant SOD1 mice and in an environmental model of ALS/PDC (mice are fed with washed cycad flour containing BMAA, which causes an ALS-like phenotype (Wilson et al. 2002)) (Wilson et al. 2003). Although the loss of EAAT2 in ALS is apparent, it remains unclear whether this loss of EAAT2 proceeds or follows the loss of motor neurons.

3.2 Reducing Glutamate Transporters in ALS

To assess whether the loss of glutamate transport or the loss of EAAT2 specifically results in motor neuron loss, pharmacological and genetic tools have been employed. To begin, research conducted by pharmacologically inhibiting glutamate transport in the rat spinal cord failed to show any motor neuron loss despite the increased levels of glutamate (Tovar et al. 2009). This experiment has not (yet) been performed in ALS mice, leaving it to be elucidated whether decreased glutamate transport pharmacologically is detrimental for motor neuron survival in ALS. In contrast, a similar experiment has been performed to address whether EAAT2 loss specifically would induce motor neuron loss. EAAT2 null mice have been generated that live for approximately 6 weeks before they succumb to epileptic seizures and show increased vulnerability to acute brain injury (Tanaka et al. 1997). The heterozygous mice (containing 1 allele of EAAT2) were used to assess the effect of approximately 40 % knockdown of EAAT2 in the spinal cord in ALS mice (Pardo et al. 2006). This described knockdown resulted in a nonsignificant decrease of symptom onset and in significant, but moderate, decrease of life span in ALS mice (Pardo et al. 2006).

3.3 Increasing Glutamate Transporters in ALS

To assess the expected beneficial role of EAAT2 in ALS, transgenic mice overexpressing human EAAT2 in astrocytes only were crossbred with mutant SOD1 mice. Although glutamate uptake is increased in the mice and is protective on primary cortical neurons in culture, these effects were unable to affect

symptom onset or life span (Guo et al. 2003). Possibly, the expression levels were too low to induce an effect, or human EAAT2 is not as efficient as murine EAAT2 in mouse, as administration of ceftriaxone (a β-lactam antibiotic) or GPI-1046 (a synthetic, non-immunosuppressive derivative of FK506) increases EAAT2 protein levels and extends life span of ALS mice (Ganel et al. 2006; Rothstein et al. 2005). In addition, EAAT2 is also expressed by other cell types than astrocytes alone (Anderson and Swanson 2000), which were not targeted with this genetic experimental design. The beneficial effect of EAAT2 is often used as an explanation of beneficial effects found by cell transfers in ALS model rodents. For instance, the systemic transplantation of c-kit-positive cells from bone marrow in mutant SOD1 mice increases survival, which is, at least in part, attributed to increased EAAT2 expression induced by the transferred cells (Corti et al. 2010). The same holds true for the prolonged survival of ALS rats when treated with focal transplantation-based astrocyte replacement with wild-type glial-restricted precursors (GRPs) (Lepore et al. 2008). This study, interestingly, also focussed on the precise role of EAAT2 by also transplanting EAAT2 overexpressing GRPs and EAAT2 null GRPs. ALS mice treated with EAAT2 overexpressing GRPs show no additional increase of life span compared to wild-type GRP-treated ALS mice (which is already increased compared to controls). Intriguingly, this positive effect of transplantation of the wild-type GRPs is diminished in the mice transplanted with the EAAT2 null GRPs (Lepore et al. 2008). In addition, cocultures of human adipose-derived stem cells with astrocytes induce higher levels of EAAT2 in the astrocytes (Gu et al. 2010), though this treatment has not (yet) been shown to effect motor neuron survival in vitro or in vivo.

4 Conclusion

Excitotoxicity plays a major role in the pathophysiology of ALS. Excitotoxins can cause neurodegeneration and ALS/PDC. In addition, the only drug that prolongs survival in patients targets excitotoxicity. The specific vulnerability of motor neurons to excitotoxic insults may be explained by their poor calcium buffering capacity and their expression of calcium-permeable receptors. Cell death in ALS can be postponed by blocking the AMPA receptor, and applying AMPA receptor agonists induces neuronal death. The AMPA receptor subunit responsible for calcium impermeability, GluR2, is less expressed by motor neurons in comparison to other neuronal subtypes, and knockdown of this subunit prolongs survival in ALS mice. Moreover, the pre-RNA editing to obtain normal GluR2 is diminished in motor neurons and in ALS patients. In accordance, increasing unedited GluR2 in mice induces neurodegeneration. Not only AMPA receptor calcium permeability but also glutamate transport is affected in ALS. Decreased levels of EAAT2 are present in ALS patients and ALS rodents, implying an increased vulnerability to glutamate. In addition, increased levels of EAAT2 prolong survival, and decreased levels reduce survival in ALS mice. Future research may focus further to develop therapeutic strategies targeting excitotoxicity in ALS.

References

Aizawa, H., et al. (2010). TDP-43 pathology in sporadic ALS occurs in motor neurons lacking the RNA editing enzyme ADAR2. *Acta Neuropathologica, 120*(1), 75–84.

Anderson, C. M., & Swanson, R. A. (2000). Astrocyte glutamate transport: Review of properties, regulation, and physiological functions. *Glia, 32*(1), 1–14.

Beers, D. R., et al. (2001). Parvalbumin overexpression alters immune-mediated increases in intracellular calcium, and delays disease onset in a transgenic model of familial amyotrophic lateral sclerosis. *Journal of Neurochemistry, 79*(3), 499–509.

Bendotti, C., et al. (2001). Transgenic SOD1 G93A mice develop reduced GLT-1 in spinal cord without alterations in cerebrospinal fluid glutamate levels. *Journal of Neurochemistry, 79*(4), 737–746.

Bensimon, G., Lacomblez, L., & Meininger, V. (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *The New England Journal of Medicine, 330*(9), 585–591.

Bogaert, E., et al. (2010). VEGF protects motor neurons against excitotoxicity by upregulation of GluR2. *Neurobiology of Aging, 31*(12), 2185–2191.

Bogaert, E., et al. (2012). Polymorphisms in the GluR2 gene are not associated with amyotrophic lateral sclerosis. *Neurobiology of Aging, 33*(2), 418–420.

Boillee, S., Vande Velde, C., & Cleveland, D. W. (2006a). ALS: A disease of motor neurons and their nonneuronal neighbors. *Neuron, 52*(1), 39–59.

Boillee, S., et al. (2006b). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science, 312*(5778), 1389–1392.

Brene, S., et al. (2000). Regulation of GluR2 promoter activity by neurotrophic factors via a neuron-restrictive silencer element. *The European Journal of Neuroscience, 12*(5), 1525–1533.

Brownson, D. M., Mabry, T. J., & Leslie, S. W. (2002). The cycad neurotoxic amino acid, beta-*N*-methylamino-L-alanine (BMAA), elevates intracellular calcium levels in dissociated rat brain cells. *Journal of Ethnopharmacology, 82*(2–3), 159–167.

Bruijn, L. I., Miller, T. M., & Cleveland, D. W. (2004). Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annual Review of Neuroscience, 27*, 723–749.

Brusa, R., et al. (1995). Early-onset epilepsy and postnatal lethality associated with an editing-deficient GluR-B allele in mice. *Science, 270*(5242), 1677–1680.

Buenz, E. J., & Howe, C. L. (2007). Beta-methylamino-alanine (BMAA) injures hippocampal neurons in vivo. *Neurotoxicology, 28*(3), 702–704.

Burnashev, N., et al. (1992). Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. *Neuron, 8*(1), 189–198.

Canton, T., et al. (2001). RPR 119990, a novel alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid antagonist: Synthesis, pharmacological properties, and activity in an animal model of amyotrophic lateral sclerosis. *The Journal of Pharmacology and Experimental Therapeutics, 299*(1), 314–322.

Carriedo, S. G., Yin, H. Z., & Weiss, J. H. (1996). Motor neurons are selectively vulnerable to AMPA/kainate receptor- mediated injury in vitro. *The Journal of Neuroscience, 16*(13), 4069–4079.

Chang, Y. C., Chiu, S. J., & Kao, K. P. (1993). Beta-*N*-methylamino-L-alanine (L-BMAA) decreases brain glutamate receptor number and induces behavioral changes in rats. *The Chinese Journal of Physiology, 36*(2), 79–84.

Chase, R. A., et al. (1985). Comparative toxicities of alpha- and beta-*N*-oxalyl-L-alpha, beta-diaminopropionic acids to rat spinal cord. *Neuroscience Letters, 55*(1), 89–94.

Cid, C., et al. (2003). Low concentrations of glutamate induce apoptosis in cultured neurons: Implications for amyotrophic lateral sclerosis. *Journal of the Neurological Sciences, 206*(1), 91–95.

Corona, J. C., & Tapia, R. (2007). Ca^{2+}−permeable AMPA receptors and intracellular Ca^{2+} determine motoneuron vulnerability in rat spinal cord in vivo. *Neuropharmacology, 52*(5), 1219–1228.

Corti, S., et al. (2010). Systemic transplantation of c-kit + cells exerts a therapeutic effect in a model of amyotrophic lateral sclerosis. *Human Molecular Genetics, 19*(19), 3782–3796.

Debonnel, G., Beauchesne, L., & de Montigny, C. (1989). Domoic acid, the alleged "mussel toxin," might produce its neurotoxic effect through kainate receptor activation: An electrophysiological study in the dorsal hippocampus. *Canadian Journal of Physiology and Pharmacology, 67*(1), 29–33.

Dejesus-Hernandez, M., et al. (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron, 72*(2), 245–256.

Del Bo, R., et al. (2009). TARDBP (TDP-43) sequence analysis in patients with familial and sporadic ALS: Identification of two novel mutations. *European Journal of Neurology, 16*(6), 727–732.

Deng, H. X., et al. (2011). Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature, 477*(7363), 211–215.

Fecto, F., et al. (2011). SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. *Archives of Neurology, 68*(11), 1440–1446.

Feldmeyer, D., et al. (1999). Neurological dysfunctions in mice expressing different levels of the Q/R site-unedited AMPAR subunit GluR-B. *Nature Neuroscience, 2*(1), 57–64.

Fiszman, M. L., et al. (2010). In vitro neurotoxic properties and excitatory aminoacids concentration in the cerebrospinal fluid of amyotrophic lateral sclerosis patients. Relationship with the degree of certainty of disease diagnoses. *Acta Neurologica Scandinavica, 121*(2), 120–126.

Ganel, R., et al. (2006). Selective up-regulation of the glial Na^+ −dependent glutamate transporter GLT1 by a neuroimmunophilin ligand results in neuroprotection. *Neurobiology of Disease, 21*(3), 556–567.

Gill, D. A., et al. (2010). Neonatal exposure to low-dose domoic acid lowers seizure threshold in adult rats. *Neuroscience, 169*(4), 1789–1799.

Greig, A., et al. (2000). Characterization of the AMPA-activated receptors present on motoneurons. *Journal of Neurochemistry, 74*(1), 179–191.

Gu, R., et al. (2010). Human adipose-derived stem cells enhance the glutamate uptake function of GLT1 in SOD1(G93A)-bearing astrocytes. *Biochemical and Biophysical Research Communications, 393*(3), 481–486.

Guo, H., et al. (2003). Increased expression of the glial glutamate transporter EAAT2 modulates excitotoxicity and delays the onset but not the outcome of ALS in mice. *Human Molecular Genetics, 12*(19), 2519–2532.

Gurney, M. E., et al. (1994). Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science, 264*(5166), 1772–1775.

Heath, P. R., et al. (2002). Quantitative assessment of AMPA receptor mRNA in human spinal motor neurons isolated by laser capture microdissection. *NeuroReport, 13*(14), 1753–1757.

Hideyama, T., et al. (2010). Induced loss of ADAR2 engenders slow death of motor neurons from Q/R site-unedited GluR2. *The Journal of Neuroscience, 30*(36), 11917–11925.

Higuchi, M., et al. (2000). Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. *Nature, 406*(6791), 78–81.

Howland, D. S., et al. (2002). Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proceedings of the National Academy of Sciences of the United States of America, 99*(3), 1604–1609.

Hudson, A. J. (1991). Amyotrophic lateral sclerosis/parkinsonism/dementia: Clinico-pathological correlations relevant to Guamanian ALS/PD. *The Canadian Journal of Neurological Sciences, 18*(Suppl 3), 387–389.

Jaarsma, D., et al. (2008). Neuron-specific expression of mutant superoxide dismutase is sufficient to induce amyotrophic lateral sclerosis in transgenic mice. *The Journal of Neuroscience, 28*(9), 2075–2088.

Johnson, J. O., et al. (2010). Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron, 68*(5), 857–864.

Kawahara, Y., et al. (2003). Human spinal motoneurons express low relative abundance of GluR2 mRNA: An implication for excitotoxicity in ALS. *Journal of Neurochemistry, 85*(3), 680–689.
Kawahara, Y., et al. (2004). Glutamate receptors: RNA editing and death of motor neurons. *Nature, 427*(6977), 801.
Kotchoubey, B., et al. (2003). Cognitive processing in completely paralyzed patients with amyotrophic lateral sclerosis. *European Journal of Neurology, 10*(5), 551–558.
Kuner, R., et al. (2005). Late-onset motoneuron disease caused by a functionally modified AMPA receptor subunit. *Proceedings of the National Academy of Sciences of the United States of America, 102*(16), 5826–5831.
Kunig, G., et al. (1994). Excitotoxins L-beta-oxalyl-amino-alanine (L-BOAA) and 3,4,6-trihydroxyphenylalanine (6-OH-DOPA) inhibit [^{3}H] alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) binding in human hippocampus. *Neuroscience Letters, 169*(1–2), 219–222.
Kwiatkowski, T. J., Jr., et al. (2009). Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science, 323*(5918), 1205–1208.
Laake, J. H., et al. (1995). Glutamine from glial cells is essential for the maintenance of the nerve terminal pool of glutamate: Immunogold evidence from hippocampal slice cultures. *Journal of Neurochemistry, 65*(2), 871–881.
Lacomblez, L., et al. (1996). Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet, 347*(9013), 1425–1431.
Lepore, A. C., et al. (2008). Focal transplantation-based astrocyte replacement is neuroprotective in a model of motor neuron disease. *Nature Neuroscience, 11*(11), 1294–1301.
Lips, M. B., & Keller, B. U. (1998). Endogenous calcium buffering in motoneurones of the nucleus hypoglossus from mouse. *The Journal of Physiology, 511*(Pt 1), 105–117.
Liu, X. Q., et al. (2010). Selective death of cholinergic neurons induced by beta-methylamino-L-alanine. *Neuroreport, 21*(1), 55–58.
Lobsiger, C. S., et al. (2009). Schwann cells expressing dismutase active mutant SOD1 unexpectedly slow disease progression in ALS mice. *Proceedings of the National Academy of Sciences of the United States of America, 106*(11), 4465–4470.
Logroscino, G., et al. (2010). Incidence of amyotrophic lateral sclerosis in Europe. *Journal of Neurology, Neurosurgery, and Psychiatry, 81*(4), 385–390.
Maessen, M., et al. (2010). Requests for euthanasia: Origin of suffering in ALS, heart failure, and cancer patients. *Journal of Neurology, 257*(7), 1192–1198.
Maruyama, H., et al. (2010). Mutations of optineurin in amyotrophic lateral sclerosis. *Nature, 465*(7295), 223–226.
Mayer, M. L. (2005). Glutamate receptor ion channels. *Current Opinion in Neurobiology, 15*(3), 282–288.
Miller, R. G., et al. (2007). Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *The Cochrane Database of Systematic Reviews*, (1), CD001447.
Muha, N., & Ramsdell, J. S. (2011). Domoic acid induced seizures progress to a chronic state of epilepsy in rats. *Toxicon, 57*(1), 168–171.
Murch, S. J., et al. (2004). Occurrence of beta-methylamino-L-alanine (BMAA) in ALS/PDC patients from Guam. *Acta Neurologica Scandinavica, 110*(4), 267–269.
Obal, I., Engelhardt, J. I., & Siklos, L. (2006). Axotomy induces contrasting changes in calcium and calcium-binding proteins in oculomotor and hypoglossal nuclei of Balb/c mice. *The Journal of Comparative Neurology, 499*(1), 17–32.
Paizs, M., et al. (2010). Hypoglossal motor neurons display a reduced calcium increase after axotomy in mice with upregulated parvalbumin. *The Journal of Comparative Neurology, 518*(11), 1946–1961.
Palecek, J., Lips, M. B., & Keller, B. U. (1999). Calcium dynamics and buffering in motoneurones of the mouse spinal cord. *The Journal of Physiology, 520*(Pt 2), 485–502.
Pardo, A. C., et al. (2006). Loss of the astrocyte glutamate transporter GLT1 modifies disease in SOD1(G93A) mice. *Experimental Neurology, 201*(1), 120–130.

Purdie, E. L., et al. (2009). Effects of the cyanobacterial neurotoxin beta-*N*-methylamino-L-alanine on the early-life stage development of zebrafish (*Danio rerio*). *Aquatic Toxicology, 95*(4), 279–284.

Rao, S. D., et al. (2006). BMAA selectively injures motor neurons via AMPA/kainate receptor activation. *Experimental Neurology, 201*(1), 244–252.

Reaume, A. G., et al. (1996). Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nature Genetics, 13*(1), 43–47.

Renton, A. E., et al. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron, 72*(2), 257–268.

Rothstein, J. D., et al. (2005). Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature, 433*(7021), 73–77.

Rutherford, N. J., et al. (2008). Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. *PLoS Genetics, 4*(9), e1000193.

Sala, G., et al. (2005). Impairment of glutamate transport and increased vulnerability to oxidative stress in neuroblastoma SH-SY5Y cells expressing a Cu, Zn superoxide dismutase typical of familial amyotrophic lateral sclerosis. *Neurochemistry International, 46*(3), 227–234.

Sasaki, S., et al. (2001). EAAT1 and EAAT2 immunoreactivity in transgenic mice with a G93A mutant SOD1 gene. *Neuroreport, 12*(7), 1359–1362.

Scholin, C. A., et al. (2000). Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature, 403*(6765), 80–84.

Seeburg, P. H., et al. (2001). Genetic manipulation of key determinants of ion flow in glutamate receptor channels in the mouse. *Brain Research, 907*(1–2), 233–243.

Shefner, J. M., et al. (1999). Mice lacking cytosolic copper/zinc superoxide dismutase display a distinctive motor axonopathy. *Neurology, 53*(6), 1239–1246.

Shi, S. H., et al. (1999). Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science, 284*(5421), 1811–1816.

Siniscalchi, A., et al. (1999). Neuroprotective effects of riluzole: An electrophysiological and histological analysis in an in vitro model of ischemia. *Synapse, 32*(3), 147–152.

Spencer, P. S., et al. (1986). Lathyrism: Evidence for role of the neuroexcitatory amino acid BOAA. *Lancet, 2*(8515), 1066–1067.

Spreux-Varoquaux, O., et al. (2002). Glutamate levels in cerebrospinal fluid in amyotrophic lateral sclerosis: A reappraisal using a new HPLC method with coulometric detection in a large cohort of patients. *Journal of the Neurological Sciences, 193*(2), 73–78.

Staats, K. A., & Van Den Bosch, L. (2009). Astrocytes in amyotrophic lateral sclerosis: Direct effects on motor neuron survival. *Journal of Biological Physics, 35*, 337–346.

Steele, J. C., & Guzman, T. (1987). Observations about amyotrophic lateral sclerosis and the parkinsonism-dementia complex of Guam with regard to epidemiology and etiology. *The Canadian Journal of Neurological Sciences, 14*(3 Suppl), 358–362.

Streifler, M., & Cohn, D. F. (1981). Chronic central nervous system toxicity of the chickling pea (*Lathyrus sativus*). *Clinical Toxicology, 18*(12), 1513–1517.

Sun, H., et al. (2006). Slow and selective death of spinal motor neurons in vivo by intrathecal infusion of kainic acid: implications for AMPA receptor-mediated excitotoxicity in ALS. *Journal of Neurochemistry, 98*(3), 782–791.

Takuma, H., et al. (1999). Reduction of GluR2 RNA editing, a molecular change that increases calcium influx through AMPA receptors, selective in the spinal ventral gray of patients with amyotrophic lateral sclerosis. *Annals of Neurology, 46*(6), 806–815.

Tanaka, K., et al. (1997). Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science, 276*(5319), 1699–1702.

Tateno, M., et al. (2004). Calcium-permeable AMPA receptors promote misfolding of mutant SOD1 protein and development of amyotrophic lateral sclerosis in a transgenic mouse model. *Human Molecular Genetics, 13*(19), 2183–2196.

Teitelbaum, J. S., et al. (1990). Neurologic sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. *The New England Journal of Medicine, 322*(25), 1781–1787.

Tortarolo, M., et al. (2004). Expression of SOD1 G93A or wild-type SOD1 in primary cultures of astrocytes down-regulates the glutamate transporter GLT-1: Lack of involvement of oxidative stress. *Journal of Neurochemistry, 88*(2), 481–493.

Tortarolo, M., et al. (2006). Glutamate AMPA receptors change in motor neurons of SOD1(G93A) transgenic mice and their inhibition by a noncompetitive antagonist ameliorates the progression of amyotrophic lateral sclerosis-like disease. *Journal of Neuroscience Research, 83*(1), 134–146.

Tovar, Y. R. L. B., et al. (2009). Chronic elevation of extracellular glutamate due to transport blockade is innocuous for spinal motoneurons in vivo. *Neurochemistry International, 54*(3–4), 186–191.

Van Damme, P., et al. (2002). GluR2-dependent properties of AMPA receptors determine the selective vulnerability of motor neurons to excitotoxicity. *Journal of Neurophysiology, 88*(3), 1279–1287.

Van Damme, P., et al. (2003). The AMPA receptor antagonist NBQX prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis. *Neuroscience Letters, 343*(2), 81–84.

Van Damme, P., et al. (2005). GluR2 deficiency accelerates motor neuron degeneration in a mouse model of amyotrophic lateral sclerosis. *Journal of Neuropathology and Experimental Neurology, 64*, 605–612.

Van Damme, P., et al. (2007). Astrocytes regulate GluR2 expression in motor neurons and their vulnerability to excitotoxicity. *Proceedings of the National Academy of Sciences of the United States of America, 104*(37), 14825–14830.

Van Deerlin, V. M., et al. (2008). TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: A genetic and histopathological analysis. *Lancet Neurology, 7*, 409–416.

Van Den Bosch, L., et al. (2000). Ca^{2+}-permeable AMPA receptors and selective vulnerability of motor neurons. *Journal of the Neurological Sciences, 180*(1–2), 29–34.

Van Den Bosch, L., et al. (2002a). Protective effect of parvalbumin on excitotoxic motor neuron death. *Experimental Neurology, 174*(2), 150–161.

Van Den Bosch, L., et al. (2002b). An alpha-mercaptoacrylic acid derivative (PD150606) inhibits selective motor neuron death via inhibition of kainate-induced Ca^{2+} influx and not via calpain inhibition. *Neuropharmacology, 42*(5), 706–713.

Vance, C., et al. (2009). Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science, 323*(5918), 1208–1211.

von Lewinski, F., & Keller, B. U. (2005). Ca^{2+}, mitochondria and selective motoneuron vulnerability: Implications for ALS. *Trends in Neurosciences, 28*(9), 494–500.

Warita, H., et al. (2002). Tardive decrease of astrocytic glutamate transporter protein in transgenic mice with ALS-linked mutant SOD1. *Neurological Research, 24*(6), 577–581.

Weiss, J. H., Koh, J. Y., & Choi, D. W. (1989). Neurotoxicity of beta-*N*-methylamino-L-alanine (BMAA) and beta-*N*-oxalylamino-L-alanine (BOAA) on cultured cortical neurons. *Brain Research, 497*(1), 64–71.

Willis, C. L., et al. (1994). Neuroprotective effect of free radical scavengers on beta-*N*-oxalylamino-L-alanine (BOAA)-induced neuronal damage in rat hippocampus. *Neuroscience Letters, 182*(2), 159–162.

Wilson, J. M., et al. (2002). Behavioral and neurological correlates of ALS-parkinsonism dementia complex in adult mice fed washed cycad flour. *Neuromolecular Medicine, 1*(3), 207–221.

Wilson, J. M., et al. (2003). Decrease in glial glutamate transporter variants and excitatory amino acid receptor down-regulation in a murine model of ALS-PDC. *Neuromolecular Medicine, 3*(2), 105–118.

Wootz, H., et al. (2010). Reduced VGLUT2 expression increases motor neuron viability in Sod1(G93A) mice. *Neurobiology of Disease, 37*(1), 58–66.

Yamanaka, K., et al. (2008). Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nature Neuroscience, 11*(3), 251–253.

Zhao, P., et al. (2008). Altered presymptomatic AMPA and cannabinoid receptor trafficking in motor neurons of ALS model mice: Implications for excitotoxicity. *The European Journal of Neuroscience, 27*(3), 572–579.

Excitotoxicity and Axon Degeneration

Anna E. King and James C. Vickers

Contents

Abstract

Excitotoxicity has been implicated as a key pathogenic pathway in a number of neurodegenerative diseases and conditions including Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, brain trauma, and stroke. While acute excitotoxicity can result in the initiation of cell death pathways, chronic or low levels of excitotoxin exposure may result in a more slowly progressing pathological cascade. In this respect, there is emerging evidence that excitotoxicity can result in axonal degeneration and pathology, a key pathological feature of many of these neurodegenerative conditions. Recent evidence supports the notion that axon degeneration can be a separate and independent

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process from cell death, and thus mechanisms involved need to be understood in order to provide axonal protection in neurological disease. While axon degeneration following transection (Wallerian degeneration) has been well documented, less is known about axon degeneration following other insults such as excitotoxicity and the mechanistic relationships they bear to Wallerian degeneration. In particular, how a primarily somatodendritic insult, such as excitotoxicity, results in a pathological cascade within the axon is unclear. This chapter reviews our current understanding of the pathological changes and mechanisms of excitotoxin-induced axon degeneration with particular reference to our understanding of other forms of axonal degeneration and potential mechanisms involved. An increased understanding of the mechanisms of axon degeneration in neurological disease is essential to the development of therapeutic agents targeting axon protection.

List of Abbreviations

Aß	Beta amyloid
ALS	Amyotrophic lateral sclerosis
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
Cdk5	Cyclin-dependent kinase 5
CNP	2′3′-Cyclic nucleotide 3′-phosphodiesterase
CNS	Central nervous system
CRMP2	Collapsin response mediator protein 2
MAG	Myelin-associated glycoprotein
MPP	1-Methyl-4-phenylpyridine
NAD	Nicotinamide adenine dinucleotide
NFH	Neurofilament heavy chain
NFL	Neurofilament light chain
NFM	Neurofilament medium chain
NMDA	*N*-methyl-D-aspartate
Plp	Proteolipid protein
PNS	Peripheral nervous system
TTX	Tetrodotoxin
Wlds	Wallerian degeneration slow

1 Introduction

The toxicity of excitatory amino acids such as glutamate to neurons ("excitotoxicity") has been known since the 1950s (Lucas and Newhouse. 1957) and has since been implicated in the pathogenesis of a number of neurodegenerative conditions (Faden et al. 1989; Aarts et al. 2002; Hynd et al. 2004; Bogaert et al. 2010). Excitotoxicity was demonstrated by Olney (1969) to occur through overactivation of excitatory amino acid receptors and, in a variety of disease states, may also occur through the

impaired ability of cells to deal with sub-toxic levels of glutamate. In this regard, a characteristic of most neurodegenerative diseases is the slowly progressing cellular pathology and degeneration that occurs prior to overt cell death. Although excitotoxicity is generally considered a somatodendritic insult, there is also emerging evidence that excitotoxicity can play a role in axon degeneration, the latter a key feature of a number of neurodegenerative diseases including Alzheimer's disease, multiple sclerosis, and amyotrophic lateral sclerosis (ALS). The evidence for excitotoxic "axonopathy" and proposed mechanisms are discussed in this chapter.

2 Axon Degeneration in Neurodegenerative Disease

Axon degeneration is a prominent feature of neurodegenerative disease (Vickers et al. 2009) pathologically characterized by axonal swelling, axonal dystrophy, and distal axon dieback. It potentially represents one of the earliest findings in many diseases, and it has been proposed that it may be the cause of cellular dysfunction and degeneration through a dying back process. *ALS* is characterized by substantial early axonal degeneration in both the upper and lower motor neurons pathologically presenting as degeneration of the corticospinal tract and disconnection of the neuromuscular junctions and large axonal swelling referred to as "spheroids" in the proximal axons of lower motor neurons (Wood et al. 2003; Fischer et al. 2004). Causes of axonal degeneration have not been fully determined but are thought to involve axonal transport disruption. Axon transport disruption has also been implicated in axonal pathology in Alzheimer's disease, the latter which consists primarily of dystrophic neurites linked with Aß plaque formation (Vickers et al. 2009). Axon pathology linked with dystrophic neurite formation is accompanied by substantial demyelination (Mitew et al. 2010). Interestingly, putative demyelinating conditions, such as multiple sclerosis, are also associated with axonopathy and neuronal cytoskeletal changes indicative of axon transport disruption (Petzold et al. 2008). The precise cause of axonal degeneration in all of these conditions has not been determined and may be multifactorial in nature. However, there is substantial evidence for a role of excitotoxicity in the pathogenesis of these diseases, leaving the possibility that axonal degeneration results from excitotoxic processes.

3 Mechanisms of Axon Degeneration

Axon degeneration following axonal transection has been very well defined and involves a process termed "Wallerian degeneration". A number of reviews describe the time course of Wallerian degeneration including the distinct phases of retraction of cut ends, formation of swollen end bulbs, latency period, axonal beading, sudden axonal fragmentation, and clearing of axonal segments by glial cells (e.g., Coleman 2005; Luo and O'Leary 2005; Wang et al. 2012,). Mechanistically, Wallerian degeneration has been shown to involve calcium influx (Knoferle et al. 2010), activation and loss of calpains (Glass et al. 2002; AraujoCouto et al. 2004),

activation of autophagy (Knoferle et al. 2010) and microtubule disruption (Zhai et al. 2003). Caspase activity and other apoptotic mechanism are not generally associated with Wallerian degeneration (Finn et al. 2000). Much of our understanding of the nature of axonal degeneration comes from a mutant mouse model, the slow Wallerian degeneration (Wlds) mouse, in which Wallerian degeneration is substantially delayed, suggesting that axon degeneration is an active process. Analysis of the genetic mutation that is responsible for this delayed degeneration revealed a splicing of two mRNAs to form a fusion protein consisting of Nmnat1 (an NAD+ salvage enzyme) and the N terminal protein of Ube4b (an E4 ubiquitin ligase) (Mack et al. 2001). Current research supports the notion that Nmnat1 activity is important for axonal protection in Wlds mice, and Ube4b may be responsible for directing it to the axon instead of the nucleus (Conforti et al. 2009). However, the protective role of Nmnat1 remains unclear (Sasaki et al. 2009, reviewed in Coleman and Freeman 2010). Despite the increasing knowledge on mechanisms of Wallerian degeneration, it remains unclear if other mechanisms of axonal degeneration exist. One type of axon degeneration that has been described that does not appear to involve Wallerian degeneration is developmental axon retraction, which occurs when a small shortening of the axon is required, for example, during neurite outgrowth and synapse formation. Axon retraction involves preservation of the axonal cytoplasm through gradual retrograde transport of materials to more proximal axon segments (reviewed in Luo and O'Leary 2005).

Axon degeneration that occurs in neurodegenerative disease, however, is not usually associated with direct severing of the axon, although it frequently shares many similarities with Wallerian degeneration, such as axon thinning, beading, and fragmentation (for review, see Saxena and Caroni 2007). In contrast, in a number of diseases and conditions, axon degeneration may occur by a dying back axonopathy, beginning at the distal axon (Cavanagh 1964), or may involve formation of large axonal swellings or spheroids (Trapp et al. 1998; King et al. 2011). Furthermore, the protective effect of the Wlds gene in some but not all (for review, see Coleman 2005) mouse models of disease suggests that different mechanisms may be involved. Pertinent to the current topic is whether axon degeneration induced by excitotoxicity occurs via a Wallerian-type degeneration or by a different mechanism.

4 Morphological Characteristics of Excitotoxin-Induced Axon Degeneration

Despite its clear relevance to neurological diseases, few studies have examined the consequence of excitotoxicity to the axonal compartment. In vitro studies suggest that axonal changes may occur rapidly, prior to the hallmarks of neuronal cell death, and within 2–6 h of exposure to glutamate receptor agonists (King et al. 2007; Hou et al. 2009). Axonal degeneration also occurs following exposure to application of both NMDA (Chung et al. 2005; King et al. 2007) and non- NMDA (King et al. 2007) agonists. However, the type of degeneration occurring following such insults may be specific to both the particular pattern of receptor activation and the cell

type (King et al. 2007). In cultured rodent cortical neurons, glutamate, NMDA, and kainic acid exposure result in axonal retraction, beading, thinning, and subsequent fragmentation of the axon (Chung et al. 2005; King et al. 2007; Hou et al. 2009; Hosie et al. 2012) that resembles the morphological changes to axons undergoing Wallerian degeneration. However, cultured motor neurons may respond in an entirely different manner, particularly following kainic acid exposure, with a proportion of motor neuron axons displaying distinct axonopathic features characterized by large swollen structures in distal axon segments (King et al. 2007) that may show more resemblance to distal axon dieback described in a number of neurological diseases. These types of distal motor axon swellings have been described in mouse models of spinal muscular atrophy (Cifuentes-Diaz et al. 2002) although they may not be characteristic of all types of distal axon dieback, which in some cases may be more associated with distal axon atrophy and thinning (Schaefer et al. 2005). Distal axon degeneration was also more prominent than proximal degeneration in an in vivo rat model of somatodendritic NMDA exposure to retinal ganglion cells in a glaucoma model (Saggu et al. 2008, 2010).

5 The Role of the Cytoskeleton in Excitotoxin-Induced Axon Degeneration

Axonal degeneration involves a breakdown of the components of the neuronal cytoskeleton. The neuronal cytoskeleton is composed of three distinct types of filaments, namely, microtubules, intermediate filaments (including neurofilaments), and microfilaments, all of which can undergo posttranslational modifications that affect their stability and function. Although distinct, the components of the cytoskeleton are dynamically connected with each other, and there are a vast number of associated proteins for linking and regulation and to enable intracellular transport. Neurofilaments, consisting of light (NFL, 61 KDa), medium (NFM 90 KDa), and heavy (NFH 115 KDa) chains (Lariviere and Julien 2004), are fibrous subunits that are only present in some eukaryotic organisms including vertebrates, nematodes, and mollusks and are likely to have broader functions than just serve as a structural scaffold (reviewed in PerroneCapano et al. 2001).

Cytoskeletal breakdown has been previously characterized for classic forms of Wallerian degeneration. In the initial stages, there is a depolymerization of the *microtubules*, which occurs within 4 h in in vitro models (Zhai et al. 2003). Depolymerization of microtubules is not accompanied by tubulin degradation, but is followed at 8–12 h by loss of neurofilament subunits (Zhai et al. 2003). Both microtubule depolymerization and loss of neurofilaments are dependent on degradation by the ubiquitin-proteasome system (Zhai et al. 2003). Our studies have examined the cytoskeletal changes in cultured cortical neurons following excitotoxic stimulation following a range of glutamate concentrations. A chronic low-dose treatment resulted in specific loss of NFH, with a relative sparing of the NFM and NFL subunits at 24 h posttreatment (Fig. 1. Chung et al. 2005). While beading of NFM and NFL were apparent at this time point with higher glutamate

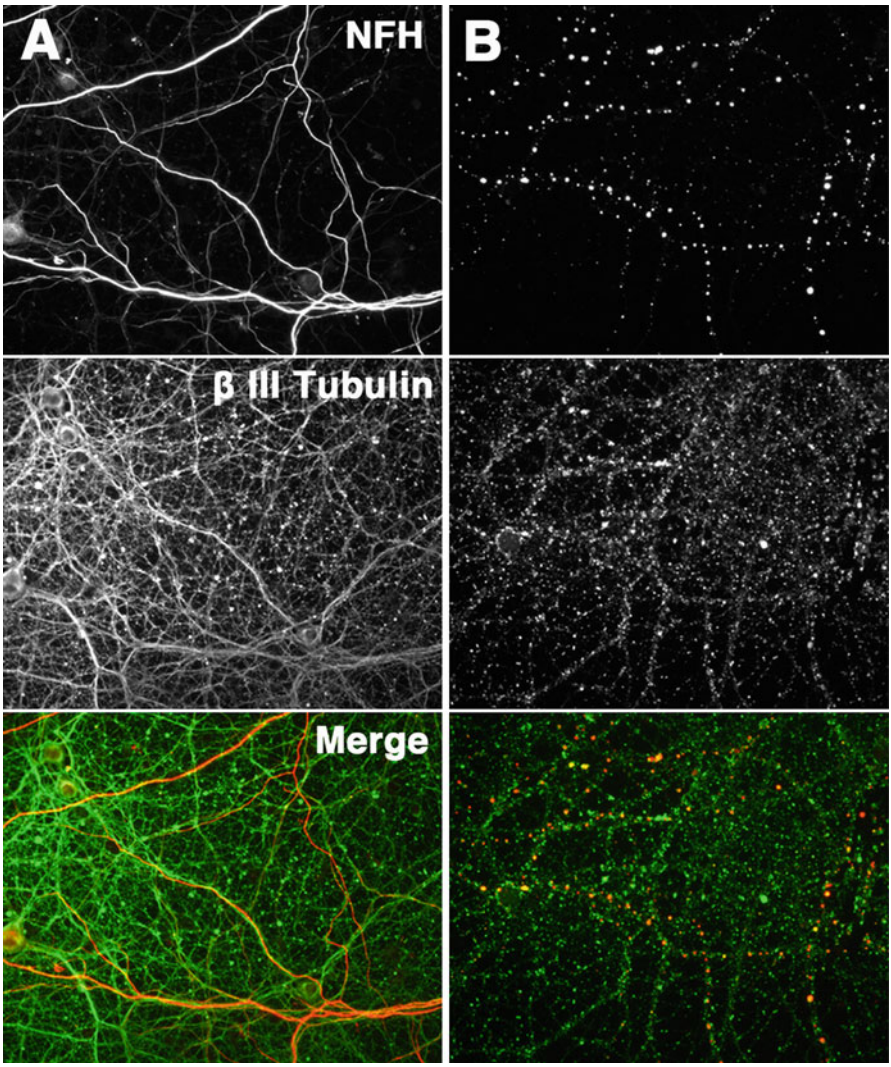

Fig. 1 Cytoskeletal architecture in primary cultured rat cortical neurons at 21 DIV. (**a**) In untreated cultures NF-H immunoreactivity was found primarily in thick axonal processes, and co-localized with βIII-tubulin. (**b**) Following treatment with 10 μM glutamate fragmentation of microtubule proteins was present accompanied by beading and loss of neurofilament proteins, particularly the NFH subunit (Modified with permission from Chung et al. 2005)

treatments (100 μm), their degradation was delayed relative to the NFH subunit. The differential vulnerability of neurofilament subunits may reflect their temporal expression during development, with the NFH subunit being the last to be expressed. In a similar fashion to Wallerian degeneration, tubulin immunoreactivity remained intact until later stages indicating that tubulin subunits were not degraded.

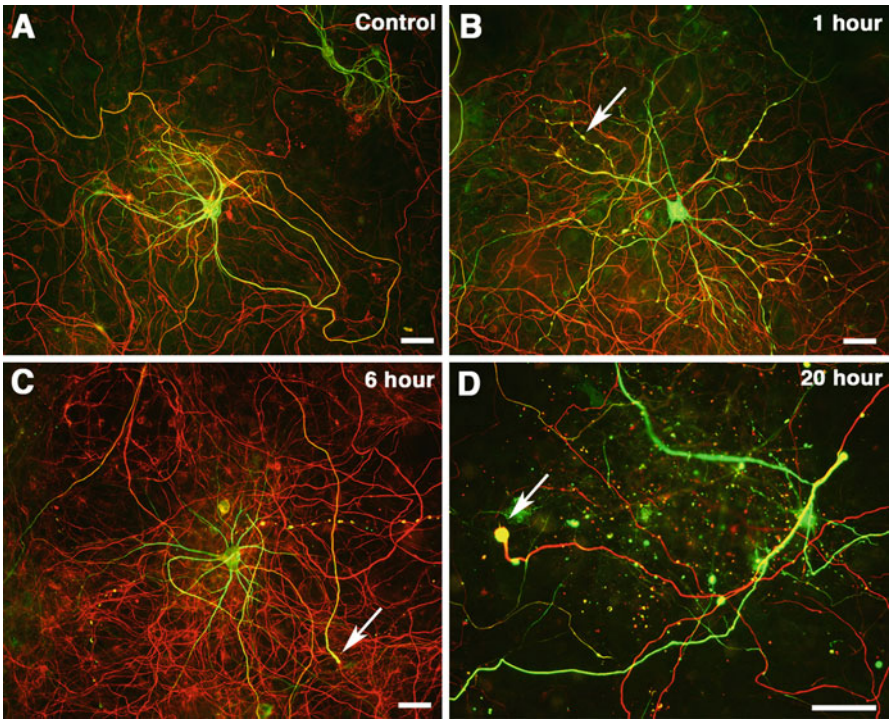

Fig. 2 Time-course of pathological changes following 100 μM kainic acid exposure in cultured motor neurons at 21 DIV. (**a**) Untreated motor neuron immunolabelled for dephosphorylated neurofilament protein (*green*) and phosphorylation-independent NFM (*red*). (**b**) Following 1 h of kainic acid exposure, beading was present in dendrites (*arrow*). (**c**) Axon swellings (e.g., *arrow*) developed following 6 h of kainic acid exposure and were frequently associated with expression of de-phosphorylated neurofilament protein (*green*) in the axonal network. (**d**) Following 20 h kainic acid exposure the distal portions of axonal segments frequently expanded to form large bulbous structures (*arrows*). Scale Bars: 50 μm (Modified with permission from King et al. 2007)

Distal axon degeneration induced by low-dose kainic acid in cultured spinal motor neurons, on the other hand, was characterized by a distinct set of cytoskeletal changes (King et al. 2007). In these neurons, dephosphorylated neurofilament protein (SMI32 immunoreactivity) is normally localized to the somatodendritic compartment and the proximal axon (King et al. 2007). However, following kainic acid treatment, there was a mislocalization of non-phosphorylated neurofilament to the more distal axon compartment, which was accompanied by accumulation of neurofilament proteins and mitochondria in axonal swellings (Fig. 2). Swollen, neurofilament-rich distal axon segments may be caused by a disruption of retrograde axonal transport mechanisms and have been shown to occur in a mouse model of Kennedy's disease due to decreased expression of the retrograde transport motor protein dynactin 1 (Katsuno et al. 2006). The presence of multiple cytoskeletal components and organelles in these swellings suggests a nonspecific disruption

of retrograde transport. Alterations in neurofilament phosphorylation have been reported in other models of kainic acid toxicity, with decreased phosphorylated and increased non-phosphorylated epitopes of NFH in a rat model of kainic acid exposure to the hippocampus (Wang et al. 1992) and in cultured spinal neurons exposed to sublethal kainic acid (Vartiainen et al. 1999). In contrast, blockade of non-NMDA receptors in cultured spinal neurons (Vartiainen et al. 1999) or glutamate exposure of dorsal root ganglion neurons (Kesavapany et al. 2007) caused increased neurofilament phosphorylation, which was localized to the soma. The significance of the changes in localization of these neurofilament epitopes remains unclear; however, dephosphorylation has been implicated in slowing of axonal transport through irregular and fused crossbridges (Gotow 2000). Conversely, in cultured cortical neurons, glutamate toxicity has been linked to slowing of anterograde transport through increased neurofilament phosphorylation and resultant dissociation of neurofilament from kinesin anterograde motors (Yabe et al. 2001). Thus, it is possible that both phosphorylation and dephosphorylation of neurofilament proteins can affect transport within the axon.

6 Molecular Mechanisms of Cytoskeletal Disruption in Excitotoxin-Induced Axon Degeneration (Calpains Versus Caspases)

The role of specific proteases in cytoskeletal breakdown following excitotoxic insult remains unclear. Calpains, calcium-activated cysteine proteases, have been implicated in cytoskeletal breakdown following traumatic brain injury and axon transection (Kampfl et al. 1996) and in focal axonal beading (Kilinc et al. 2009). Furthermore, a study by Higuchi et al. (2005) demonstrated the protective effect of calpain, but not caspase, inhibition on the degeneration of neuronal processes and downstream subacute cell death, following kainic acid exposure in vivo, although acute cell death was not affected. In this model, calpain activation was associated with increased tau phosphorylation through cleavage of the CDK5 activator p35 to p25 (Nath et al. 2000; Kilinc et al. 2009). Calpain activation was higher in the somatodendritic compartment leading to the hypothesis that tau phosphorylation occurs in the soma, resulting in depletion of axonal tau, with microtubule disruption-induced axonal degeneration occurring later than somatodendritic changes. Detailed analysis of the axonal processes in this model was not performed, and the role of calpain in axonal beading, a common feature of both excitotoxic and Wallerian axon degeneration, was not determined. However, in a cell culture model of glutamate excitotoxicity in cortical neurons, calpain inhibition was protective against axonal beading and thinning that occurred from 2 h post-glutamate exposure (Hou et al. 2009). The authors showed that, in addition to calpain, CaMKII is activated, and the two proteins affect the microtubule-associated protein (Fukata et al. 2002) collapsin response mediator protein 2 (CRMP2). CaMKII causes phosphorylation of CRMP2, which is protective against calpain-mediated proteolysis, although

CaMKII inhibition is also protective against axonal beading, suggesting a central modulatory role of CRMP2 in axon integrity (Hou et al. 2009). Furthermore, overexpression of CRMP2 protects against axonal beading and subsequent degeneration. It is interesting to note that phosphorylation of neurofilaments has also been shown to protect these proteins from calpain-mediated proteolysis (Goldstein et al. 1987). Thus, dephosphorylation of neurofilaments, as occurs in motor neuron axons following kainic acid exposure (King et al. 2007), may make these proteins more vulnerable to proteolysis. Neurofilament proteolysis is involved in removing excess neurofilaments under physiological conditions (reviewed in Liu et al. 2004), and thus, it is unclear whether dephosphorylation of neurofilament is a protective mechanism to remove excess neurofilament from the axon terminal following transport disruption or a consequence of the pathological cascade (King et al. 2007).

Caspase activation, which can occur by a number of different mechanisms including both intrinsic pathways such as mitochondrial release of cytochrome c and extrinsic pathways such as activation of death receptors, is thought not to be involved in axon degeneration following transection (Wallerian degeneration) despite its activation in the soma (Finn et al. 2000). Similarly, in models of neurodegenerative disease such as ALS, caspase inhibition fails to protect against axon degeneration despite protection from neuronal cell death (Gould et al. 2006). Conversely, caspase activation has been implicated in axon degeneration following a number of insults including psychosine toxicity (a sphingolipid involved in Krabbe's disease, Smith et al. 2011), amyloid toxicity (Nikolaev et al. 2009), and MPP toxicity (Morfini et al. 2007). Reports of the role of caspase activation following growth factor deprivation are controversial, with some authors finding that it is involved (Schoenmann et al. 2010) and others finding no involvement (Finn et al. 2000). Our studies using cortical neurons in compartmented chambers to allow application of toxins and inhibitors to either the axon or somatodendritic compartment of the cell indicated that caspase inactivation with the pan-caspase inhibitor Z-FAD-FMK to either the somatodendritic compartment or the axon provides some protection against axon degeneration induced by somatodendritic excitotoxicity, with an overall decrease in axon degeneration of 42 % and 33 %, respectively (Hosie et al. 2012). This suggests that axon degeneration following excitotoxicity involves degenerative processes occurring in both the axon and the soma. Elucidating the role of calpains and caspases in excitotoxic axon degeneration in specific cell types is an area of future investigation.

7 How Is Degeneration Initiated in the Axon Following Excitotoxic Insult?

Following excitotoxic insult, degeneration is initiated in the axon, and the type of degeneration is cell type specific. However, it remains unclear how the insult is transferred from glutamate receptors, the mediators of the insult, to the axonal

compartment. Glutamate receptors involved in synaptic signaling are predominantly located on the postsynaptic densities of glutamatergic synapses and at extrasynaptic sites on the somatodendritic compartment of neurons. Increase in calcium influx in this region is unlikely to spread to the axon terminals. It is possible that axon degeneration results only from cell death; however, as discussed, protection from cell death does not protect the axon, and axon degeneration is apparent prior to cell death (Chung et al. 2005; Higuchi et al. 2005; King et al. 2007; Hou et al. 2009). Axon degeneration may be initiated through a number of different mechanisms that will be examined in more detail: (1) non-apoptotic somatic changes resulting in axon transport disruption and subsequent axon starvation, (2) soma-axon signaling, and (3) glutamate receptor expression on the axonal compartment (Fig. 3).

7.1 Axon Starvation

A potential mechanism of initiation of axon degeneration through excitotoxic insult is through transport disruption, which results in reduced delivery of mitochondria or cytoplasmic components to the axon. Slowing of the transport of neurofilament proteins has been demonstrated following glutamate exposure to primary cortical neurons in culture (Ackerley et al. 2000). Transport disruption was accompanied by an increased phosphorylation of the neurofilament subunits (Ackerley et al. 2000), which has previously been associated with decreased transport rates (Lewis and Nixon 1988). Accumulation of phosphorylated neurofilament subunits in the soma of dorsal root ganglia following exposure to glutamate has also been reported (Kesavapany et al. 2007). As described earlier, aberrant phosphorylation of microtubule-associated proteins such as tau may prevent their transport into the axon leading to microtubule disassembly. The differential role of phosphorylation of cytoskeletal proteins in the soma in axon degeneration in different neuronal populations has not been investigated. More recently experiments in cultured hippocampal neurons have demonstrated disruption of motor proteins themselves following excitotoxic stimulation. A C-terminally truncated form of the retrograde dynein/dynactin motor complex p150Glued was formed after glutamate exposure and was also present in tissue from Alzheimer's disease cases (Fujiwara and Morimoto 2012). Overexpression of the wild-type protein was protective against neuritic beading and knockout of the protein exacerbated beading (Fujiwara and Morimoto 2012). Collectively, these data suggest a role for transport failure in excitotoxin-induced degeneration; however, the mechanisms by which axon transport failure results in axon degeneration remain unclear. Recent data indicates that axon transport disruption alone may not be sufficient to induce axon degeneration, at least on a rapid scale (Marinkovic et al. 2012). Furthermore, the links between failure of axon transport in the soma and axonal blebbing or distal axonal neurofilament accumulations, which occur rapidly following excitotoxin exposure in cell culture models, remain to be elucidated.

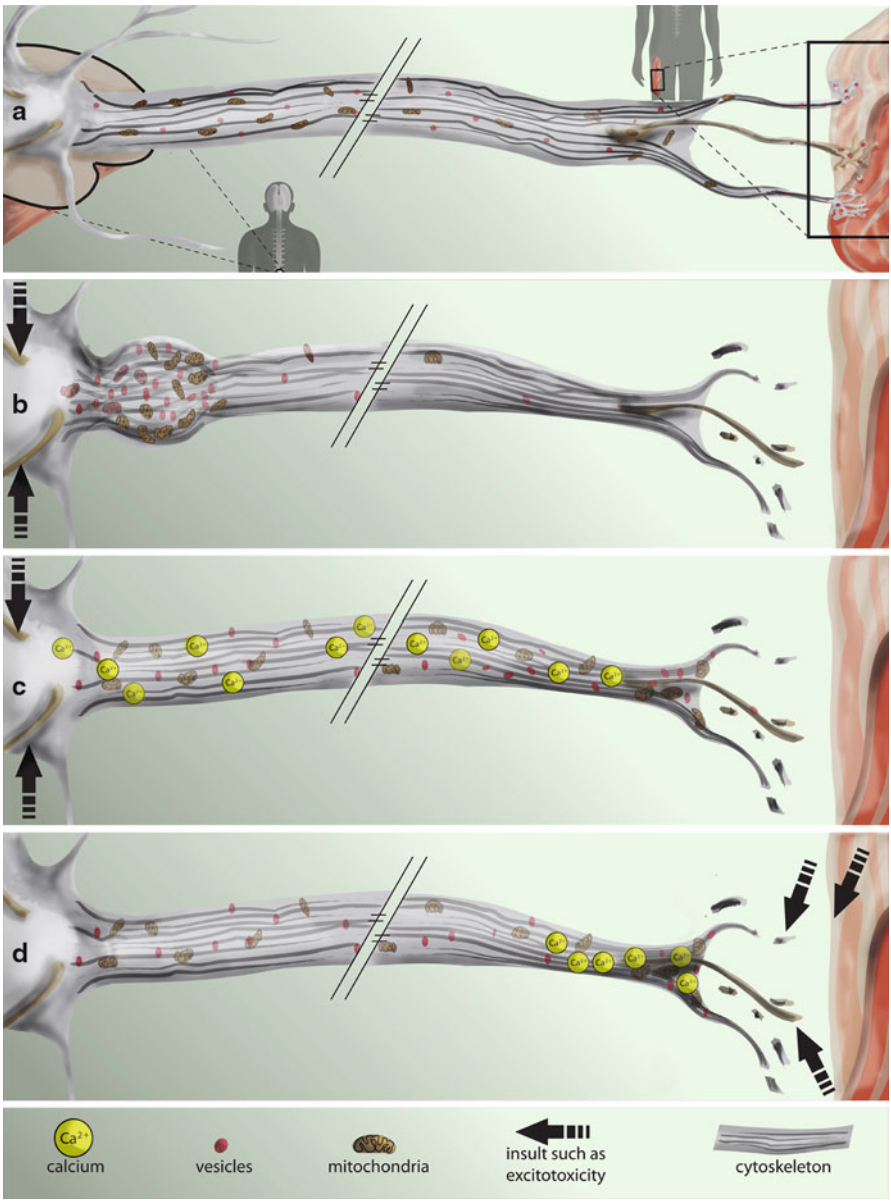

Fig. 3 Potential mechanisms of axon degeneration induced by excitotoxicity. (**a**) Motor axons are spatially separated from the somatodendritic compartment. (**b**) Excitotoxicity to the somatodendritic compartment may result in transport blockage and withering of the distal axon. (**c**) Somatodendritic exposure may result in a signal (such as calcium, or newly translated protein) being transferred to the distal axon regions. (**d**). Distal axon exposure to excitotoxicity may directly result in degeneration (Figure courtesy of Graeme McCormack)

7.2 Axon-Soma Signaling

An alternative mechanism of initiation of axon degeneration following somatodendritic glutamate receptor stimulation is production of an axon destruction signal and/or loss of an axon maintenance signal. There is little information on this type of initiation of axon destruction instigated specifically by excitotoxicity. However, excitotoxic mechanisms of degeneration are calcium dependent; therefore, studies examining changes in calcium influx following nontoxic glutamate exposure may give an indication of changes that could occur following excitotoxicity. A study by Yamada and colleagues (2008), in developing granule neuron cultures, demonstrated that growth cone modulation by glutamate in immature neurons occurs through perikaryal AMPA receptor-mediated glutamate exposure, suggesting soma to distal axon signaling. Despite the absence of action potentials in these immature neurons, signaling was the result of a sweep of calcium along the axon. Both perikaryal and distal axon glutamate exposure resulted in a fast calcium transient throughout the whole cell, but only perikaryal exposure resulted in a subsequent calcium sweep from proximal to distal axon. The calcium sweep could also be initiated by depolarization and perikaryal calcium increase and involved release of calcium from intracellular stores. A study by Christie and Jahr (2008) examined the contribution of NMDA receptor stimulation to axonal calcium increase in stellate cells. They demonstrated that bath application of NMDA to cerebellar slice cultures, in the presence of TTX to block action potentials, caused increases in intracellular calcium concentrations in both axons and dendrites. Axonal calcium increases particularly occurred at axonal boutons, thought to be the sites of en passant presynaptic terminals. However, calcium increase was higher in boutons closer to the soma and lower in distal axon segments, suggesting that the increase in calcium arose from somatic NMDA receptor activation, with a passive propagation of depolarization to axonal voltage-gated calcium channels. Local activation of dendritic NMDA receptors confirmed this somatodendritic transfer (Christie and Jahr 2008). In addition to passive transfer of calcium currents, action potentials caused large increases in axonal calcium concentrations in boutons (Christie and Jahr 2008). It is currently unclear if these calcium increases are sufficient to cause activation of axonal degeneration mechanisms or if axonal beading corresponds to positions of boutons in axons. However, together these data suggest that somatodendritic glutamate exposure can result in axonal calcium influx even without action potential. In the event that axon potentials are initiated in hyperexcitable neurons, it has been shown that calcium entry can occur through the reverse operation of the sodium calcium exchanger that can be the result of persistent sodium influx (Stys et al. 1992).

Aside from calcium influx, studies investigating other intracellular signaling mechanisms in excitotoxin-induced axon degeneration are lacking. In terms of axon degeneration, inhibition of protein synthesis in the soma results in axon degeneration without cell loss and proteasome inhibition following axon

transection is protective against axon degeneration, supporting the hypothesis that a protein/proteins synthesized in the soma are required for axon maintenance (Gilley and Coleman 2010). These authors have identified the NAD+ synthesizing enzyme Nmnat2 (an isoform of the Wlds protein Nmnat1) as a likely candidate for an axonal survival signal. Involvement of these processes in excitotoxin-induced axon degeneration and distal axon dieback has not yet been investigated.

7.3 Axonal Expression of Glutamate Receptor Proteins

Although the prime site of glutamate receptor expression is on the postsynaptic density of the somatodendritic compartment, where they are involved in synaptic transmission, recent advances have demonstrated other roles for glutamate receptors (Araque and Perea 2004), and their expression has been demonstrated at a number of non-synaptic locations. Their presence at extrasynaptic sites on the somatodendritic compartment has been well documented (Passafaro et al. 2001; Tovar and Westbrook 2002; Kane-Jackson and Smith 2003) where they are thought to play a modulatory role (Diamond and Jahr 2000; Jourdain et al. 2007) in addition to acting as a reserve supply for synaptic receptors (for recent review, see Newpher and Ehlers 2008). The axonal expression of glutamate receptors has been less well documented, although they have been reported to be present in immature neurons (Yamada et al. 2008) on growth cones (Zheng, et al. 1996; King et al. 2006) and are present in presynaptic terminals (recently reviewed in Pinheiro and Mulle 2008). Recently AMPA/kainate receptors have been demonstrated to be present on myelinated axons (Ouardouz et al. 2009a, b). The functional activity of axonal receptors is currently disputed (e.g., Berretta and Jones 1996; Huang and Bordey 2004; Christie and Jahr 2008). In many excitotoxic models, particularly in cell culture, excitotoxins are bath applied, leaving the possibility that excitotoxin-induced axon degeneration occurs directly through stimulation of axonal glutamate receptors. To directly test the hypothesis that axonal glutamate exposure can result in axon degeneration, we have recently utilized compartmented microfluidic chambers (Taylor et al. 2005) to allow specific exposure of the axonal compartment to glutamate. Our findings demonstrate that axon degeneration characterized by beading and fragmentation resulted from prolonged (24 h), but not acute (Underhill and Goldberg 2007), exposure of the axons to glutamate without subsequent apoptosis of the soma, even after 72 h (Fig. 4, Hosie et al. 2012). Western blot analysis confirmed the presence of AMPA/kainate receptors in the axons, although NMDA receptors could not be detected by this method. In this study, caspase inhibition was protective against axonal excitotoxin-induced axon degeneration. Interestingly protection was higher when caspase activation in the soma was prevented, suggesting that axon-soma-axon signaling is involved in degeneration rather than direct degeneration resulting from calcium influx in the axon (Hosie et al. 2012).

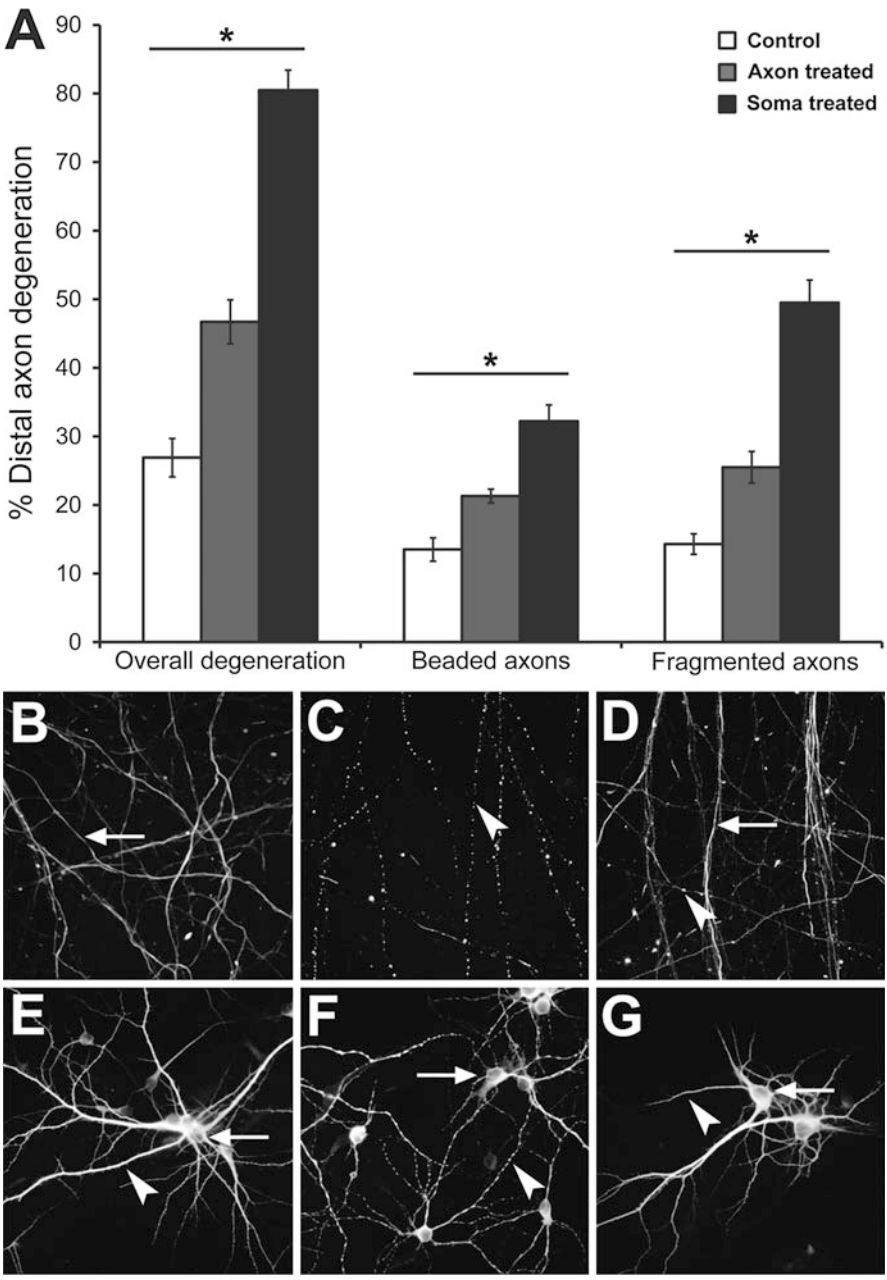

Fig. 4 Axon degeneration in cortical neurons grown in compartmentalized microfluidic chambers (Taylor et al. 2005) and exposed to 100 μM glutamate to either the somatodendritic compartment or the isolated axon compartment. (**a**) Quantitation of axonal beading and fragmentation following glutamate exposure indicated a significant threefold increase in axon degeneration (beading plus fragmentation) following somatodendritic exposure, and a significant 1.5-fold increase following

8 White Matter Excitotoxicity

In addition to excitotoxicity directly acting on the neuron to induce axon degeneration, there is increasing evidence that axon degeneration can be initiated in white matter indirectly through disturbed neuron-glia interactions. White matter tracts are vulnerable to damage following brain trauma (Kraus et al. 2007; Sharp and Ham 2011) and hypoxia/ischemia (Pantoni et al. 1996; Yam et al. 1998), and white matter excitotoxicity has been particularly implicated in multiple sclerosis (Pitt et al. 2000; Smith et al. 2000). Direct application of excitotoxins to white matter tracts of the optic nerve (Matute 1998) or internal capsule (Fowler et al. 2003) confirmed the role of excitotoxicity in the degeneration of the white matter tracts as a distinct process from somatodendritic exposure. Damage is characterized by injury to both the axonal process and surrounding glial cells. Axonal damage included swollen and bulbous profiles, cytoskeletal disruption such as loss of neurofilament (Fowler et al. 2003; Cuthill et al. 2006), and disruption to axonal electrical activity (Stys et al. 1992). Nonneuronal damage included damage to the myelin sheath (Li and Stys 2000) and focal swelling and cytoskeletal breakdown of oligodendrocytes and astrocytes (Pantoni et al. 1996; Li and Stys 2000). Although damage to axons following hypoxia can occur through non-excitotoxic pathways (Underhill and Goldberg 2007), non-NMDA (Agrawal and Fehlings 1997; Li and Stys 2000; Tekkok and Goldberg 2001) receptor antagonists are protective against white matter degeneration both in vitro and in vivo, suggesting a role for excitotoxicity in this damage, specifically mediated through non-NMDA receptors. The role of NMDA receptors remains less clear (Yam et al. 2000; Li and Stys 2000), although recent reports similarly suggest a role for NMDA receptor stimulation (Bakiri et al. 2008). In acute spinal cord slices, white matter degeneration occurs via a calcium-dependent mechanism (Li and Stys 2000).

White matter excitotoxicity is thought to occur predominantly through *oligodendrocytes*, which possess both AMPA and NMDA receptors, localized to the soma and the myelin sheath (Li and Stys 2000; Brand-Schieber and Werner 2003; Karadottir et al. 2005; Salter and Fern 2005; Micu et al. 2006) and have been shown to be vulnerable to excitotoxicity both in vitro (Yoshioka et al. 1996; Sanchez-Gomez and Matute 1999) and in vivo (Matute 1998). Toxicity can occur both through direct stimulation of the receptors and indirect mechanisms involving transporters (Oka et al. 1993), and the mechanisms involved are dependent on the type and duration of insult. For a recent review on white matter excitotoxicity and

Fig. 4 (continued) exposure to the isolated axon compartment. Representative immunocytochemical images of axons (NFM, **b**–**d**) and somatodendritic compartment (MAP2, **e**–**g**) in control (**b**, **e**), soma treated (**c**, **f**) and axon treated (**d**, **g**) cultures. Axons demonstrated increased beading and fragmentation following treatments. *Arrows* indicate whole axons, arrowheads indicate fragmented and beaded axons. MAP2 immunoreactive dendrites demonstrated increased beading following somatodendric exposure (**f**) but not following axonal exposure (**g**). *Arrows* indicate soma, arrowheads indicate dendrites. Scale bar: 50 μm (Reproduced with permission, from Hosie et al. 2012)

its role in disease, see reviews by Matute et al. 2007; Matute and Ransom 2012. The question remains to as to how oligodendrocyte damage following glutamate exposure results in axonopathy.

Recent advances have detailed interactions between oligodendrocytes and axons that extend far beyond the role of passive insulation. Signaling between the myelin sheath and the axon is essential in maintaining fine-tuning of myelination, which is important in regulation of precise neuronal activity within a network (Wake et al. 2011). A type of axo-myelinic synapse has been proposed with glutamate receptors on both the axon and innermost compact myelin sheath surrounding the axon (Stys 2011). It is hypothesized that this type of myelin-axon signaling could be responsible for activity-dependent signaling that allows optimization of axonal sheath thickness. In terms of axon support, it has been proposed that oligodendrocytes and other glia (Magistretti 2006) may provide neurotrophic support to the axon in times of energy shortage from the soma (reviewed in Nave 2010). Specific myelin/oligodendrocyte proteins additionally may promote axonal integrity and survival. Mouse models with knockout of the oligodendrocyte proteins plp and CNP have frequent axonopathy characterized by axonal swelling and cytoskeletal disruption (Lappe-Siefke et al. 2003; Edgar et al. 2004). These axon disruptions occur in the absence of gross myelin loss highlighting the importance of specific proteins in myelin-axon signaling to axonal maintenance and function. Myelin has been shown to have a protective effect on axons in injury paradigms and is protective against mild stretch injury (Staal and Vickers 2011). This protection may be through myelin-associated glycoprotein (MAG) signaling to the axon, as knockout of MAG in mice results in increased axon degeneration in both the CNS and PNS and increased axonal vulnerability to insults such as acrylamide toxicity (Nguyen et al. 2009). Protective effects of MAG may be through cytoskeletal stabilization as it has been shown to affect the phosphorylation of neurofilament heavy and medium chains (NFH and NFM), which in turn affects the caliber of the axon (Hsieh et al. 1994; Dashiell et al. 2002; Garcia et al. 2003). Myelination and specific myelin protein may also affect axon transport mechanisms. Plp has been shown to play a role in axonal transport (Edgar et al. 2004).

The importance of demyelination and loss of glial support of the axon is highlighted in multiple sclerosis, in which immune attack of the myelin sheath results in axonopathy (reviewed in Dutta and Trapp 2011). However, the role of excitotoxic mechanisms in white matter injury remains an area for future research. Axon degeneration may result directly from loss of interaction with myelin, specific oligodendrocyte proteins, loss of neurotrophic support, or alternatively direct excitotoxicity on the axon shaft (Hosie et al. 2012).

9 Conclusion

A number of diseases are characterized by axon degeneration, and excitotoxicity has been implicated as a pathogenic mechanism in these diseases. Axon degeneration may occur through a number of different excitotoxic mechanisms including somatodendritic glutamate exposure, direct axonal glutamate exposure, and glial

glutamate toxicity. A better knowledge of the mechanisms involved in specific diseases may aid in directing therapeutic interventions. For example, excitotoxicity is implicated in both multiple sclerosis and ALS, and both have evidence of increased glutamate within the cerebrospinal fluid (Shaw et al. 1995; Barkhatova et al. 1998) and involve axon degeneration. However, the mechanisms of axon degeneration are likely to be different, with ALS potentially involving somatodendritic exposure to the spinal motor neurons and subsequent axonal degeneration and multiple sclerosis involving degeneration through loss of myelin proteins. Therapeutics may need to target specific complements of glutamate receptors, or downstream signaling pathways, that are expressed on the axon, on glial cells, or at synaptic or non-synaptic sites on neurons.

An alternative mechanism of therapeutic intervention would be to target the pathways of axonal degeneration, which may show common features between various disorders. Cytoskeletal degeneration, in particular, is an attractive target, and trials of drugs that stabilize the microtubule network, such as taxol and epothilone, are having positive outcomes in a number of mouse models involving axonopathy including tau transgenic mouse models of AD pathology (Brunden et al. 2010; Barten et al. 2012; Zhang et al. 2012) and spinal cord injury (Hellal et al. 2011). In either scenario, drug targets that are well defined to specific components of the pathological cascade leading to axonal and neuronal degeneration are more likely to lead to successful treatment strategies. Thus, increasing our knowledge of mechanisms of axon degeneration in specific neurodegenerative conditions is fundamental to developing appropriate therapeutic agents.

References

Aarts, M., Liu, Y., Liu, L., Besshoh, S., Arundine, M., Gurd, J. W., Wang, Y. T., Salter, M. W., & Tymianski, M. (2002). Treatment of ischemic brain damage by perturbing NMDA receptor-PSD-95 protein interactions. *Science, 298*, 846–850.

Ackerley, S., Grierson, A. J., Brownlees, J., Thornhill, P., Anderton, B. H., Leigh, P. N., Shaw, C. E., & Miller, C. C. (2000). Glutamate slows axonal transport of neurofilaments in transfected neurons. *The Journal of Cell Biology, 150*, 165–176.

Agrawal, S. K., & Fehlings, M. G. (1997). Role of NMDA and non-NMDA ionotropic glutamate receptors in traumatic spinal cord axonal injury. *Journal of Neuroscience, 17*, 1055–1063.

Araque, A., & Perea, G. (2004). Glial modulation of synaptic transmission in culture. *GLIA, 47*, 241–248.

AraujoCouto, L., SampaioNarciso, M., Hokoc, J. N., & Blanco Martinez, A. M. (2004). Calpain inhibitor 2 prevents axonal degeneration of opossum optic nerve fibers. *Journal of Neuroscience Research, 77*, 410–419.

Bakiri, Y., Hamilton, N. B., Karadottir, R., & Attwell, D. (2008). Testing NMDA receptor block as a therapeutic strategy for reducing ischaemic damage to CNS white matter. *GLIA, 56*, 233–240.

Barkhatova, V. P., Zavalishin, I. A., Askarova, L., Shavratskii, V., & Demina, E. G. (1998). Changes in neurotransmitters in multiple sclerosis. *Neuroscience and Behavioral Physiology, 28*, 341–344.

Barten, D. M., Fanara, P., Andorfer, C., Hoque, N., Wong, P. Y., Husted, K. H., Cadelina, G. W., Decarr, L. B., Yang, L., Liu, V., Fessler, C., Protassio, J., Riff, T., Turner, H., Janus, C. G., Sankaranarayanan, S., Polson, C., Meredith, J. E., Gray, G., Hanna, A., Olson, R. E., Kim, S. H., Vite, G. D., Lee, F., & Albright, C. F. (2012). Hyperdynamic microtubules,

cognitive deficits, and pathology are improved in tau transgenic mice with low doses of the microtubule-stabilizing agent BMS-241027. *Journal of Neuroscience, 32*, 7137–7145.

Berretta, N., & Jones, R. S. (1996). Tonic facilitation of glutamate release by presynaptic N-methyl-D-aspartate autoreceptors in the entorhinal cortex. *Neuroscience, 75*, 339–344.

Bogaert, E., d'Ydewalle, C., & Van Den Bosch, L. (2010). Amyotrophic lateral sclerosis and excitotoxicity: From pathological mechanism to therapeutic target. *CNS & Neurological Disorders Drug Targets, 9*, 297–304.

Brand-Schieber, E., & Werner, P. (2003). AMPA/kainate receptors in mouse spinal cord cell-specific display of receptor subunits by oligodendrocytes and astrocytes and at the nodes of Ranvier. *GLIA, 42*, 12–24.

Brunden, K. R., Zhang, B., Carroll, J., Yao, Y., Potuzak, J. S., Hogan, A. M., Iba, M., James, M. J., Xie, S. X., Ballatore, C., Smith, A. B., 3rd, Lee, V. M., & Trojanowski, J. Q. (2010). Epothilone D improves microtubule density, axonal integrity, and cognition in a transgenic mouse model of tauopathy. *Journal of Neuroscience, 30*, 13861–13866.

Cavanagh, J. B. (1964). The significance of the "dying back" process in experimental and human neurological disease. *International Review of Experimental Pathology, 3*, 219–267.

Christie, J. M., & Jahr, C. E. (2008). Dendritic NMDA receptors activate axonal calcium channels. *Neuron, 60*, 298–307.

Chung, R. S., McCormack, G. H., King, A. E., West, A. K., & Vickers, J. C. (2005). Glutamate induces rapid loss of axonal neurofilament proteins from cortical neurons in vitro. *Experimental Neurology, 193*, 481–488.

Cifuentes-Diaz, C., Nicole, S., Velasco, M. E., Borra-Cebrian, C., Panozzo, C., Frugier, T., Millet, G., Roblot, N., Joshi, V., & Melki, J. (2002). Neurofilament accumulation at the motor endplate and lack of axonal sprouting in a spinal muscular atrophy mouse model. *Human Molecular Genetics, 11*, 1439–1447.

Coleman, M. (2005). Axon degeneration mechanisms: Commonality amid diversity. *Nature Reviews Neuroscience, 6*, 889–898.

Coleman, M. P., Freeman, M. R. (2010). Wallerian degeneration, wld(s), and nmnat. *Annual Review of Neuroscience 33*, 245–267.

Conforti, L., Wilbrey, A., Morreale, G., Janeckova, L., Beirowski, B., Adalbert, R., Mazzola, F., Di Stefano, M., Hartley, R., Babetto, E., Smith, T., Gilley, J., Billington, R. A., Genazzani, A. A., Ribchester, R. R., Magni, G., & Coleman, M. (2009). Wld S protein requires Nmnat activity and a short N-terminal sequence to protect axons in mice. *The Journal of Cell Biology, 184*, 491–500.

Cuthill, D. J., Fowler, J. H., McCulloch, J., & Dewar, D. (2006). Different patterns of axonal damage after intracerebral injection of malonate or AMPA. *Experimental Neurology, 200*(2), 509–520.

Dashiell, S. M., Tanner, S. L., Pant, H. C., & Quarles, R. H. (2002). Myelin-associated glycoprotein modulates expression and phosphorylation of neuronal cytoskeletal elements and their associated kinases. *Journal of Neurochemistry, 81*, 1263–1272.

Diamond, J. S., & Jahr, C. E. (2000). Synaptically released glutamate does not overwhelm transporters on hippocampal astrocytes during high-frequency stimulation. *J Neurophysiol, 83*, 2835–2843.

Dutta, R., & Trapp, B. D. (2011). Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. *Progress in Neurobiology, 93*, 1–12.

Edgar, J. M., McLaughlin, M., Yool, D., Zhang, S. C., Fowler, J. H., Montague, P., Barrie, J. A., McCulloch, M. C., Duncan, I. D., Garbern, J., Nave, K. A., & Griffiths, I. R. (2004). Oligodendroglial modulation of fast axonal transport in a mouse model of hereditary spastic paraplegia. *The Journal of Cell Biology, 166*, 121–131.

Faden, A. I., Demediuk, P., Panter, S. S., & Vink, R. (1989). The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science, 244*, 798–800.

Finn, J. T., Weil, M., Archer, F., Siman, R., Srinivasan, A., & Raff, M. C. (2000). Evidence that Wallerian degeneration and localized axon degeneration induced by local neurotrophin deprivation do not involve caspases. *Journal of Neuroscience, 20*, 1333–1341.

Fischer, L. R., Culver, D. G., Tennant, P., Davis, A. A., Wang, M., Castellano-Sanchez, A., Khan, J., Polak, M. A., & Glass, J. D. (2004). Amyotrophic lateral sclerosis is a distal axonopathy: Evidence in mice and man. *Experimental Neurology, 185*, 232–240.

Fowler, J. H., McCracken, E., Dewar, D., & McCulloch, J. (2003). Intracerebral injection of AMPA causes axonal damage in vivo. *Brain Research, 991*, 104–112.

Fukata, Y., Itoh, T. J., Kimura, T., Menager, C., Nishimura, T., Shiromizu, T., Watanabe, H., Inagaki, N., Iwamatsu, A., Hotani, H., & Kaibuchi, K. (2002). CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. *Nature Cell Biology, 4*, 583–591.

Fujiwara, T., & Morimoto, K. (2012). Cooperative effect of p150Glued and microtubule stabilization to suppress excitotoxicity-induced axon degeneration. *Biochemical and Biophysical Research Communications, 424*, 82–88.

Garcia, M. L., Lobsiger, C. S., Shah, S. B., Deerinck, T. J., Crum, J., Young, D., Ward, C. M., Crawford, T. O., Gotow, T., Uchiyama, Y., Ellisman, M. H., Calcutt, N. A., & Cleveland, D. W. (2003). NF-M is an essential target for the myelin-directed "outside-in" signaling cascade that mediates radial axonal growth. *The Journal of Cell Biology, 163*, 1011–1020.

Gilley, J., & Coleman M. P. (2010). Endogenous Nmnat2 is an essential survival factor for maintenance of healthy axons. *PLoS Biol, 8*(1), e1000300

Glass, J. D., Culver, D. G., Levey, A. I., & Nash, N. R. (2002). Very early activation of m-calpain in peripheral nerve during Wallerian degeneration. *Journal of the Neurological Sciences, 196*, 9–20.

Goldstein, M. E., Sternberger, N. H., & Sternberger, L. A. (1987). Phosphorylation protects neurofilaments against proteolysis. *Journal of Neuroimmunology, 14*, 149–160.

Gotow, T. (2000). Neurofilaments in health and disease. *Medical Electron Microscopy, 33*, 173–199.

Gould, T. W., Buss, R. R., Vinsant, S., Prevette, D., Sun, W., Knudson, C. M., Milligan, C. E., & Oppenheim, R. W. (2006). Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *Journal of Neuroscience, 26*, 8774–8786.

Hellal, F., Hurtado, A., Ruschel, J., Flynn, K. C., Laskowski, C. J., Umlauf, M., Kapitein, L. C., Strikis, D., Lemmon, V., Bixby, J., Hoogenraad, C. C., & Bradke, F. (2011). Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. *Science, 331*, 928–931.

Higuchi, M., Zhang, B., Forman, M. S., Yoshiyama, Y., Trojanowski, J. Q., & Lee, V. M. (2005). Axonal degeneration induced by targeted expression of mutant human tau in oligodendrocytes of transgenic mice that model glial tauopathies. *Journal of Neuroscience, 25*, 9434–9443.

Hosie, K. A., King, A. E., Blizzard, C. A., Vickers, J. C., & Dickson, T. C. (2012). Chronic excitotoxin-induced axon degeneration in a compartmented neuronal culture model. *ASN Neuro, 4*(1).

Hou, S. T., Jiang, S. X., Aylsworth, A., Ferguson, G., Slinn, J., Hu, H., Leung, T., Kappler, J., & Kaibuchi, K. (2009). CaMKII phosphorylates collapsin response mediator protein 2 and modulates axonal damage during glutamate excitotoxicity. *Journal of Neurochemistry, 111*, 870–881.

Hsieh, S. T., Kidd, G. J., Crawford, T. O., Xu, Z., Lin, W. M., Trapp, B. D., Cleveland, D. W., & Griffin, J. W. (1994). Regional modulation of neurofilament organization by myelination in normal axons. *Journal of Neuroscience, 14*, 6392–6401.

Huang, H., & Bordey, A. (2004). Glial glutamate transporters limit spillover activation of presynaptic NMDA receptors and influence synaptic inhibition of Purkinje neurons. *Journal of Neuroscience, 24*, 5659–5669.

Hynd, M. R., Scott, H. L., & Dodd, P. R. (2004). Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochemistry International, 45*, 583–595.

Jourdain, P., Bergersen, L. H., Bhaukaurally, K., Bezzi, P., Santello, M., Domercq, M., Matute, C., Tonello, F., Gundersen, V., & Volterra, A. (2007). Glutamate exocytosis from astrocytes controls synaptic strength. *Nature Neuroscience, 10*, 331–339.

Kampfl, A., Posmantur, R., Nixon, R., Grynspan, F., Zhao, X., Liu, S. J., Newcomb, J. K., Clifton, G. L., & Hayes, R. L. (1996). mu-calpain activation and calpain-mediated cytoskeletal proteolysis following traumatic brain injury. *Journal of Neurochemistry, 67*, 1575–1583.

Kane-Jackson, R., & Smith, Y. (2003). Pre-synaptic kainate receptors in GABAergic and glutamatergic axon terminals in the monkey globus pallidus. *Neuroscience, 122*, 285–289.

Karadottir, R., Cavelier, P., Bergersen, L. H., & Attwell, D. (2005). NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. *Nature, 438*, 1162–1166.

Katsuno, M., Adachi, H., Minamiyama, M., Waza, M., Tokui, K., Banno, H., Suzuki, K., Onoda, Y., Tanaka, F., Doyu, M., & Sobue, G. (2006). Reversible disruption of dynactin 1-mediated retrograde axonal transport in polyglutamine-induced motor neuron degeneration. *Journal of Neuroscience, 26*, 12106–12117.

Kesavapany, S., Patel, V., Zheng, Y. L., Pareek, T. K., Bjelogrlic, M., Albers, W., Amin, N., Jaffe, H., Gutkind, J. S., Strong, M. J., Grant, P., & Pant, H. C. (2007). Inhibition of Pin1 reduces glutamate-induced perikaryal accumulation of phosphorylated neurofilament-H in neurons. *Molecular Biology of the Cell, 18*(9), 3645–3655.

Kilinc, D., Gallo, G., & Barbee, K. A. (2009). Mechanical membrane injury induces axonal beading through localized activation of calpain. *Experimental Neurology, 219*, 553–561.

King, A. E., Chung, R. S., Vickers, J. C., & Dickson, T. C. (2006). Localization of glutamate receptors in developing cortical neurons in culture and relationship to susceptibility to excitotoxicity. *The Journal of Comparative Neurology, 498*, 277–294.

King, A. E., Dickson, T. C., Blizzard, C. A., Foster, S. S., Chung, R. S., West, A. K., Chuah, M. I., & Vickers, J. C. (2007). Excitotoxicity mediated by non-NMDA receptors causes distal axonopathy in long-term cultured spinal motor neurons. *European Journal of Neuroscience, 26*, 2151–2159.

King, A. E., Dickson, T. C., Blizzard, C. A., Woodhouse, A., Foster, S. S., Chung, R. S., & Vickers, J. C. (2011). Neuron-glia interactions underlie ALS-like axonal cytoskeletal pathology. *Neurobiology of Aging, 32*, 459–469.

Knoferle, J., Koch, J. C., Ostendorf, T., Michel, U., Planchamp, V., Vutova, P., Tonges, L., Stadelmann, C., Bruck, W., Bahr, M., & Lingor, P. (2010). Mechanisms of acute axonal degeneration in the optic nerve in vivo. *Proceedings of the National Academy of Sciences of the U S A, 107*, 6064–6069.

Kraus, M. F., Susmaras, T., Caughlin, B. P., Walker, C. J., Sweeney, J. A., & Little, D. M. (2007). White matter integrity and cognition in chronic traumatic brain injury: A diffusion tensor imaging study. *Brain, 130*, 2508–2519.

Lappe-Siefke, C., Goebbels, S., Gravel, M., Nicksch, E., Lee, J., Braun, P. E., Griffiths, I. R., & Nave, K. A. (2003). Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. *Nature Genetics, 33*, 366–374.

Lariviere, R. C., & Julien, J. P. (2004). Functions of intermediate filaments in neuronal development and disease. *Journal of Neurobiology, 58*, 131–148.

Lewis, S. E., & Nixon, R. A. (1988). Multiple phosphorylated variants of the high molecular mass subunit of neurofilaments in axons of retinal cell neurons: Characterization and evidence for their differential association with stationary and moving neurofilaments. *The Journal of Cell Biology, 107*, 2689–2701.

Li, S., & Stys, P. K. (2000). Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. *Journal of Neuroscience, 20*, 1190–1198.

Liu, Q., Xie, F., Siedlak, S. L., Nunomura, A., Honda, K., Moreira, P. I., Zhua, X., Smith, M. A., & Perry, G. (2004). Neurofilament proteins in neurodegenerative diseases. *Cellular and Molecular Life Sciences, 61*, 3057–3075.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Arch Ophthalmology, 58*, 193–201.

Luo, L., & O'Leary, D. D. (2005). Axon retraction and degeneration in development and disease. *Annual Review of Neuroscience, 28*, 127–156.

Mack, T. G., Reiner, M., Beirowski, B., Mi, W., Emanuelli, M., Wagner, D., Thomson, D., Gillingwater, T., Court, F., Conforti, L., Fernando, F. S., Tarlton, A., Andressen, C.,

Addicks, K., Magni, G., Ribchester, R. R., Perry, V. H., & Coleman, M. P. (2001). Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. *Nature Neuroscience, 4*, 1199–1206.

Magistretti, P. J. (2006). Neuron-glia metabolic coupling and plasticity. *J Exp Biol*, *209*, 2304–2311

Marinkovic, P., Reuter, M. S., Brill, M. S., Godinho, L., Kerschensteiner, M., & Misgeld, T. (2012). Axonal transport deficits and degeneration can evolve independently in mouse models of amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the U S A, 109*, 4296–4301.

Matute, C. (1998). Characteristics of acute and chronic kainate excitotoxic damage to the optic nerve. *Proceedings of the National Academy of Sciences of the U S A, 95*, 10229–10234.

Matute, C., Alberdi, E., Domercq, M., Sanchez-Gomez, M. V., Perez-Samartin, A., Rodriguez-Antiguedad, A., & Perez-Cerda, F. (2007). Excitotoxic damage to white matter. *Journal of Anatomy, 210*, 693–702.

Matute, C., & Ransom, B. R. (2012). Roles of white matter in central nervous system pathophysiologies. *ASN Neuro, 4*.

Micu, I., Jiang, Q., Coderre, E., Ridsdale, A., Zhang, L., Woulfe, J., Yin, X., Trapp, B. D., McRory, J. E., Rehak, R., Zamponi, G. W., Wang, W., & Stys, P. K. (2006). NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. *Nature, 439*, 988–992.

Mitew, S., Kirkcaldie, M. T., Halliday, G. M., Shepherd, C. E., Vickers, J. C., & Dickson, T. C. (2010). Focal demyelination in Alzheimer's disease and transgenic mouse models. *Acta Neuropathologica, 119*, 567–577.

Morfini, G., Pigino, G., Opalach, K., Serulle, Y., Moreira, J. E., Sugimori, M., Llinas, R. R., & Brady, S. T. (2007). 1-Methyl-4-phenylpyridinium affects fast axonal transport by activation of caspase and protein kinase C. *Proceedings of the National Academy of Sciences of the U S A, 104*, 2442–2447.

Nath, R., Davis, M., Probert, A. W., Kupina, N. C., Ren, X., Schielke, G. P., & Wang, K. K. (2000). Processing of cdk5 activator p35 to its truncated form (p25) by calpain in acutely injured neuronal cells. *Biochemical and Biophysical Research Communications, 274*, 16–21.

Nave, K. A. (2010). Myelination and support of axonal integrity by GLIA. *Nature, 468*, 244–252.

Newpher, T. M., & Ehlers, M. D. (2008). Glutamate receptor dynamics in dendritic microdomains. *Neuron, 58*, 472–497.

Nguyen, T., Mehta, N. R., Conant, K., Kim, K. J., Jones, M., Calabresi, P. A., Melli, G., Hoke, A., Schnaar, R. L., Ming, G. L., Song, H., Keswani, S. C., & Griffin, J. W. (2009). Axonal protective effects of the myelin-associated glycoprotein. *Journal of Neuroscience, 29*, 630–637.

Nikolaev, A., McLaughlin, T., O'Leary, D. D., & Tessier-Lavigne, M. (2009). APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. *Nature, 457*, 981–989.

Oka, A., Belliveau, M. J., Rosenberg, P. A., & Volpe, J. J. (1993). Vulnerability of oligodendroglia to glutamate: Pharmacology, mechanisms, and prevention. *Journal of Neuroscience, 13*, 1441–1453.

Olney, J. W. (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science, 164*, 719–721.

Ouardouz, M., Coderre, E., Basak, A., Chen, A., Zamponi, G. W., Hameed, S., Rehak, R., Yin, X., Trapp, B. D., & Stys, P. K. (2009a). Glutamate receptors on myelinated spinal cord axons: I. GluR6 kainate receptors. *Annals of Neurology, 65*, 151–159.

Ouardouz, M., Coderre, E., Zamponi, G. W., Hameed, S., Yin, X., Trapp, B. D., & Stys, P. K. (2009b). Glutamate receptors on myelinated spinal cord axons: II. AMPA and GluR5 receptors. *Annals of Neurology, 65*, 160–166.

Pantoni, L., Garcia, J. H., & Gutierrez, J. A. (1996). Cerebral white matter is highly vulnerable to ischemia. *Stroke, 27*, 1641–1646; discussion 1647.

Passafaro, M., Piech, V., & Sheng, M. (2001). Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nature Neuroscience, 4*, 917–926.

PerroneCapano, C., Pernas-Alonso, R., & di Porzio, U. (2001). Neurofilament homeostasis and motoneurone degeneration. *Bioessays, 23*, 24–33.

Petzold, A., Gveric, D., Groves, M., Schimierer, K., Grant, D., Chapman, M., Keir, G., Cuzner, L., & Thompson, E. J. (2008). Phosphorylation and compactness of neurofilaments in multiple sclerosis: Indicators of axonal pathology. *Experimental Neurology, 213*, 326–335.

Pinheiro, P. S., & Mulle, C. (2008). Presynaptic glutamate receptors: Physiological functions and mechanisms of action. *Nature Reviews Neuroscience, 9*, 423–436.

Pitt, D., Werner, P., & Raine, C. S. (2000). Glutamate excitotoxicity in a model of multiple sclerosis. *Nature Medicine, 6*, 67–70.

Saggu, S. K., Chotaliya, H. P., Blumbergs, P. C., & Casson, R. J. (2010). Wallerian-like axonal degeneration in the optic nerve after excitotoxic retinal insult: An ultrastructural study. *BMC Neuroscience, 11*, 97.

Saggu, S. K., Chotaliya, H. P., Cai, Z., Blumbergs, P., & Casson, R. J. (2008). The spatiotemporal pattern of somal and axonal pathology after perikaryal excitotoxic injury to retinal ganglion cells: A histological and morphometric study. *Experimental Neurology, 211*, 52–58.

Salter, M. G., & Fern, R. (2005). NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. *Nature, 438*, 1167–1171.

Sanchez-Gomez, M. V., & Matute, C. (1999). AMPA and kainate receptors each mediate excitotoxicity in oligodendroglial cultures. *Neurobiology of Disease, 6*, 475–485.

Sasaki, Y., Vohra, B. P., Lund, F. E., & Milbrandt, J. (2009). Nicotinamide mononucleotide adenylyl transferase-mediated axonal protection requires enzymatic activity but not increased levels of neuronal nicotinamide adenine dinucleotide. *Journal of Neuroscience, 29*, 5525–5535.

Saxena, S., & Caroni, P. (2007). Mechanisms of axon degeneration: From development to disease. *Progress in Neurobiology, 83*, 174–191.

Schaefer, A. M., Sanes, J. R., & Lichtman, J. W. (2005). A compensatory subpopulation of motor neurons in a mouse model of amyotrophic lateral sclerosis. *The Journal of Comparative Neurology, 490*, 209–219.

Schoenmann, Z., Assa-Kunik, E., Tiomny, S., Minis, A., Haklai-Topper, L., Arama, E., & Yaron, A. (2010). Axonal degeneration is regulated by the apoptotic machinery or a NAD+ -sensitive pathway in insects and mammals. *Journal of Neuroscience, 30*, 6375–6386.

Sharp, D. J., & Ham, T. E. (2011). Investigating white matter injury after mild traumatic brain injury. *Current Opinion in Neurology, 24*, 558–563.

Shaw, P. J., Forrest, V., Ince, P. G., Richardson, J. P., & Wastell, H. J. (1995). CSF and plasma amino acid levels in motor neuron disease: Elevation of CSF glutamate in a subset of patients. *Neurodegeneration, 4*, 209–216.

Smith, B., Galbiati, F., Castelvetri, L. C., Givogri, M. I., Lopez-Rosas, A., & Bongarzone, E. R. (2011). Peripheral neuropathy in the Twitcher mouse involves the activation of axonal caspase 3. *ASN Neuro, 3*, e00066.

Smith, T., Groom, A., Zhu, B., & Turski, L. (2000). Autoimmune encephalomyelitis ameliorated by AMPA antagonists. *Nature Medicine, 6*, 62–66.

Staal, J. A., & Vickers, J. C. (2011). Selective vulnerability of non-myelinated axons to stretch injury in an in vitro co-culture system. *Journal of Neurotrauma, 28*, 841–847.

Stys, P. K. (2011). The axo-myelinic synapse. *Trends in Neurosciences, 34*, 393–400.

Stys, P. K., Waxman, S. G., & Ransom, B. R. (1992). Ionic mechanisms of anoxic injury in mammalian CNS white matter: Role of Na+ channels and Na(+)-Ca^{2+} exchanger. *Journal of Neuroscience, 12*, 430–439.

Taylor, A. M., Blurton-Jones, M., Rhee, S. W., Cribbs, D. H., Cotman, C. W., & Jeon, N. L. (2005). A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nature Methods, 2*, 599–605.

Tekkok, S. B., & Goldberg, M. P. (2001). Ampa/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. *Journal of Neuroscience, 21*, 4237–4248.

Tovar, K. R., & Westbrook, G. L. (2002). Mobile NMDA receptors at hippocampal synapses. *Neuron, 34*, 255–264.

Trapp, B. D., Peterson, J., Ransohoff, R. M., Rudick, R., Mork, S., & Bo, L. (1998). Axonal transection in the lesions of multiple sclerosis. *The New England Journal of Medicine, 338*, 278–285.

Underhill, S. M., & Goldberg, M. P. (2007). Hypoxic injury of isolated axons is independent of ionotropic glutamate receptors. *Neurobiology of Disease, 25*, 284–290.

Vartiainen, N., Tikka, T., Keinanen, R., Chan, P. H., & Koistinaho, J. (1999). Glutamatergic receptors regulate expression, phosphorylation and accumulation of neurofilaments in spinal cord neurons. *Neuroscience, 93*, 1123–1133.

Vickers, J. C., King, A. E., Woodhouse, A., Kirkcaldie, M. T., Staal, J. A., McCormack, G. H., Blizzard, C. A., Musgrove, R. E., Mitew, S., Liu, Y., Chuckowree, J. A., Bibari, O., & Dickson, T. C. (2009). Axonopathy and cytoskeletal disruption in degenerative diseases of the central nervous system. *Brain Research Bulletin, 80*, 217–223.

Wake, H., Lee, P. R., & Fields, R. D. (2011). Control of local protein synthesis and initial events in myelination by action potentials. *Science, 333*, 1647–1651.

Wang, J. T., Medress, Z. A., & Barres, B. A. (2012). Axon degeneration: Molecular mechanisms of a self-destruction pathway. *The Journal of Cell Biology, 196*, 7–18.

Wang, S., Hamberger, A., Ding, M., & Haglid, K. G. (1992). In vivo activation of kainate receptors induces dephosphorylation of the heavy neurofilament subunit. *Journal of Neurochemistry, 59*, 1975–1978.

Wood, J. D., Beaujeux, T. P., & Shaw, P. J. (2003). Protein aggregation in motor neurone disorders. *Neuropathology and Applied Neurobiology, 29*, 529–545.

Yabe, J. T., Chylinski, T., Wang, F. S., Pimenta, A., Kattar, S. D., Linsley, M. D., Chan, W. K., & Shea, T. B. (2001). Neurofilaments consist of distinct populations that can be distinguished by C-terminal phosphorylation, bundling, and axonal transport rate in growing axonal neurites. *Journal of Neuroscience, 21*, 2195–2205.

Yam, P. S., Dewar, D., & McCulloch, J. (1998). Axonal injury caused by focal cerebral ischemia in the rat. *Journal of Neurotrauma, 15*, 441–450.

Yam, P. S., Dunn, L. T., Graham, D. I., Dewar, D., & McCulloch, J. (2000). NMDA receptor blockade fails to alter axonal injury in focal cerebral ischemia. *J Cereb Blood Flow Metab, 20*, 772–779

Yamada, R. X., Sasaki, T., Ichikawa, J., Koyama, R., Matsuki, N., & Ikegaya, Y. (2008). Long-range axonal calcium sweep induces axon retraction. *Journal of Neuroscience, 28*, 4613–4618.

Yoshioka, A., Bacskai, B., & Pleasure, D. (1996). Pathophysiology of oligodendroglialexcitotoxicity. *Journal of Neuroscience Research, 46*, 427–437.

Zhai, Q., Wang, J., Kim, A., Liu, Q., Watts, R., Hoopfer, E., Mitchison, T., Luo, L., & He, Z. (2003). Involvement of the ubiquitin-proteasome system in the early stages of Wallerian degeneration. *Neuron, 39*, 217–225.

Zhang, B., Carroll, J., Trojanowski, J. Q., Yao, Y., Iba, M., Potuzak, J. S., Hogan, A. M., Xie, S. X., Ballatore, C., Smith, A. B., 3rd, Lee, V. M., & Brunden, K. R. (2012). The microtubule-stabilizing agent, epothilone D, reduces axonal dysfunction, neurotoxicity, cognitive deficits, and Alzheimer-like pathology in an interventional study with aged tau transgenic mice. *Journal of Neuroscience, 32*, 3601–3611.

Zheng, J. Q., Wan, J. J., & Poo, M. M. (1996). Essential role of filopodia in chemotropic turning of nerve growth cone induced by a glutamate gradient. *Journal of Neuroscience, 16*, 1140–1149.

Excitotoxicity in HIV Associated Neurocognitive Disorders

Belinda Cruse and Bruce J. Brew

Contents

Abstract

HAND (HIV associated neurocognitive disorders) represents a group of disorders, encompassing HIV associated asymptomatic neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorders (MND), and HIV associated dementia (HAD). Even in the era of highly-active antiretroviral therapy, the prevalence of HAND is thought to be around 40 %, with milder forms making up a greater proportion of cases. HAND are clinically manifest as subcortical dementing

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processes; correlating with the preferential involvement of the basal ganglia, subcortical white matter, frontal lobe and hippocampus in neuropathological studies.

A central mechanism leading to synaptic dysfunction and loss, neuronal dysfunction and apoptosis in HAND is excitotoxicity mediated through the excessive activation of neuronal (and probably astrocytic) NMDA receptors (NMDAR). There is direct and indirect evidence that in HAND, increased neuronal NMDAR activation occurs due to elevated extracellular glutamate; a result of both increased presynaptic release and impaired clearance from the synaptic space. In addition, high levels of quinolinic acid (QUIN) (an NMDAR agonist) released by activated macrophages (and to a lesser extent microglia) lead to excessive NMDAR activation and excitotoxicity. Furthermore, HIV related proteins (particularly gp120 and Tat) can both activate the NMDAR, and augment receptor activation by glutamate and other agonists.

In vitro evidence supports the role of NMDAR antagonists in the prevention of neuronal loss induced by HIV related proteins. Clinical studies have demonstrated that the NMDAR antagonist memantine is safe and tolerable in HAND patients; so far clinical trials have failed to demonstrate a clinically beneficial effect of this therapy on neurocognitive function in HAND patients, however trials are ongoing.

Keywords

Dementia • Excitotoxicity • Glutamate • HIV • Neurocognitive disorders • NMDAR

1 Introduction

This chapter focuses the reader on the role of excitotoxicity in the pathogenesis of HIV-associated neurocognitive disorders (HAND) after first considering the epidemiology of this group of conditions and briefly outlining the pathological and clinical features of HAND. An overview of the multiple neuropathogenic pathways that contribute to HAND is presented, with a particular emphasis on changes in clinical, pathological, and possibly pathophysiological aspects of HAND that are taking place in the era of highly active antiretroviral therapy (HAART). Evidence for, and mechanisms of, NMDA receptor (NMDAR)-mediated excitotoxicity in HAND is then described in detail, followed by a discussion of current evidence regarding the use of adjunctive therapies (and more specifically NMDAR antagonists) in the treatment of HAND.

2 Epidemiology of HAND

HAND represents a group of disorders, encompassing HIV-associated asymptomatic neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorders (MND), and HIV-associated dementia (HAD) (Antinori et al. 2007).

Despite the considerable prognostic improvement achieved in the HIV-infected population with the introduction of highly active antiretroviral therapy (HAART), it so far has failed to fully protect treated individuals from HAND, even in those patients in whom virologic suppression is achieved in the plasma and CSF (Simioni et al. 2010; Robertson et al. 2007; Cysique and Brew 2011; Cysique et al. 2006). Epidemiological studies in the post-HAART era indicate the prevalence of these disorders at around 40 % (similar to that in the pre-HAART era), despite a decrease in the prevalence of HAD after HAART introduction (Cysique et al. 2004; Heaton et al. 2010; Dore et al. 1999). Therefore, while milder forms of HAND (ANI and MND) make up a greater proportion of cases, these disorders impact significantly on affected individuals' quality of life and independence (Antinori et al. 2007) and mortality (Ellis et al. 1997) and clearly impact socioeconomically. HAND also appears to impact HIV-infected individuals at higher CD4 T-cell counts in the post-HAART era (Sacktor et al. 2001; Brew 2004).

3 Pathological Features

HIV probably enters the central nervous system (CNS) via infected monocytes from the periphery. These cells cross the blood–brain barrier (BBB) both as part of the usual process of perivascular macrophage turnover and because the permeability of the BBB is pathologically increased in the context of HIV infection (by the effects of systemic inflammation and the direct effects of gp120 and tat proteins (reviewed by Ivey et al. 2009; Langford and Masliah 2001; Toborek et al. 2003; Annunziata 2003)). While both of these processes are shown to contribute to the entry of HIV into the CNS, it is currently unclear which factor is of greater importance in the early pathogenesis of HAND or in fact which occurs first. Furthermore, transmigration across the BBB by infected and uninfected monocytes and macrophages is accelerated by inflammatory cytokines (produced in the periphery and centrally, as a result of neuroinflammation) (Persidsky et al. 2000), with resultant amplification of monocyte/macrophage chemotaxis and HIV delivery to the CNS. Within the CNS HIV productively infects monocytes and microglial cells and infects a smaller proportion of astrocytes in a restricted, non-cytolytic state (Churchill et al. 2009; Wang et al. 2004; Thompson et al. 2004).

Pathological changes, neuronal dysfunction and apoptosis, and corresponding volume loss on neuroimaging preferentially affect subcortical deep grey structures, the hippocampus, and the frontal cortex (Lindl et al. 2010; Bell 2004; Boissé et al. 2008; Moore et al. 2006).

Microscopically, HIV infection is associated with inflammatory infiltrates of mononuclear cells, with microglial and astrocyte activation. The neuroinflammatory changes in HIV infection are termed encephalitis (HIVE). Infiltration of blood-derived macrophages, together with microglial cells and, virtually pathognomonic of HIVE, multinucleated giant cells, are seen. Microglial nodules, multinucleated giant cells, and HIV antigens are frequently located in the basal ganglia and in a subset of patients in the subcortical white matter (termed HIV

leukoencephalitis) (Petito 2003). There is also a widespread reactive astrocytosis and a variable degree of myelin damage (Bell 2004) and a sparse lymphocytic infiltrate in leptomeninges and perivascular spaces (Petito 2003). Neurons are not infected by HIV; however, they are clearly affected, with diffuse or focal neuronal cell death in the grey matter in severe cases (Petito 2003). Furthermore diffuse synaptic and dendritic loss, together with neuronal loss, is reported in HIV-infected individuals (Everall et al. 1999; Bell 2004).

The clinical features that predominate in HAND are those of a subcortical dementing process, with effects on cognitive slowing and impairment, behavioral and emotional change (usually apathy, especially in the HAD stage), together with prominent gross and fine motor slowing and gait disturbance (McArthur et al. 2005; Staekenborg et al. 2008). While currently the exact clinicopathological correlate is not fully understood in HAND (Gray and Keohane 2003), neuronal degeneration (i.e., neuronal degradation and death) underlies the neuropsychological impairment in HIV infection (Moore et al. 2006). Massive neuronal death, however, is not commonly seen and is not the main pathological counterpart of HAND (Adle-Biassette et al. 1999). In fact milder forms of HAND appear to correlate more closely with reduced synaptic and dendritic density rather than overt neuronal loss (Everall et al. 1999). Importantly, some of these changes are thought to be reversible (Gray and Keohane 2003), offering potential therapeutic targets.

4 Neuropathogenesis of HAND

Neuronal and astrocyte loss in HAND have historically been found in close anatomical proximity to the aforementioned inflammatory infiltrates and activated microglia (Brew et al. 1995; Adle-Biassette et al. 1999; Kaul et al. 2005). These neuropathological findings imply that neurotoxicity in HAND occurs through indirect mechanisms, specifically through toxins produced by activated mononuclear cells and microglia, rather than as a direct effect of HIV. Further supporting this widely held view is the fact that cognitive changes observed clinically in HAND are more closely associated with neuronal loss, decreased synaptic and dendritic density, intensity of local inflammation (such as the number of microglial cells and markers of tumor necrosis factor-α (TNF-α) expression by microglia and astrocytes), and evidence of excitotoxins, as opposed to the number of HIV-infected cells or concentration of HIV proteins (reviewed in Kaul et al. 2001; Adle-Biassette et al. 1999).

Monocytes and microglial cells are activated by HIV infection; exposure to extracellular HIV proteins, cytokines, and chemokines liberated from other similarly activated microglial cells (reviewed by Bol et al. 2011); and other factors including quinolinic acid (QUIN), glutamate and L-cysteine, arachidonic acid, platelet-activating factor (PAF), NTox, superoxide anions, and TNF-α. Monocytes and microglial cells are considered central to the pathogenesis of HAND, both by virtue of their role as the principle cells infected by HIV and as a major source of neurotoxins (Valle et al. 2004). Importantly, however, abnormalities of astrocyte

function due to neurotoxic actions of some of the above cytokines and chemokines, astrocyte activation and astrocytosis, and astrocyte loss have also been implicated (Kaul et al. 2001) and will be further discussed. Indeed, while there is evidence of a direct role of some HIV proteins in the neuropathogenesis, the observed dissociation between the HIV viral burden in the brain and clinical deficits points to relatively more important roles of toxins produced by activated monocytes and microglial cells, most of which are uninfected with HIV (Brew et al. 1995).

A central mechanism leading to synaptic dysfunction and loss, neuronal dysfunction, and apoptosis in HAND is excitotoxicity mediated through the excessive activation of neuronal (and probably astrocytic) NMDAR and the subsequent, stereotyped cascade of intracellular events driven by intracellular Ca^{2+} overload and shared by many neuroinflammatory and neurodegenerative disorders. The other two classes of ionotropic glutamate receptors (AMPA and kainate) appear less relevant in HAND and will not be discussed in further detail in this review. NMDAR are heterotetrameric structures that are subtyped depending upon the subunit structure of the receptor. There are three NMDAR subunit groups: NMDAR subunit 1 (NR1), NR2, and the more recently described NR3, which appears to have roles in NMDAR regulation. In most receptors one NR1 subunit (of which there are eight splice variants, NR1a–h) is combined with one NR2 subunit (of which there are four (NR2A–D) differing by the gene or origin), forming NR1/NR2 heterodimers, two of which make up a functional NMDAR (Köhr 2006; Guillemin 2005a; Dingledine et al. 1999; Chatterton et al. 2002). Neurons expressing a high proportion of NMDARs which contain the NR2A and NR2B subunits (i.e., hippocampal, forebrain, and basal ganglia neurons) appear particularly sensitive to NMDAR-mediated excitotoxicity and indeed are particularly vulnerable in HIV infection (O'Donnell et al. 2006).

NMDAR activation in HAND is a result of several mechanisms, some of which are unique to HIV infection when compared with other neuroinflammatory and neurodegenerative disorders. Firstly, neuronal NMDAR activation follows elevated extracellular glutamate as a result of both increased presynaptic release and impaired clearance from the synaptic space. Secondly, high levels of QUIN (an NMDAR agonist) released by activated macrophages (and to a lesser extent microglia) lead to NMDAR activation and excitotoxicity. Thirdly, there are the direct effects of some HIV-related proteins which can both activate the NMDAR and augment receptor activation by glutamate and other agonists. Furthermore, astrocytes play an important homeostatic role in protecting cells from excitotoxic cell death by efficient uptake of glutamate and other neurotransmitters from the extracellular environment in healthy and diseased brains (reviewed by Sofroniew and Vinters 2010), a process which is disturbed in HAND and contributes to excitotoxicity.

Importantly, oxidative stress and lipid peroxidation also contribute to HAND and may occur purely because of downstream effects of NMDAR-mediated excitotoxicity, although there are direct effects of cytokines/chemokines or HIV proteins (particularly gp120 and tat) (reviewed by Mattson et al. 2005). The relative significance of these multiple pathways to cell death and dysfunction in HAND is difficult to establish with certainty, particularly as oxidative stress, inflammation,

and excitotoxicity share common final pathways to cell death by caspase activation and apoptosis. Furthermore, some of these pathways are sequential (rather than convergent) and therefore pathophysiologically inseparable. Additionally, caspases, p53 and other enzymes, and transduction systems that mediate apoptosis seem also to play a role in neuronal dysfunction, injury, and dendritic degeneration in the absence of cell death (Garden et al. 2002, 2004).

Alternative pathological mechanisms have been recently advanced and may act simultaneously with excitotoxicity to produce HAND. Of note in particular, HIV has been shown to additionally infect neural progenitor cells and adversely impact on adult neurogenesis (ANG) (the process of neuron generation from neural progenitor cells possibly occurring in adults), likely by perturbing neurotrophic support from astrocytes and altered metabolic processes in particular insulin homeostasis in the CNS (reviewed by Lindl et al. 2010; Mattson et al. 2005). Neuronal autophagy (a protective process by which terminally differentiated neurons process and degrade aged proteins within the cytosol ultimately leading to cell death) is also inhibited by HIV-infected microglial cells (Alirezaei et al. 2008a, b). Inhibition of autophagy is associated with the development of neurodegenerative disorders. HIV-induced inflammation also leads to increased levels of ubiquitinated proteins as a result of disruption of the immunoproteasome function, and thus disturbance in neuronal and synaptic function, hypothesized to contribute to neurocognitive impairment (Nguyen et al. 2010).

5 Neuropathogenesis of HAND in the Era of HAART

Importantly, there have been relatively few histopathological studies examining HAND-affected individuals, particularly in the pre-AIDS stage of illness (Bell 2004) and in the era of HAART. After the introduction of antiretroviral therapy (but before widespread use of HAART), HIVE remained at high levels, for example, 26 % in one autopsy study (Masliah et al. 2000). Despite its efficacy in the prevention of opportunistic infections, HAART does not fully protect from HIVE, and "burnt-out" forms of HIVE have been observed in autopsy studies in those treated with HAART, displaying neuronal loss and leukoencephalopathy, which correlate with worsening cognitive function prior to death (Gray et al. 2003). An important issue that follows from these observations is whether the clinical disorder of HAND in the current era is pathologically indeed the same disorder as that observed before the widespread use of HAART as several clinical and pathological changes have been observed. While an association between the presence and severity of HAD and HIV proviral load in the brain (Bell et al. 1998) and CSF, or markers of inflammation (such as beta-2 microglobulin), has been historically described, it is not consistently demonstrated in HAART-treated patients (Brew et al. 2007). Use of antiretroviral agents with high CSF penetrance and activity against resting monocyte-lineage cells in the CNS has not been demonstrated in human trials to consistently yield beneficial effects on neuropsychological function,

though this may be related to trial design issues (Cysique et al. 2011; Brew et al. 2007). While further evaluation of this therapeutic approach is required, this implicates processes that are independent of HIV itself as responsible for ongoing HAND neuropathogenesis. In addition, cognitive changes are now not as closely correlated with basal ganglia abnormalities, with changes in the mesial temporal lobe possibly more relevant (reviewed by Brew 2004). Furthermore, HAART itself leads to upregulation of lymphocytes with immune reconstitution (Hirsch et al. 2004), which may alter the neuropathology in HIV-infected individuals on treatment (Bell 2004). Additionally, as the HAART-treated population ages, superimposed or synergistic effects of other comorbidities may also impact on the clinical expression of HAND, particularly vascular dementia, Alzheimer's dementia (Brew 2004), and other medical disorders, or toxicity arising from HAART itself. Therefore, while excitotoxicity in HAND has definitely been demonstrated, clearly ongoing research is required to establish its relative importance currently and with further therapeutic advances.

6 Mechanisms of Excitotoxicity in HAND

6.1 Disturbed Glutamate Homeostasis

Excessive extracellular glutamate concentrations occur in HAND patients, reflected by increased concentrations of glutamate in the CSF (Ferrarese et al. 2001) and reduced total parietal glutamate (due to reduced intracellular (neuronal) glutamate levels) in HAND-affected individuals (Ernst et al. 2010). Increased extracellular glutamate could occur as a result of impaired clearance of glutamate from the synaptic space and/or increased glutamate release presynaptically.

The process of glutamate transport (and thus clearance) from the synaptic space occurs primarily through the actions of excitatory amino acid transporter 2 (EAAT2) located on pre- and postsynaptic neuronal membranes and astrocytes sheathing synapses in the CNS (Sheldon and Robinson 2007). Reduced astrocyte clearance of glutamate from the extracellular space has been demonstrated in HIV infection, firstly due to impaired transporter function, following dysregulated release of glutamate by astrocytes, and as a consequence of overt astrocyte loss.

Gp120 activates astrocytic Na+/H+ antiporters in rat and human astrocytes in vitro, leading to depletion of intracellular H+, indirect induction of outward K+ movement due to intracellular alkalinization, and a reduction in the Na+ gradient across the astrocytic membrane. These changes are thought to be responsible for reduced astrocyte uptake (and therefore excessive extracellular levels) of glutamate (Patton et al. 2000). Furthermore, gp120 induces transcriptional downmodulation of the EAAT2 transporter gene in both infected and uninfected human astrocytes and attenuates glutamate transport by the cells in vitro (Wang et al. 2004). HIV-infected astrocytes also demonstrate higher levels of astrocyte-elevated gene-1 (AEG-1) expression, known to decrease EAAT2 promoter activity

(and thus EAAT2 expression) (Sheldon and Robinson 2007 and references therein). In addition to adversely impacting on astrocyte uptake of glutamate, gp120 can stimulate the release of glutamate from astrocytes into the extracellular space (Benos et al. 1994).

QUIN (further detailed below) also impairs the function of EAAT2 on astrocytes and adversely impacts the glutamate-glutamine cycle within human fetal astrocytes (by inhibition of glutamine synthase (GS) enzyme) and therefore likely further contributes to the accumulation of glutamate in the extracellular environment (Ting et al. 2009). There is also evidence from rat models that QUIN impairs the uptake of glutamate (but not inhibitory neurotransmitters) by synaptic vesicles, representing another avenue leading to excessive extracellular glutamate (Tavares et al. 2000).

Loss of astrocytes in HIV infection clearly also can contribute to increased extracellular glutamate and therefore excitotoxicity and more broadly to the loss of trophic support, synaptic homeostasis, CNS metabolism, and maintenance of the blood–brain barrier (reviewed by Sofroniew and Vinters 2010). QUIN induces apoptosis of human astrocytes in vitro at concentrations comparable found in patients with HAND, in a dose-dependent manner (Guillemin et al. 2005b).

Astrocytes also display functional NMDAR that can be activated by QUIN (Braidy et al. 2009; Lee et al. 2010). Furthermore, astrocytes can support restricted HIV infection and sustain production of tat and nef, both of which can independently adversely impact on neuronal function and lead to neuronal death, as detailed below.

6.2 Role of the Kynurenine Pathway and Quinolinic Acid in Excitotoxicity

QUIN is an intermediate in the kynurenine pathway (KP), the major route of L-tryptophan catabolism (ending in the production of nicotinamide adenine dinucleotide (NAD)), and is implicated in the pathogenesis of many neuroinflammatory disorders, including HAND. QUIN is predominantly produced in the CNS by macrophages/microglia, after induction of the KP by proinflammatory cytokines, some interferons (particularly IFN-γ), and possibly by direct induction by HIV proteins tat and nef (Smith et al. 2001) produced within the CNS. Furthermore, BBB endothelial cells and pericytes can be activated across an intact BBB by inflammatory signals (IFN-γ in particular) from the periphery to produce QUIN precursors. These intermediaries can be further metabolized to QUIN by perivascular macrophages and microglia even in the absence of CNS HIV infection (Owe-Young et al. 2008).

QUIN causes dendritic injury at low concentrations (consistent however with levels found in HAND-affected individuals) and neuronal death at higher concentrations (Smith et al. 2001; Kerr et al. 1998) through activation of NMDAR. Neurons of the hippocampus, striatum, and neocortex are particularly sensitive to QUIN (reviewed in Guillemin et al. 2005a). QUIN is an endogenous competitive

partial agonist of NMDAR containing NR1 and NR2A or NR2B subunits (Priestley et al. 1995), the very neurons that are particularly vulnerable to HIV-induced neurotoxicity (O'Donnell et al. 2006).

Indeed, several lines of evidence support a significant contribution of QUIN to the NMDAR-mediated excitotoxicity that occurs in HAND (reviewed by Kandanearatchi and Brew 2012 and by Guillemin et al. 2005a). CSF QUIN levels are elevated in HAND-affected patients and correlate with the severity of HIV-associated cognitive impairment in some studies (Heyes et al. 1991) but not all (Valle et al. 2004). Furthermore, atrophy of brain regions that are particularly vulnerable to excitotoxic injury (but not regions established to be resistant to excitotoxic injury) correlates with elevations in CSF QUIN concentrations in HIV-infected patients (Heyes et al. 2001).

Animal models have also demonstrated neuronal loss in cell cultures following exposure to QUIN (Whetsell and Schwarcz 1989) and regional susceptibility to effects of QUIN in the brain that parallel those regions preferentially affected in HAND (reviewed in Guillemin et al. 2005a). Furthermore, pharmacological blockade of NMDAR activation and the KP upstream of QUIN production (Kerr et al. 1997) have been shown to attenuate the neuronal damage and loss by macrophage supernatants.

HAART appears to reduce CSF levels of QUIN in treated individuals, and mathematical modelling indicates that QUIN decay patterns parallel CSF HIV decrement and improvement in neurological performance (Valle et al. 2004). QUIN also directly activates astrocytes, thus amplifying the inflammatory response and astrogliosis in neuroinflammatory disorders including HAND (Ting et al. 2009 and reviewed in Guillemin et al. 2005a).

6.3 HIV Proteins as Excitotoxins

HIV proteins can adversely impact all resident cells in the CNS (including neurons) and blood-derived cells without requiring direct infection. Defective virions and viral particles, viral particles released by cell death, together with viral coat proteins shed by HIV upon cellular infection, are present in the extracellular environment. HIV-infected cells also express some HIV proteins in their surface and through cell-cell interactions impact on uninfected adjacent cells (Nath 2002). Furthermore, gp120, Tat, and Vpr have all been shown to be present in the serum of HIV-infected patients, and Tat (Schwarze et al. 1999) and gp120 (Annunziata 2003) can cross the (often defective) blood–brain barrier, representing another pathway by which CNS neurons and other cells may be exposed to HIV proteins in the absence of direct infection.

There is evidence that gp120 (and Tat to a lesser extent) acts directly as excitotoxins through NMDAR activation in HAND, further detailed below. Other HIV proteins have also been studied in isolation; however, currently there is a lack of convincing evidence to support a significant contribution to excitotoxicity. Of note, gp41 has been demonstrated to induce the release of glutamate from

astrocytes in vitro (Nath 2002 and references therein). Nef is toxic to human neurons and microglial cells in vitro (Trillo-Pazos et al. 2000) (possibly through adverse impacts on neuronal potassium currents across the neuronal membrane (Werner et al. 1991)). In vivo nef exposure also leads to monocyte/macrophage recruitment, increased production of TNF-α and astrogliosis, and behavioral change in rats (Mordelet et al. 2004). Vpr has not been established as a contributor to excitotoxicity; however, it has been shown experimentally to induce neuronal apoptosis possibly through activation of caspase-8 (Patel et al. 2000) and neuronal lysis through disturbance of membrane permeability with subsequent depolarization (Piller et al. 1998, 1999). Rev, Vpu, and other HIV proteins have been studied much less than the above proteins, and at this stage there is insufficient evidence describing a direct role of these proteins in the pathogenesis of HAND.

6.3.1 Gp120

As part of the HIV life cycle, gp120 forms virion spikes and interacts with receptors on target cells (Reitz and Gallo 2009). In addition, gp120 induces neuronal cell death of midbrain dopaminergic, hippocampal, cortical, and cerebellar granule neurons in vitro and within rodent brains in vivo (Bachis et al. 2003 and references therein). While experimental evidence suggests that gp120 has the capacity to induce neuronal apoptosis through cytokine receptor activation directly (reviewed in Gonzalez-Scarano and Martin-Garcia 2005) and involvement of the caspase system of enzymes (Garden et al. 2002), the relative importance of this effect pathophysiologically is not certain.

The bulk of evidence suggests that gp120 contributes to neuronal apoptosis ultimately through NMDA receptor-mediated excitotoxicity; however, again the relative importance of this protein overall in the excitotoxicity in HAND is not established. It has been demonstrated experimentally that HIV requires the presence of non-neuronal cells (microglia and macrocytes) to induce excitotoxicity (Meucci and Miller 1996; Kaul et al. 2001, 2005 and references therein), and gp120 has been implicated in the activation of these cells. Indeed, in vivo studies using transgenic gp120 positive mice have demonstrated that gp120 in the absence of HIV itself is sufficient to induce excitotoxic neuronal injury, reduced dendritic density, neuronal death, and behavioral changes consistent with HAND, effects that are at least partly ameliorated by the NMDAR antagonist treatment (Kaul et al. 2005 and references therein). The requirement of non-neuronal cells, however, for neurotoxicity in HIV implies a microglial cell-derived intermediate at play (QUIN, e.g., has been proposed as at least one of these intermediaries as detailed above as well as arachidonic acid (AA)). AA is released by astrocytes and macrophages in the presence of gp120. AA is known to reduce the reuptake of glutamate and other excitatory amino acids by astrocytes and nerve endings via EAA transporters in vitro. AA can also potentiate neuronal NMDAR activation by glutamate, increasing channel open time and mean open probability after activation (Miller et al. 1992; Lipton et al. 1994). There is also some evidence, however, that gp120 itself directly interacts with the NMDAR at its glycine binding site, potentiating its activation by endogenous agonists (Li et al. 2005; Kaul et al. 2001).

6.3.2 Tat

Tat is actively released extracellularly by HIV-1-infected lymphoid and glial cells and astrocytes and can be taken up by most cell types (including neurons) or interact with cell surfaces, thereby exerting toxicity to both infected and uninfected cells. Tat also appears to evade proteolysis by neurons and enter axonal transport systems and therefore can cause neuronal damage and microglial activation at sites distant from cells infected with HIV (reviewed by Li et al. 2009). Cells particularly sensitive to the neurotoxic effects of Tat are those in the striatum, dentate gyrus, and CA3 region of the hippocampus (Rumbaugh and Nath 2006).

Experimental evidence supports a role of Tat in NMDAR-mediated excitotoxicity by potentiating its activation by endogenous agonists (Haughey et al. 2001; Li et al. 2005; Mattson et al. 2005). The pathway of receptor potentiation involves phosphorylation of NMDAR subunits NR2A and NR2B and modulation of Zn-binding sites on the NMDAR (Haughey et al. 2001). This potentiation may explain the observed synergy between Tat and gp120 (Nath et al. 2000; Ferris et al. 2008), as well as with glutamate itself (Li et al. 2009 and references therein), in inducing excitotoxic neuronal cell death. Furthermore, Tat uniquely activates the NMDAR, at a site distinct from glutamate. In vitro rat hippocampal neurons are activated by Tat binding to a distinct modulatory site on the NR1 subunit of NMDARs at concentrations compatible to those in the brains of HIV-infected individuals (Song et al. 2003). The relative importance of this interaction with the NMDAR is uncertain, however, in the pathogenesis of HAND.

Like gp120, there is extensive evidence of Tat involvement in the widespread glial cell activation characteristic of HIVE (particularly monocytes and macrophages), with subsequent induction of a wide range of cytokines (Nath 2002; Mattson et al. 2005; Rumbaugh and Nath 2006). Indeed, Tat increases NO production by microglial cells through inducible NOS gene transactivation, an effect that is amplified in the presence of IFN-γ (Polazzi et al. 1999). Similar to gp120, Tat has also been demonstrated to bind and activate some chemokine receptors directly (Albini et al. 1998). Furthermore, Tat interferes with multiple molecular mechanisms in microglial cells experimentally, potentially further contributing to neuropathology (reviewed by Minghetti et al. 2004). Importantly, however, the relative importance of these multiple pathways to neuronal death induced by Tat experimentally is uncertain. Interestingly, astrocytes transfected with Tat are protected from oxidative stress and cellular injury experimentally, despite the extracellular release of Tat inducing neurite trimming and neuron death (Chauhan et al. 2003).

7 Adjunctive Therapeutic Approaches: The Role of NMDAR Antagonists

Current HAART fails to protect HIV-infected individuals from HAND even when viral suppression is achieved and immune system function optimized. Therefore, therapies that augment the partially protective effects of HAART are actively being sought. Multiple avenues for alternative therapies have been explored.

The following discussion will focus on agents that reduce excitotoxicity at the level of NMDAR activation; however, the associated rise in intracellular calcium with activation of NOS and apoptotic pathways and/or the impacts of locally and peripherally produced cytokines are also acknowledged as potential targets (Wallace 2006). For example, both lithium and valproate modulate apoptotic protein function; lithium also stabilizes Na+/K+ channels and reduces inflammation.

Notably, studies have shown preliminary efficacy of valproate in preventing neuronal dysfunction in animal models of HIVE (Dou et al. 2003, 2005) and in the treatment of HAND-affected individuals (Schifitto et al. 2009; Letendre et al. 2006).

The recognition that NMDAR activation plays a central role in neuronal degeneration and death in HAND has been facilitated by the use of NMDAR antagonists experimentally. Therapeutic trials of such antagonists have naturally progressed from these findings, however as yet their effectiveness in improving cognitive function or slowing the progression of HAND has not been established. Additionally, while changes to glutamate receptor functioning in astrocytes and microglial cells have been implicated in excitotoxic cell death cascades, the consequences of therapeutic manipulation of glutamate transporters in models of other neurodegenerative disorders have yielded conflicting results (Sheldon and Robinson 2007) and as yet have not been evaluated in the setting of HAND.

Memantine is a noncompetitive, low-affinity, open-channel blocker of the NMDAR (pharmacology reviewed by Lipton 2004), with preferential action on extrasynaptic NMDAR (those containing NR2B subunits) (Shin et al. 2011). It has an acceptable side effect profile due to its ability to prevent NMDAR activation at pathophysiological levels without compromising normal neuronal function; indeed, many experimental agents have failed in this regard with resultant excessive toxicity (Lipton 2004). It is used widely in the treatment of Alzheimer's dementia; however, as yet therapeutic trials examining its use in HAND have not led to recommendations for its use in this setting thus far. In addition, memantine is likely only to be effective when used preventatively or to slow dementia progression in affected patients, as it would have no impact on neuronal impairment once established (Wallace 2006).

Specifically, in vitro studies have demonstrated that neurons are protected from injury and death induced by gp120 (Lipton 1992) and gp120 and tat cotreatment (Nath et al. 2000) in the presence of memantine. Gp120-induced reductions in dendritic and presynaptic terminal density are also diminished in gp120+ transgenic mouse models when treated with memantine (Toggas et al. 1996). Furthermore, memantine has been demonstrated experimentally to prevent Tat-induced death of rat hippocampal neurons in culture. Interestingly, while failing to protect from Tat-induced synapse loss in this study, when administered after significant synapse loss had occurred, memantine induced a recovery of synapse number. The authors hypothesize that NMDA receptor subtypes differentially contribute to synaptic death and recovery and that synaptic NMDA receptor subtypes undergo pharmacological change during the neurotoxic process (Shin et al. 2011).

Memantine treatment in the setting of mild to severe forms of HAND has so far been demonstrated to be safe and tolerable in a double-blind 16-week trial

(followed by 20-week washout period), with proton magnetic resonance spectroscopy (MRS) data (measuring metabolites associated with glia and neuronal function in vivo) across the treatment period consistent with a neuroprotective effect in treated individuals (Schifitto et al. 2007). However, the open-label phase of this same study extended to 60 weeks of therapy yielded no statistically significant improvement in neuropsychological performance in those receiving memantine (Zhao et al. 2010). Importantly, however, study limitations necessitate further evaluation to more accurately assess the efficacy of memantine in those affected by HAND (Zhao et al. 2010).

8 Conclusion

So far current therapy for HIV infection has failed to protect treated patients from the development of HAND despite apparent efficacy in virologic control, as least as measured outside of the brain. While current evidence supports the contribution of multiple pathophysiological pathways in the development of HAND, a central role of NMDAR-mediated excitotoxicity is supported by both direct and indirect evidence. Indeed, HIV impacts on this pathway in some ways that are unique among other neuroinflammatory and neurodegenerative disorders, as outlined. Clearly further research is required in the area of HAND pathogenesis to establish the relative importance of NMDAR-mediated excitotoxicity in the development of these disorders (especially in the era of HAART). This would assist in the development of adjunctive therapies in the prevention and treatment of HAND.

References

Adle-Biassette, H., Chretien, F., Wingertsmann, L., Ereau, H., Scaravilli, F., Tardieu, M., & Gray, F. (1999). Neuronal apoptosis does not correlate with dementia in HIV infection but is related to microglial activation and axonal damage. *Neuropathology and Applied Neurobiology, 25*, 123–133.

Albini, A., Ferrini, S., Benelli, R., Sforzini, S., Giuncinglio, D., Aluigi, M., Proudfoot, A., Alouani, S., Wells, T., Mariani, G., Rabin, R., Farber, J., & Noonan, D. (1998). HIV-1 Tat protein mimicry of chemokines. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 13153–13158.

Alirezaei, M., Kiosses, W., Flynn, H., Brady, N., & Fox, H. (2008a). Disruption of neuronal autophagy by infected microglia results in neurodegeneration. *PloS One, 3*(8).

Alirezaei, M., Kiosses, W., & Fox, H. S. (2008b). Decreased neuronal autophagy in HIV dementia: A mechanism of indirect neurotoxicity. *Autophagy, 4*(7), 963–966.

Annunziata, P. (2003). Blood–brain barrier changes during invasion of the CNS by HIV-1: Old and new insights into the mechanism. *Journal of Neurology, 250*, 901–906.

Antinori, A., Arendt, G., Becker, J. T., Brew, B. J., Byrd, D. A., Cherner, M., Clifford, D. B., Cinque, P., Epstein, L. G., Goodkin, K., Gisslen, M., Grant, I., Heaton, R. K., Joseph, J., Marder, K., Marra, C. M., McArthur, J. C., Nunn, M., Price, R. W., Pulliam, L., Robertson, K. R., Sacktor, N., Valcour, V., & Wojna, V. E. (2007). Updated research nosology for HIV-associated neurocognitive disorders. *Neurology, 69*, 1789–1799.

Bachis, A., Major, E., & Mocchetti, I. (2003). Brain-derived neurotrophic factor inhibits human immunodeficiency virus-1/gp120-mediated cerebellar granule cell death by preventing gp120 internalization. *The Journal of Neuroscience, 23*(13), 5715–5722.

Bell, J. (2004). An update on the neuropathology of HIV in the HAART era. *Histopathology, 45*, 549–559.

Bell, J., Brettle, R., Chiswick, A., & Simmonds, P. (1998). HIV encephalitis, proviral load and dementia in drug users and homosexuals with AIDS: Effect of neocortical involvement. *Brain, 121*, 2043–2052.

Benos, D., Hahn, B., Bubien, J., Ghosh, S., Mashburn, N., Chaikin, M., Shaw, G., & Benvenistes, E. (1994). Envelope glycoprotein gpl20 of human immunodeficiency virus type 1 alters ion transport in astrocytes: Implications for AIDS dementia complex. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 494–498.

Boissé, L., Gill, J., & Power, C. (2008). HIV infection of the central nervous system: Clinical features and neuropathogenesis. *Neurologic Clinics, 26*, 799–819.

Bol, S., Cobos-Jiménez, V., Kootstra, N., & van't Wout, A. (2011). HIV-1 and the macrophage. *Future Virology, 6*(2), 187–208.

Braidy, N., Grant, R., Adams, S., Brew, B., & Guillemin, G. (2009). Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons. *Neurotoxicity Research, 16*, 77–86.

Brew, B. (2004). Evidence for a change in AIDS dementia complex in the era of highly active antiretroviral therapy and the possibility of new forms of AIDS dementia complex. *AIDS, 18* (Suppl 1), S75–S78.

Brew, B., Rosenblum, M., Cronin, K., & Price, R. (1995). AIDS dementia complex and HIV-1 brain infection: Clinical-virological correlations. *Annals of Neurology, 38*, 563–570.

Brew, B., Halman, M., Catalan, J., Sacktor, N., Price, R., Brown, S., Atkinson, H., Clifford, D., Simpson, D., Torres, G., Hall, C., Power, C., Marder, K., Mc Arthur, J., Symonds, W., & Romero, C. (2007). Factors in AIDS dementia complex trial design: Results and lessons from the abacavir trial. *PLoS Clinical Trials, 2*(3), e13.

Chatterton, J. E., Awobuluyi, M., Premkumar, L. S., Takahashi, H., Talantova, M., Shin, Y., Cui, J., Tu, S., Sevarino, K. A., Nakanishi, N., Tong, G., Lipton, S., & Zhang, D. (2002). Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature, 415*(6873), 793–798.

Chauhan, A., Turchan, J., Pocernich, C., Bruce-Keller, A., Roth, S., Butterfield, D., Major, E., & Nath, A. (2003). Intracellular human immunodeficiency virus Tat expression in astrocytes promotes astrocyte survival but induces potent neurotoxicity at distant sites via axonal transport. *The Journal of Biological Chemistry, 278*(15), 13512–13519.

Churchill, M., Wesselingh, S., Cowley, M., Pardo, C., McArthur, J., Brew, B. J., & Gorry, P. (2009). Extensive astrocyte infection is prominent in human immunodeficiency virus–associated dementia. *Annals of Neurology, 66*(2), 253–258.

Cysique, L. A., & Brew, B. J. (2011). Prevalence of non-confounded HIV-associated neurocognitive impairment in the context of plasma HIV RNA suppression. *Journal of Neurovirology, 17*(2), 176–183.

Cysique, L., Maruff, P., & Brew, B. J. (2004). Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus-infected/acquired immune deficiency syndrome (HIV/AIDS) patients across pre and post highly active antiretroviral therapy eras: A combined study of two cohorts. *Journal of Neurovirology, 10*(6), 350–357.

Cysique, L. A., Maruff, P., & Brew, B. J. (2006). Variable benefit in neuropsychological function in HIV-infected HAART-treated patients. *Neurology, 66*(9), 1447–1450.

Cysique, L. A., Waters, E. K., & Brew, B. J. (2011). Central nervous system antiretroviral efficacy in HIV infection: A qualitative and quantitative review and implications for future research. *BMC Neurology, 11*, 148.

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacological Reviews, 51*(1), 7–61.

Dore, G., Correll, P., Li, Y., Kaldor, J., Cooper, D., & Brew, B. (1999). Changes to the AIDS dementia complex in the era of highly active antiretroviral therapy. *AIDS, 13*, 1249–1253.

Dou, H., Birusingh, K., Faraci, J., Gorantla, S., Poluektova, L., Maggirwar, S., Dewhurst, S., Gelbard, H., & Gendelman, H. (2003). Neuroprotective activities of sodium valproate in

a murine model of human immunodeficiency virus-1 encephalitis. *The Journal of Neuroscience, 23*(27), 9162–9170.

Dou, H., Ellison, B., Bradley, J., Kasiyanov, A., Poluektova, L., Xiong, H., Maggiwir, S., Dewhurst, S., Gelbard, H., & Gendelman, H. (2005). Neuroprotective mechanisms of lithium in murine human immunodeficiency virus-1 encephalitis. *Journal of Neuroscience, 25*(37), 8375–8385.

Ellis, R., Deutsch, R., Heaton, R., Marcotte, T., McCutchan, J., Nelson, J., Abramson, I., Thal, L., Atkinson, J., Wallace, M., & Grant, I. (1997). Neurocognitive impairment is an independent risk factor for death in HIV infection. *Archives of Neurology, 54*(4), 416–424.

Ernst, T., Jiang, C., Nakama, H., Buchthal, S., & Chang, L. (2010). Lower brain glutamate is associated with cognitive deficits in HIV patients: A new mechanism for HIV-associated neurocognitive disorder. *Journal of Magnetic Resonance Imaging, 32*, 1045–1053.

Everall, I., Heaton, R., Marcotte, T., Ellis, R., McCutchan, J., Atkinson, J., Grant, I., Mallory, M., Masliah, E., & The HNRC Group. (1999). Cortical synaptic density is reduced in mild to moderate human immunodeficiency virus neurocognitive disorder. *Brain Pathology, 9*, 209–217.

Ferrarese, C., Aliprandi, A., Tremolizzo, L., Stanzani, L., De Micheli, A., Dolara, A., & Frattola, L. (2001). Increased glutamate in CSF and plasma of patients with HIV dementia. *Neurology, 57*(4), 671–675.

Ferris, M., Mactutus, C., & Booze, R. (2008). Neurotoxic profiles of HIV, psychostimulant drugs of abuse, and their concerted effect on the brain: Current status of dopamine system vulnerability in NeuroAIDS. *Neuroscience and Biobehavioral Reviews, 32*(5), 883–909.

Garden, G., Budd, S., Tsai, E., Hanson, L., Kaul, M., D'Emilia, D., Friedlander, R., Yuan, J., Masliah, E., & Lipton, S. (2002). Caspase cascades in human immunodeficiency virus-associated neurodegeneration. *The Journal of Neuroscience, 22*(10), 4015–4024.

Garden, G., Guo, W., Jayadev, S., Tun, C., Balcaitis, S., Choi, J., Montine, T., Möller, T., & Morrison, R. (2004). HIV associated neurodegeneration requires p53 in neurons and microglia. *The FASEB Journal, 18*(10), 1141–1143.

Gonzalez-Scarano, F., & Martin-Garcia, J. (2005). The neuropathogenesis of AIDS. *Nature, 5*, 69–81.

Gray, F., & Keohane, C. (2003). The neuropathology of HIV infection in the era of highly active antiretroviral therapy (HAART). *Brain Pathology, 13*, 79–83.

Gray, F., Chrétien, F., Vallat-Decouvelaere, A., & Scaravilli, F. (2003). The changing pattern of HIV neuropathology in the HAART era. *Journal of Neuropathology and Experimental Neurology, 62*(5), 429–440.

Guillemin, G. J., Kerr, S. J., & Brew, B. J. (2005a). Involvement of quinolinic acid in AIDS dementia complex. *Neurotoxicity Research, 7*(1–2), 103–123.

Guillemin, G., Wang, L., & Brew, B. (2005b). Quinolinic acid selectively induces apoptosis of human astrocytes: Potential role in AIDS dementia complex. *Journal of Neuroinflammation, 2*, 16.

Haughey, N., Nath, A., Mattson, M., Slevin, J., & Geiger, J. (2001). HIV-1 Tat through phosphorylation of NMDA receptors potentiates glutamate excitotoxicity. *Journal of Neurochemistry, 78*, 457–467.

Heaton, R. K., Clifford, D. B., Franklin, D. R., Woods, S., Ake, C., Vaida, F., Ellis, R., Letendre, S., Marcotte, T., Atkinson, J., Rivera-Mindt, M., Vigil, O., Taylor, M., Collier, A., Marra, C., Gelman, B., McArthur, J., Morgello, S., Simpson, D., McCutchan, J., Abramson, I., Gamst, A., Fennema-Notestine, C., Jernigan, T., Wong, J., & Grant, I. (2010). HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy; the CHARTER Study. *Neurology, 75*, 2087–2096.

Heyes, M., Brew, B., Martin, A., Price, R., Salazar, A., Sidtis, J., Yergey, J., Mouradian, M., Sadler, A., Keilp, J., Rubinow, D., & Markey, S. (1991). Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: Relationship to clinical and neurological status. *Annals of Neurology, 29*(2), 202–209.

Heyes, M., Ellis, R., Ryan, L., Childers, M., Grant, I., Wolfson, T., Archibald, S., Jernigan, T., & The HNRC Group. (2001). Elevated cerebrospinal fluid quinolinic acid levels are associated with region-specific cerebral volume loss in HIV infection. *Brain, 124*, 1033–1042.

Hirsch, H., Kaufmann, G., Sendi, P., & Battegay, M. (2004). Immune reconstitution in HIV-infected patients. *Clinical Infectious Diseases, 38*, 1159–1166.

Ivey, N., MacLean, A., & Lackner, A. (2009). AIDS and the blood–brain barrier. *Journal of Neurovirology, 15*(2), 111–122.

Kandanearatchi, A., & Brew, B. (2012). The kynurenine pathway and quinolinic acid: Pivotal roles in HIV associated neurocognitive disorders. *FEBS Journal, 279*(8), 1366–1374.

Kaul, M., Garden, G., & Lipton, S. (2001). Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature, 410*, 988–994.

Kaul, M., Zheng, J., Okamoto, S., Gendelmen, H., & Lipton, S. (2005). HIV-1 infection and AIDS: Consequences for the central nervous system. *Cell Death and Differentiation, 12*, 878–892.

Kerr, S., Armati, P., Pemberton, L., Smythe, G., Tattam, B., & Brew, B. (1997). Kynurenine pathway inhibition reduces neurotoxicity of HIV-1 infected macrophages. *Neurology, 49*, 1671–1681.

Kerr, S., Armati, P., Guillemin, G., & Brew, B. (1998). Chronic exposure of human neurons to quinolinic acid results in neuronal changes consistent with AIDS dementia complex. *AIDS, 12*, 355–363.

Köhr, G. (2006). NMDA receptor function: Subunit composition versus spatial distribution. *Cell and Tissue Research, 326*, 439–446.

Langford, D., & Masliah, E. (2001). Crosstalk between components of the blood brain barrier and cells of the CNS in microglial activation in AIDS. *Brain Pathology, 11*, 306–312.

Lee, M., Ting, K., Adams, S., Brew, B., Chung, R., & Guillemin, G. (2010). Characterisation of the expression of NMDA receptors in human astrocytes. *PloS One, 5*(11), e14123.

Letendre, S., Woods, S., Ellis, R., Atkinson, J., Masliah, E., van den Brande, G., Durelle, J., Grant, I., Everall, I., & The HNRC Group. (2006). Lithium improves HIV-associated neurocognitive impairment. *AIDS, 20*, 1885–1888.

Li, W., Galey, D., Mattson, M., & Nath, A. (2005). Molecular and cellular mechanisms of neuronal cell death in HIV dementia. *Neurotoxicity Research, 8*(1–2), 119–134.

Li, W., Li, G., Steiner, J., & Nath, A. (2009). Role of Tat protein in HIV neuropathogenesis. *Neurotoxicity Research, 16*, 205–220.

Lindl, K., Marks, D., Kolson, D., & Jordan-Sciutto, K. (2010). HIV-associated neurocognitive disorderd pathogenesis and therapeutic opportunities. *Journal of Neuroimmune Pharmacology, 5*, 294–309.

Lipton, S. (1992). Memantine prevents HIV coat protein- induced neuronal injury in vitro. *Neurology, 42*, 1403–1405.

Lipton, S. (2004). Failures and successes of NMDA receptor antagonists: Molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. *NeuroRx: The Journal of the American Society for Experimental NeuroTherapeutics, 1*, 101–110.

Lipton, S., Yeh, M., & Dreyer, E. (1994). Update on current models of HIV-related neuronal injury: Platelet activating factor, arachidonic acid and nitric oxide. *Advances in Neuroimmunology, 4*, 181–188.

Masliah, E., DeTeresa, R., Mallory, M., & Hansen, L. (2000). Changes in pathological findings at autopsy in AIDS cases for the last 15 years. *AIDS, 14*, 69–74.

Mattson, M., Haughey, N., & Nath, A. (2005). Cell death in HIV dementia. *Cell Death and Differentiation, 12*, 893–904.

McArthur, J., Brew, B., & Nath, A. (2005). Neurological complications of HIV infection. *Lancet Neurology, 4*, 543–555.

Meucci, O., & Miller, R. (1996). gp120-induced neurotoxicity in hippocampal pyramidal neuron cultures: Protective action of TGF-β1. *The Journal of Neuroscience, 16*(13), 4080–4088.

Miller, B., Sarantis, M., Traynelis, S., & Attwell, D. (1992). Potentiation of NMDA receptor currents by arachidonic acid. *Nature, 355*, 722–725.

Minghetti, L., Visentin, S., Patrizio, M., Franchini, L., Ajmone-Cat, M., & Levi, G. (2004). Multiple actions of the human immunodeficiency virus type-1 Tat protein on microglial cell functions. *Neurochemical Research, 29*(5), 965–978.

Moore, D., Masliah, E., Rippeth, J., Gonzalez, R., Carey, C., Cherner, M., Ellis, R., Achim, C., Marcotte, T., Heaton, R., Grant, I., & The HNRC group. (2006). Cortical and subcortical neurodegeneration is associated with HIV neurocognitive impairment. *AIDS, 20*, 879–887.

Mordelet, E., Kissa, K., Cressant, A., Gray, F., Ozden, S., Vidal, C., Charneau, P., & Granon, S. (2004). Histopathological and cognitive defect induced by Nef in the brain. *The FASEB Journal, 18*, 1851–1861.

Nath, A. (2002). Human immunodeficiency virus (HIV) proteins in neuropathogenesis of HIV dementia. *The Journal of Infectious Diseases, 186*(Suppl 2), S193–S198.

Nath, A., Haughey, N., Jones, M., Anderson, C., Bell, J., & Geiger, J. (2000). Synergistic neurotoxicity by human immunodeficiency virus proteins tat and gp120: Protection by memantine. *Annals of Neurology, 47*, 186–194.

Nguyen, T., Soukup, V., & Gelman, B. (2010). Persistent hijacking of brain proteasomes in HIV-associated dementia. *The American Journal of Pathology, 176*(2), 893–902.

O'Donnell, L., Agrawal, A., Jordan-Sciutto, K., Dichter, M., Lynch, D., & Kolson, D. (2006). Human immunodeficiency virus (HIV)-induced neurotoxicity: Roles for the NMDA receptor subtypes 2A and 2B and the calcium-activated protease Calpain by a CSF-derived HIV-1 strain. *The Journal of Neuroscience, 26*(3), 981–990.

Owe-Young, R., Webster, N., Mukhtar, M., Pomerantz, R., Smythe, G., Walker, D., Armati, P., Crowe, S., & Brew, B. (2008). Kynurenine pathway metabolism in human blood–brain-barrier cells: Implications for immune tolerance and neurotoxicity. *Journal of Neurochemistry, 105*, 1346–1357.

Patel, C., Mukhtar, M., & Pomerantz, R. (2000). Human immunodeficiency virus type 1 Vpr induces apoptosis in human neuronal cells. *Journal of Virology, 74*(20), 9717–9726.

Patton, H., Zhou, Z., Bubien, J., Benveniste, E., & Benos, D. (2000). gp120-induced alterations of human astrocyte function: Na1/H1 exchange, K1 conductance, and glutamate flux. *American Journal of Physiology. Cell Physiology, 279*, C700–C708.

Persidsky, Y., Zheng, J., Miller, D., & Gendelman, H. (2000). Mononuclear phagocytes mediate blood–brain barrier compromise and neuronal injury during HIV-1-associated dementia. *Journal of Leukocyte Biology, 68*, 413–422.

Petito, C. (2003). Neuropathology of acquired immunodeficiency syndrome. In *Principles and practice of neuropathology* (2nd ed.). Oxford: Oxford University Press.

Piller, S., Jans, P., Gage, P., & Jans, D. (1998). Extracellular HIV-1 virus protein R causes a large inward current and cell death in cultured hippocampal neurons: Implications for AIDS pathology. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 4595–4600.

Piller, S., Ewart, G., Jans, D., Gage, P., & Cox, G. (1999). The amino-terminal region of Vpr from human immunodeficiency virus type 1 forms ion channels and kills neurons. *Journal of Virology, 73*(5), 4230–4238.

Polazzi, E., Levi, G., & Minghetti, L. (1999). Human immunodeficiency virus type 1 Tat protein stimulates inducible nitric oxide synthase expression and nitric oxide production in microglial cultures. *Journal of Neuropathology and Experimental Neurology, 58*(8), 825–831.

Priestley, T., Laughton, P., Myers, J., Le Bourdellés, B., Kerby, J., & Whiting, P. J. (1995). Pharmacological properties of recombinant human *N*-methyl-D-aspartate receptors comprising NR1a/NR2A and NR1a/NR2B subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Molecular Pharmacology, 48*(5), 841–848.

Reitz, M., & Gallo, R. (2009). Human Immunodeficiency Viruses. In G. L. Mandell, J. E. Bennett, R. Dolin, & G. V. Kelvin (Eds.), *Mandell, Douglas and Bennett's principles and practice of infectious diseases* (7th ed.). Philadelphia, PA: Churchill Livingston.

Robertson, K. R., Smurzynski, M., Parsons, T. D., Wu, K., Bosch, R. J., Wu, J., McArthur, J. C., Collier, A. C., Evans, S. R., & Ellis, R. J. (2007). The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS, 21*, 1915–1921.

Rumbaugh, J., & Nath, A. (2006). Developments in HIV neuropathogenesis. *Current Pharmaceutical Design, 12*, 1023–1044.

Sacktor, N., Lyles, R., Skolasky, R., Kleeberger, C., Selnes, O., Miller, E., Becker, J., Cohen, B., McArthur, J., & Multicenter AIDS Cohort Study. (2001). HIV associated neurologic disease incidence changes: Multicenter AIDS Cohort Study, 1990–1998. *Neurology, 56*(2), 257–260.

Schifitto, G., Navia, B., Yiannoutsos, C., Marra, C., Chang, L., Ernst, T., Jarvik, J., Miller, E., Singer, E., Ellis, R., Kolson, D., Simpson, D., Nath, A., Berger, J., Shriver, S., Millar, L., Colquhoun, D., Lenkinski, R., Gonzalez, G., Lipton, S., & The Adult AIDS Clinical Trial Group (ACTG) 301 and 700 Teams and the HIV MRS Consortium. (2007). Memantine and HIV-associated cognitive impairment: A neuropsychological and proton magnetic resonance spectroscopy study. *AIDS, 21*, 1877–1886.

Schifitto, G., Zhong, J., Gill, D., Peterson, D., Gaugh, M., Zhu, T., Tivarus, M., Cruttenden, K., Maggirwar, S., Gendelman, H., Dewhurst, S., & Gelbard, H. (2009). Lithium therapy for human immunodeficiency virus type-1 associated neurocognitive impairment. *Journal of Neurovirology, 15*, 176–186.

Schwarze, S., Ho, A., Vocero-Akbani, A., & Dowdy, S. (1999). In vivo protein transduction: Delivery of a biologically active protein into the mouse. *Science, 285*, 1569–1572.

Sheldon, A., & Robinson, M. (2007). The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. *Neurochemistry International, 51*(6–7), 333–355.

Shin, A., Kim, H., & Thayer, S. (2011). Subtype selective NMDA receptor antagonists induce recovery of synapses lost following exposure to HIV-1 Tat. *British Journal of Pharmacology, 166*(3), 1002–1017.

Simioni, S., Cavassini, M., Annoni, J., Abraham, A. R., Bourquin, I., Schiffer, V., Calmy, A., Chave, J., Giacobini, E., Hirschel, B., & Du Pasquier, R. A. (2010). Cognitive dysfunction in HIV patients despite long-standing suppression of viremia. *AIDS, 24*, 1243–1250.

Smith, D., Guillemin, G., Pemberton, L., Kerr, S., Nath, A., Smythe, G., & Brew, B. (2001). Quinolinic acid is produced by macrophages stimulated by platelet activating factor, Nef and Tat. *Journal of Neurovirology, 7*, 56–60.

Sofroniew, M., & Vinters, H. (2010). Astrocytes: Biology and pathology. *Acta Neuropathologica, 119*, 7–35.

Song, L., Nath, A., Geiger, J., Moore, A., & Hochman, S. (2003). Human immunodeficiency virus type-1 Tat protein directly activates neuronal N-methyl-D-aspartate receptors at an allosteric zinc-sensitive site. *Journal of Neurovirology, 9*, 399–403.

Staekenborg, S. S., van der Flier, W. M., van Straaten, E. C., Lane, R., Barkhof, F., & Scheltens, P. (2008). Neurological signs in relation to type of cerebrovascular disease in vascular dementia. *Stroke, 39*, 317–322.

Tavares, R., Tasca, C., Santos, C., Wajner, M., Souza, M., & Dutra-Filho, C. (2000). Quinolinic acid inhibits glutamate uptake into synaptic vesicles from rat brain. *Neuroreport, 11*, 249–253.

Thompson, K., Churchill, M., Gorry, P., Sterjovski, J., Oelrichs, R., Wesselingh, S., & McLean, C. (2004). Astrocyte specific viral strains in HIV dementia. *Annals of Neurology, 56*, 873–877.

Ting, K., Brew, B., & Guillemin, G. (2009). Effect of quinolinic acid on human astrocytes morphology functions: Implications in Alzheimer's disease. *Journal of Neuroinflammation, 6*, 36.

Toborek, M., Lee, Y., Pu, H., Malecki, A., Flora, G., Garrido, R., Hennig, B., Bauer, H., & Nath, A. (2003). HIV-Tat protein induces oxidative and inflammatory pathways in brain endothelium. *Journal of Neurochemistry, 84*, 169–179.

Toggas, S., Masliah, E., & Mucke, L. (1996). Prevention of HIV-1 gp120-induced neuronal damage in the central nervous system of transgenic mice by the NMDA receptor antagonist memantine. *Brain Research, 706*, 303–307.

Trillo-Pazos, G., McFarlane-Abdulla, E., Campbell, I., Pilkington, G., & Everall, I. (2000). Recombinant nef HIV-IIIB protein is toxic to human neurons in culture. *Brain Research, 864*(2), 315–326.

Valle, M., Price, R., Nilsson, A., Heyes, D., & Verotta, D. (2004). CSF quinolinic acid levels are determined by local HIV infection: Cross-sectional analysis and modelling of dynamics following antiretroviral therapy. *Brain, 127*, 1047–1060.

Wallace, D. (2006). HIV neurotoxicity: Potential therapeutic interventions. *Journal of Biomedicine and Biotechnology, 2006*, 1–10.

Wang, Z., Trillo-Pazos, G., Kim, S., Canki, M., Morgello, S., Sharer, L., Gelbard, H., Su, Z., Kang, D., Brooks, A., Fisher, P., & Volsky, D. (2004). Effects of human immunodeficiency virus type 1 on astrocyte gene expression and function: Potential role in neuropathogenesis. *Journal of Neurovirology, 10*(Suppl 1), 25–32.

Werner, T., Ferroni, S., Saermark, T., Brack-Werner, R., Banati, R., Mager, R., Steinaa, L., Kreutzberg, G., & Erfle, V. (1991). HIV-1 Nef protein exhibits structural and functional similarity to scorpion peptides interacting with K+ channels. *AIDS, 5*(11), 1301–1308.

Whetsell, W., & Schwarcz, R. (1989). Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system. *Neuroscience Letters, 97*(3), 271–275.

Zhao, Y., Navia, B., Marra, C., Singer, E., Chang, L., Berger, J., Ellis, R., Kolson, D., Simpson, D., Miller, E., Lipton, S., Evans, S., Schifitto, G., & The Adult AIDS Clinical Trial Group (ACTC) 301 Team. (2010). Memantine for AIDS dementia complex: Open label report of ACTG 301. *HIV Clinical Trials, 11*(1), 59–67.

Excitotoxicity in the Pathogenesis of Autism

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Contents

Abstract

Autism is a neurodevelopmental disorder characterized by stereotyped interests and behaviors and abnormalities in verbal and nonverbal communication. Autism is reported as a multifactorial disorder resulting from interactions between genetic, environmental, and immunological factors. Excitotoxicity and oxidative stress are potential mechanisms, which are likely to serve as a converging point to these risk factors. Numerous studies suggest that excitotoxicity is a likely cause of neuronal dysfunction in autistic patients. Glutamate is the main excitatory neurotransmitter generated in the CNS, and

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overactivation of glutamate receptors triggers excitotoxicity. Hyperactivation of glutamatergic receptors, NMDA and AMPA, leads to activation of enzymes, which damage cellular structure, membrane permeability, and electrochemical gradients. The role of excitotoxicity in autistic subjects is summarized in this chapter.

Keywords
Autism • Excitotoxicity • Free radicals • Glutamatergic receptors • Ion channel • Membrane potential • Neurotransmitter

1 Introduction

Autism is a neurodevelopmental disorder associated with several cognitive manifestations including repetitive behaviors, social withdrawal, and abnormalities in verbal and nonverbal communications (Gillberg and Coleman 2000). Baron-Cohen et al. (2009) showed that autism affects 1–2 in 100 children, according to recent data on the broad array of autism spectrum disorders (ASD) (Baron-Cohen et al. 2009).

Excitotoxicity is an important pathological event mediated by neuronal excitation through overstimulation of neurons by excitatory amino acids receptors of glutamate and aspartate (Olney 1969). The pathological hallmarks of excitotoxicity include neuronal swelling, vacuolization, and neuronal dystrophy (Farooqui and Horrocks 1994). Under physiological conditions, excitatory receptors induce the influx of sodium, calcium, and potassium, which results in neuronal excitation (Lan et al. 2001). Excess levels of glutamate and other excitatory molecules result in overexcitation of the ionotropic glutamatergic receptors *N*-methyl-D-aspartate (NMDA) and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate (AMPA) and the release of other excitotoxins followed by excessive calcium levels in cytosol. The influx of intracellular calcium triggers the induction of inducible nitric oxide (iNOS) and phosphorylation of protein kinase C. Increased iNOS enhances nitric oxide (NO•) production in excess, whereas protein kinase C activates phospholipase A2 which in turn results in the generation of pro-inflammatory molecules (Babu et al. 1994; Banion 1999). The subsequent generation of free radicals can inhibit oxidative phosphorylation and damage mitochondrial enzymes involved in the electron transport chain, which mitigate energy production (Eliasson et al. 1999).

Seizures represent important pathological event associated with excitotoxicity in autistic children. Experimental studies substantiated the relationship between seizure formation and excitotoxicity (Rogawski 1995). Elevation of glutamate levels in brain is the primary cause of seizure formation (Johnston 1995; Blaylock 2003).

2 Evidence for Excitotoxicity in Autistic Patients

Biochemical analysis has shown elevated serum level of glutamate in autistic patients compared to controls (Shinohe et al. 2006). Abnormalities in immune

system regulation have also been reported in autistic patients (Cohly and Panja 2005). Higher levels of tumor necrosis factor-α (TNF-α) receptor II have been observed in the sera of children with autism spectrum disorders (Chez et al. 2007). Microglial activation can further enhance the release of TNF-α, which increases reactive oxygen and nitrogen species and inhibits the reuptake of glutamate (Hu et al. 2001).

Increased glutamate may also occur as a result of interaction between pathogens with bacterial components, viruses, and lipopolysaccharides (Fontana et al. 1996). Impaired immunity promotes viral persistence in host tissues for a greater period of time and stimulates the release of glutamate and other excitotoxins, leading to increased production of reactive oxygen species, and energy depletion in neurons (Espey et al. 1998). Peroxynitrates and other peroxidation products reduce the function of mitochondrial enzymes by impairing oxidative phosphorylation and inhibiting complex II of the electron transport chain (Fosslier 2001; Novelli et al. 1988). Moreover, lipid peroxidation products can interact with synaptic protein and impair transport of glucose and glutamate to reduce energy production and increase vulnerability to excitotoxicity (Henneberry 1989).

The formation of seizures is associated with excitotoxicity and the accumulation of glutamate (Rogawski 1995). The infant brain is more vulnerable to excitotoxic effects since the developing brain contains more synaptic glutamate receptors than at birth, and that number slowly declines during the ageing process (Johnston 1995). There is substantial evidence that excitotoxicity is responsible for the mirror focus reported in temporal lobe seizures and cognitive decline associated with epilepsy (Ekonomou and Angelatou 1999; Olney et al. 1986). Pathological changes have been observed in the hippocampus after prolonged seizures in several cases of autism. These changes include the progressive loss of hippocampal neurons in the CA1 and CA3, as well as dendritic swelling in the hilus of the fascia dentate (Ekonomou and Angelatou 1999; Olney et al. 1986).

Excitotoxic processes in the brain can also be observed using magnetic resonance imaging (MRI), usually with cortical and subcortical atrophy and progressive ventricular dilation (Baxter et al. 1996; Gospe and Hecht 1998). A magnetic resonance spectroscopy study by Page et al. (2006) demonstrated that individuals with ASD have significantly higher concentrations of glutamate in the amygdale-hippocampal region than healthy age-matched controls (Page et al. 2006). Another study demonstrated increased NMDA receptor content in several cases of temporal lobe epilepsy, suggestive of dentate granule cell hyperexcitability (Mathern et al. 1999). Postmortem studies have suggested that dysfunction in the glutamatergic system in the cerebellum may also be involved in the pathophysiology of autism. Interestingly, a recent study found that the anatomic substrate of the limbic system is considerably smaller in autistic children compared to controls (Saitoh et al. 2001).

Additionally, Zilbovicius et al. (2000) used positron emission tomography to visualize neuronal damage in 75 % of autistic children. These changes were localized to the auditory association area and temporal cortex, similar to the areas of hypoperfusion reported in a majority of cases with infantile spasms

(Zilbovicius et al. 2000). As well, experimental lesions in the temporal lobe of nonhuman primates closely resemble temporal lesions observed in autistic children (Bachevalier 1994).

3 Conclusion

Autism is a multifactorial neurodevelopmental disorder characterized by deficits in neurobehavioral and neurological dysfunction. Excitotoxicity is triggered by potential mutation in ion channels and signalling pathways, viral and bacterial pathogens, toxic metals, and free radical generation. Overexpression of glutamate receptors and increased glutamate levels lead to increased calcium influx and oxidative stress and progressive cellular degeneration and cell death. Genetic defect, such as mutation in voltage gated or ligand channels that regulate neuronal excitability, leads to defect in synaptic transmission and excitotoxic condition in autism.

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References

Babu, G. N., Bawari, M., & Ali, M. M. (1994). Lipid peroxidation potential and antioxidant status of circumventricular organs of rat brain following neonatal monosodium glutamate. *Neurotoxicology, 15*, 773–777.

Bachevalier, J. (1994). Medical temporal lobe structures and autism. A review of clinical and experimental findings. *Neuropsychologia, 32*(6), 627–648.

Baxter, P., Griffiths, P., Kelly, T., & Gardner-Medwin, D. (1996). Pyridoxine dependent seizuer; demographic clinical, MRI and psychometric feature, and affect of dose on intelligent quotient. *Dev. Med. Chil Neurol. 38*(11), 998–1006.

Baron-Cohen, S., Scott, F. J., Allison, C., Williams, J., Bolton, P., Matthews, F. E., & Brayne, C. (2009). Prevalence of autism-spectrum conditions: UK school-based population study. *The British Journal of Psychiatry, 194*, 500–509.

Blaylock, R. L. (2003). The central role of excitotoxicity in autism spectrum disorders. *The Journal of the American Nutraceutical Association, 6*, 7–19.

Chez, M. G., Burton, Q., Dowling, T., Chang, M., Khanna, P., & Kramer, C. (2007). Memantine as adjunctive therapy in children diagnosed with autistic spectrum disorders: An observation of initial clinical response and maintenance tolerability. *Journal of Child Neurology, 22*, 574–579.

Cohly, H. H., & Panja, A. (2005). Immunological findings in autism. *International Review of Neurobiology, 71*, 317–341.

Ekonomou, A., & Angelatou, F. (1999). Upregulation of NMDA receptors in hippocampus and cortex in the pentylenetetrazol-induced "kindling" model of epilepsy. *Neurochemical Research, 24*, 1515–1522.

Eliasson, M. J., Huang, Z., & Ferrante, R. J. (1999). Neuronal nitric oxide synthase activation and peroxynitrite formation in ischemic stroke linked to neural damage. *The Journal of Neuroscience, 19*, 5910–5918.

Espey, M. G., Kustova, Y., Sei, Y., & Basile, A. S. (1998). Extracellular glutamate levels are chronically elevated in the brains of LPBM5-infected mice: A mechanism of retrovirus-induced encephalopathy. *Journal of Neurochemistry, 71*, 2079–2087.

Farooqui, A. A., & Horrocks, L. A. (1994). Excitotoxicity and neurological disorders: Involvement of membrane phospholipids. *International Review of Neurobiology, 36*, 267–323.

Fontana, A., Constam, D., Frei, K., et al. (1996). Cytokines and defense against CNS infection. In R. M. Ransohoff & E. N. Beneviste (Eds.), *Cytokines and the CNS*. Boca Raton: CRC Press. ISBN ISBN-10: 0849324521.

Fosslier, E. (2001). Mitochondrial medicine-molecular pathology of defective oxidative phosphorylation. *Annals of Clinical and Laboratory Science, 31*, 25–67.

Gillberg, C., & Coleman, M. (2000). *The biology of autistic syndromes* (3rd ed.). London: Mac Keith (distributed by Cambridge University Press), New York: Mac Keith Press.

Gospe, S. M., & Hecht, S. T. (1998). Longitudinal MRI findings in pyridoxine dependent seizures. *Neurology, 51*, 74–78.

Henneberry, R. C. (1989). The role of neuronal energy in neurotoxicity of excitatory amino acids. *Neurobiology of Aging, 10*, 611–613.

Hu, H., Shao, L. R., Chavoshy, S., Gu, N., Trieb, M., Behrens, R., Laake, P., Pongs, O., Knaus, H. G., Ottersen, O. P. et al. (2001). Glutamatergic hippocampul terminals and their role in spike repolarization and regulation of transmitter nllase. *J. Neurosci*., 15:21(24), 9585–9597.

Johnston, M. V. (1995). Neurotransmitters and vulnerability of the developing brain. *Brain & Development, 17*, 301–306.

Lan, J. Y., Skeberdis, V. A., Jover, T., Grooms, S. Y., Lin, Y., Araneda, R. C., et al. (2001). Protein kinase C modulates NMDA receptor trafficking and gating. *Nature Neuroscience, 4*, 382–390.

Mathern, G. W., Pretorius, J. K., Mendoza, D., et al. (1999). Hippocampal N-methyl-D-aspartate receptor subunit mRNA levels in temporal lobe epilepsy patients. *Annals of Neurology, 46*, 343–358.

Novelli, A., Reilly, J. A., Lysko, P. G., & Henneberry, R. C. (1988). Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Research, 451*, 205–212.

O'Banion, M. K. (1999). Cyclooxygenase-2: Molecular biology, pharmacology, and neurobiology. *Critical Reviews in Neurobiology, 13*, 45–82.

Olney, J. W. (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science, 165*, 719–721.

Olney, J. W., Collias, R. C., & Sloviter, R. S. (1986). Excitotoxic mechanism of epileptic brain damage. *Advances in Neurology, 44*, 857–877.

Page, L. A., Daly, E., Schmitz, N., Simmons, A., Toal, F., Deeley, Q., Ambery, F., McAlonan, G. M., Murphy, K. C., & Murphy, D. G. M. (2006). In Vivo ^{1}H-magnetic resonance spectroscopy study of Amygdala-Hippocampal and Parietal regions in autism. *The American Journal of Psychiatry, 12*, 2189–2192.

Rogawski, M. A. (1995). Excitatory amino acids and seizures. In T. W. Stone (Ed.), *CNS neurotransmitters and neuromodulators: Glutamate* (pp. 219–237). Boca Raton: CRC Press.

Saitoh, O., Karns, C. M., & Courchesne, E. (2001). Development of the hippocampal formation from 2 to 42 years: MRI evidence of smaller area dentata in autism. *Brain, 124*, 1317–1324.

Shinohe, A., Hashimoto, K., Nakamura, K., et al. (2006). Increased serum levels of glutamate in adult patients with autism. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 30*, 1472–1477.

Zilbovicius, M., Boddaert, N., Belin, P., et al. (2000). Temporal lobe dysfunction in childhood autism: A PET study. *The American Journal of Psychiatry, 157*, 1988–1993.

Glutamate and Neurodegeneration in the Retina

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Contents

Abstract

L-Glutamate is a neurotransmitter throughout the various levels of retinal neurons (photoreceptors, bipolar cells, ganglion cells) and acts at a range of ionotropic (iGlu) and metabotropic glutamate (mGlu) receptors to exert its physiological roles. A function unique to the retina can be ascribed to mGlu6 receptors, which are expressed only by ON bipolar cells at the sign-inverting synapse formed by photoreceptors. More conventional excitatory sign-conserving synapses utilizing iGlu receptors are formed at other locations in the retina. Over-activation of iGlu receptors (and especially NMDA receptors) is known to lead to excitotoxicity in the retina, as in other areas of the nervous system, and this may be associated with excessive neuronal Ca^{2+} influx. This excitotoxicity can be prevented or countered by iGlu receptor antagonists, both in vitro and in vivo. Furthermore, in some experimental models of retinal neurodegeneration, e.g., for glaucoma or ischemia, a number of different types of iGluR antagonists have been shown to be effective in a variety of species. In particular, the uncompetitive NMDA antagonist memantine has shown promise

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in experimental studies, but clinical trials to treat glaucoma with this drug have not had a successful outcome, possibly due to the unavailability of suitable markers of disease progression and heterogeneity of the disease in humans. It is thus important that further work is carried out so as to translate preclinical findings on neurodegeneration into successful clinical treatments.

Keywords
AMPA receptor • Glaucoma • mGlu receptor • NMDA receptor • Retina

List of Abbreviations

AMPA	(S)-α-amino-3-hydroxy-5-methyl-4-iso-xazolepropionic acid
EAAT	Excitatory amino acid transporter
L-AP4	(APB) L-2-amino-4-phosphonobutyric acid
mGlu	Metabotropic glutamate
NMDA	*N*-methyl-D-aspartate
RGC	Retinal ganglion cell

1 Introduction

The excitatory amino acid L-glutamate (henceforth referred to as "glutamate") has been known to have toxic effects in the eye and the retina for over five decades, since the early work of Lucas and Newhouse (Lucas and Newhouse 1957) and Kobayashi (Kobayashi 1967), working in mice and rabbits. Since then the role of glutamate as an excitatory neurotransmitter in the central nervous system and retina has been well established, based on the pioneering work of Curtis and Watkins (Curtis and Watkins 1963) and the subsequent development of a pharmacological receptor scheme for the excitatory amino acids (Watkins and Evans 1981; Lodge 2009; Traynelis et al. 2010).

As with several other neurotransmitter receptor systems, the glutamate receptors can be divided into two families: the ionotropic receptors (Traynelis et al. 2010) and the metabotropic receptors (Niswender and Conn 2010). The ionotropic glutamate receptors, commonly referred to as NMDA receptors (NR1, NR2A-D, NR3A, NR3B), AMPA receptors (GluR1-4), and kainate receptors (GluR5, GluR6, KA1, KA2), are tetra-heteromeric ligand-gated cation channels (Lodge 2009; Traynelis et al. 2010) that can have a wide variety of characteristics depending on their subunit composition. In addition, *delta* receptors have been described that appear to form homomeric receptors that are not activated by any known agonist (Traynelis et al. 2010). A standard IUPHAR-approved naming convention has been developed that supersedes the common nomenclature (Table 1) (Traynelis et al. 2010). Eight metabotropic glutamate (mGlu) receptor subtypes (mGlu1-mGlu8) have been characterized to date, all of which can be found in the retina, with mGlu6 having a particularly unique role in the visual ON pathway (Morgans et al. 2010) (see below). The mGlu receptors can be placed into three Groups (I, II, III) on the basis

Table 1 Ionotropic glutamate receptor subunits

			Example antagonists	
Receptor	Common subunit names	IUPHAR subunit names	Competitive	Uncompetitive
AMPA	GluR1, GluRA	GluA1	CNQX	
	GluR2, GluRB	GluA2	DNQX	GYKI-52466
	GluR3, GluRC	GluA3	NBQX	GYKI-53655
	GluR4, GluRD	GluA4	LY293558	
Kainate	GluR5	GluK1	CNQX	GYKI-53655
	GluR6	GluK2	DNQX	
			NBQX	
	GluR7	GluK3	LY382884	
	KA1	GluK4		
	KA2	GluK5		
NMDA	NMDAR1, NR1	GluN1		Mg^{2+}
	NMDAR2A, NR2A	GluN2A	D-AP5	MK-801
	NMDAR2B, NR2B	GluN2B	R-CPP	Phencyclidine
	NMDAR2C, NR2C	GluN2C		Ketamine
	NMDAR2D, NR2D	GluN2D		Memantine
	NR3A	GluN3A		
	NR3B	GluN3B		
delta	δ1, GluR delta-1	GluD1		
	δ2, GluR delta-2	GluD2		

Abbreviations: *AMPA* α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, *CNQX* 6-cyano-7-nitroquinoxaline-2,3-dione, *D-AP5* D-(−)-2-amino-5-phosphonopentanoic acid, *DNQX* 6,7-dinitroquinoxaline-2,3-dione, *NBQX* 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f] quinoxaline-7-sulfonamide, *NMDA* *N*-methyl-D-aspartic acid, *R-CPP* 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid

of their sequence homology, their pharmacological characteristics, and the types of intracellular transduction cascade to which they may couple in in vitro expression systems (Niswender and Conn 2010). Group I (mGlu1, mGlu5) receptors typically couple to postsynaptic inositol phosphate metabolism, while Group II (mGlu2, mGlu3) and Group III (mGlu4, mGlu6-8) receptors may couple to an inhibitory cyclic-AMP cascade (Table 2). However, it is becoming evident that these receptors may also couple to alternative signalling pathways in a context-dependent manner and this potentially adds complexity to the possible outcomes of mGlu receptor activation (Niswender and Conn 2010).

2 Glutamate Receptors in the Retinal Circuitry

Glutamate is found throughout the stages of retinal circuitry, from the photoreceptors which release glutamate tonically in the dark to the retinal ganglion cells (RGCs) that use glutamate as their major transmitter in the retinal output to the brain (Wassle 2004; Lukasiewicz 2009; Slaughter 2010). Photoreceptors release glutamate onto

Table 2 Metabotropic glutamate receptor groups and subtypes

			Orthosteric agonists		Orthosteric antagonists		Allosteric modulators	
Group	Receptor	Transduction mechanism	Subtype selective	Group selective	Subtype selective	Group selective	Positive	Negative
I	mGlu1	IP3/Ca^{2+} cascade		DHPG	LY367385	4CPG	Ro67–4853	CPCCOEt JNJ16259685
	mGlu5		CHPG				CDDPB	MTEP, MPEP
II	mGlu2	Inhibitory	LY395756	LY354740		LY341495	BINA	Ro64-5229
	mGlu3	cAMP		LY379268	LY395756		LY487379	
		cascade		APDC				
III	mGlu4	Inhibitory	LSP4-2022	L-AP4		MAP4	VU0361737	
	mGlu6	cAMP		L-SOP		MPPG		
	mGlu7	cascade				CPPG		MMPIP, ADX71743
	mGlu8		DCPG				AZ12216052	

Abbreviations: *APDC* 2R,4R-4- aminopyrrolidine-2,4-dicarboxylate, *BINA* 3′-[[(2-cyclopentyl-2,3-dihydro-6,7-dimethyl-1-oxo-1H-inden-5-yl)oxy]methyl]-[1,1′-biphenyl]-4-carboxylic acid, *CDDPB* 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide, *CHPG* 2-chloro-5-hydroxyphenylglycine, *CPCOOEt* 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate, *4CPG* (S)-4-carboxyphenylglycine, *CPPG alpha*-cyclopropyl-4-phosphonophenylglycine, *DCPG* (S)-3,4-dicarboxyphenylglycine, *DHPG* 3,5-dihydroxyphenylglycine, L-*AP4* L-2-amino-4-phosphonobutyric acid, *L-SOP* L-serine-*O*-phosphate, *MAP4* (S)-2-amino-2-methyl-4-phosphonobutanoic acid, *MMPIP* 6-(4-methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-c]pyridin-4(5H)-one, *MPEP* 2-methyl-6-(phenylethynyl)-pyridine, *MPPG* (RS)-α-methyl-4-phosphonophenylglycine, *MTEP* 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine.

either ON or OFF bipolar cells, and it is at this point in the retinal circuitry that opposite polarity light responses are first generated from the "OFF" response of the photoreceptors which reduce their glutamate release upon light stimulation. The ON bipolar cells express mGlu6 receptors in a highly specific manner (indeed this is almost the only location in the nervous system where this receptor is expressed to any significant extent (Nakajima et al. 1993)), and it has been shown that mGlu6 is an essential component of the retinal ON pathway (Nakajima et al. 1993; Morgans et al. 2010). In the dark, constant release of glutamate from photoreceptors activates mGlu6 receptors, and conversely light stimulation leads to a reduction of mGlu6 receptor activation due to a drop in glutamate release onto bipolar cells. This light-induced deactivation of the mGlu6 signal transduction pathway results in the opening of a nonselective cation channel that is now known to be TRPM1 (Morgans et al. 2010), and this generates the ON response of the ON bipolar cells, representing a sign inversion of the synaptic input from the photoreceptors. mGlu6 receptors can be activated by the synthetic glutamate analogue L-2-amino-4-phosphonobutyric acid (L-AP4, formerly known as APB), and application of this agonist to the retina functionally blocks the ON pathway as it achieves constant activation of mGlu6 receptors, mimicking the constant release of glutamate in darkness (Slaughter 2010). By contrast, OFF bipolar cells express AMPA and kainate receptors rather than mGlu6 receptors and this results in a synaptic OFF response that follows the polarity of the photoreceptor response (Lukasiewicz 2009; Slaughter 2010).

Retinal bipolar cells are predominantly glutamatergic, although glycinergic bipolar cells have also been described (Wassle 2004). NMDA receptors appear in the retinal circuitry postsynaptic to ON bipolar cells and OFF bipolar cells on RGCs and amacrine cells where they participate in both ON and OFF signalling pathways together with AMPA and kainate receptors (Lukasiewicz 2009; Slaughter 2010). There is spatial segregation of ON bipolar cell synapses and OFF bipolar cell synapses onto ON and OFF RGCs, respectively, and NMDA receptors appear to be localized primarily peri-synaptically at ON synapses, whereas they have a more subsynaptic location at ON synapses. Furthermore, it appears that different NMDA receptor subunit types are found at these locations, with NR2B-containing receptors more prevalent at ON synapses and NR2A-containing receptors more prevalent at OFF synapses of RGCs (Fletcher et al. 2000; Zhang and Diamond 2009). In addition to these complexities, the amino acids glycine and D-serine act as co-agonists at NMDA receptors, particularly those containing the NR2B subunit (Traynelis et al. 2010), and it is possible that these co-agonists have a specific role in ON pathway signalling (Gustafson et al. 2007; Kalbaugh et al. 2009).

In addition to the highly specific role of mGlu6 receptors in the ON pathway (see above), the other mGlu receptors are also found in the retina, but specific functional roles have not been so readily ascribed (Koulen et al. 1996, 1997; Dyka et al. 2004; Quraishi et al. 2007). However, a role for mGlu8 as a presynaptic receptor located on photoreceptors and bipolar cells is beginning to emerge, and it appears that at both of these locations, it may function as an autoreceptor to regulate the release of glutamate from photoreceptors and from bipolar cells (Koulen and Brandstatter 2002; Quraishi et al. 2010).

3 Glutamate Receptors in Neurodegenerative Mechanisms

Since the initial observations on retinotoxic effects of glutamate by Lucas and Newhouse (1957), there have been a vast number of studies in many species that have investigated the neurotoxic effects of glutamate and glutamate receptor agonists and neuroprotective effects of antagonists and modulators throughout the nervous system, and these have been extensively reviewed (Choi 1988; Danysz and Parsons 1998; Doble 1999; Waxman and Lynch 2005; Casson 2006; Luo et al. 2011). There has been a substantial focus on NMDA receptors, partly due to the early pharmacological characterization of NMDA receptors (Watkins and Evans 1981) but also due to the observation that neurotoxicity was caused by large amount of Ca^{2+} influx induced by over-activation of NMDA receptors, a mechanism that was termed *excitotoxicity* (Choi 1988; Luo et al. 2011). More recently, it has become evident that the subunit composition of NMDA receptors is also critical in how they may take part in excitotoxic processes, and in particular, incorporation of NR3 subunits may have a neuroprotective effect (Nakanishi et al. 2009; Low and Wee 2010; Luo et al. 2011).

It should also be noted that activation of kainate or AMPA receptors can also lead to excitotoxicity (Choi 1992). In particular, the presence of GluR2 in AMPA receptors results in low Ca^{2+} permeability in most situations, due to RNA editing of the GluR2 subunit at the so-called Q/R site. Thus, GluR2 content and Q/R editing is critical in controlling the Ca^{2+} permeability of the AMPA receptor, and it has been suggested that increased proportion of unedited GluR2 and reduction in the total amount of overall GluR2 in AMPA receptors makes neurons more susceptible to AMPA-receptor-mediated excitotoxicity (Kim et al. 2001; Lebrun-Julien et al. 2009).

4 Glutamate Transporters in Neurodegenerative Mechanisms

It has become evident that, in addition to glutamate receptors, the uptake of glutamate by neurons and glia via specific transporters (Excitatory Amino Acid Transporters, or EAATs) is a key component of synaptic transmission and excitotoxic processes, maintaining a physiological extracellular glutamate level and thus regulating the balance between signalling and pathology (Shigeri et al. 2004; Beart and O'Shea 2007; Bringmann et al. 2009; Furuya et al. 2012). In addition, it has also been suggested that EAATs provide glutathione precursors such as glutamate and cysteine to cells and that this is crucial to prevent oxidative stress and cell death in neurons (Harada et al. 2007; Had-Aissouni 2012). There are five major subtypes of EAAT that have been termed EAAT1-5 in humans with homologues in other mammals and vertebrates (Beart and O'Shea 2007). Within the retina, EAAT1 (also known as GLAST) is localized in Müller cells (the major retinal glial cells) and astrocytes (Rauen et al. 1997). EAAT2 (also known as GLT-1) is mainly localized in cone photoreceptors, cone bipolar cells, RGCs, and Müller

cells (Rauen et al. 1997; Furuya et al. 2012). EAAT3 (also known as EAAC1) is found on Müller cells and neurons including RGCs (Kugler and Beyer 2003; Furuya et al. 2012). EAAT4 has been found in photoreceptors and astrocytes albeit at lower levels than in the brain (Ward et al. 2004; Pignataro et al. 2005). EAAT5 is present in rod photoreceptors and bipolar cells of the retina (Fyk-Kolodziej et al. 2004; Lee et al. 2012), where it may serve to regulate glutamate levels and also modulate a chloride conductance in bipolar cells (Wersinger et al. 2006).

5 Glutamate Excitotoxicity in Retinal Diseases?

The death of RGCs and other retinal neurons upon injection of glutamate agonists has been reported in numerous studies conducted in many vertebrate species (Baltmr et al. 2010; Almasieh et al. 2012). Indeed use of NMDA to lesion RGCs is often used as a model of retinal diseases such as glaucoma (Baltmr et al. 2010; Almasieh et al. 2012). The question thus arises as to whether these excitotoxic mechanisms can be related to retinal disease processes that may occur in, for example, glaucoma or retinal ischemia. There are several strands of evidence to suggest that this may be the case. The most compelling of these is that in in vivo experimental models of glaucoma or ischemia produced by a variety of different means in different species from rodent to nonhuman primate, a range of NMDA receptor antagonists (administered locally or systemically), including MK-801 (Chaudhary et al. 1998; Schuettauf et al. 2000; Guo et al. 2006), memantine (Kim et al. 2002; WoldeMussie et al. 2002; Hare et al. 2004), ifenprodil (Guo et al. 2006), and eliprodil (Kapin et al. 1999), have proven to be effective. Similarly AMPA/kainate antagonists have also been shown to be effective (Schuettauf et al. 2000). Furthermore, genetic deletion of EAATs in mice produces an RGC pathology that bears similarities to glaucoma (Harada et al. 2007).

It has also been reported that glutamate levels are elevated in the vitreous of the eye in glaucoma (Dreyer et al. 1996; Brooks et al. 1997), although these findings have been called into question (Dalton 2001), and contradictory results have been published (Carter-Dawson et al. 2002; Kwon et al. 2005). Although superficially it might be attractive to think that glutamate could be elevated in the vitreous in glaucoma, it would seem that this would be unlikely given the prevalence of uptake and compartmentalization of glutamate in neural tissue (Meldrum 2000; Salt and Cordeiro 2006; Almasieh et al. 2012). Thus, a lack of detectable glutamate increase in the vitreous of the eye should not be taken to indicate that glutamate is not taking part in neurodegenerative processes (Salt and Cordeiro 2006; Osborne 2009).

Memantine is an uncompetitive (open channel blocker) NMDA antagonist with fast kinetics that is in clinical use for neurodegenerative conditions such as Alzheimer's disease (AD) in some countries (Lipton 1993; Parsons et al. 1999; Lipton 2004). This tolerability and apparent efficacy has made it an attractive candidate for retinal neuroprotection in glaucoma (Greenfield et al. 2005; Casson 2006). However, although preclinical data in experimental glaucoma provided positive data for memantine (Kim et al. 2002; WoldeMussie et al. 2002;

Hare et al. 2004; Yucel et al. 2006), positive data from clinical trials have not been forthcoming (Allergan Inc 2008; Osborne 2009). Although disappointing, this result could be due to a number of factors. For example, in addition to NMDA receptors, other factors are likely to contribute to glaucoma (Weinreb and Khaw 2004; Osborne 2009; Almasieh et al. 2012), and blockade of NMDA receptors may not be appropriate or sufficient in every case. Furthermore, it is possible, if not likely, that the end points and methods for monitoring disease progression and benefit of therapy were not appropriate (Osborne 2009). In this respect, recent advances in imaging of different phases of retinal neurodegeneration may be of benefit in any future clinical trials (Cordeiro et al. 2010).

6 Neuroprotection via Activation of Receptors

All of the known mGlu receptors have been found in the retina (Hartveit et al. 1995; Koulen et al. 1996, 1997; Dyka et al. 2004; Quraishi et al. 2007). It is known that activation of the Group II or the Group III mGlu receptors can be neuroprotective in some experimental situations (Copani et al. 1995; Bruno et al. 2001). In particular, systemic application of Group II-selective agonists is neuroprotective in whole-brain ischemia models (Bond et al. 1998); in the retina, the Group II agonist LY354740 has been shown to be neuroprotective against RGC apoptosis in a rat glaucoma model (Guo et al. 2006) and against anoxic damage in the goldfish (Beraudi et al. 2007). The mechanism of action remains to be elucidated, but it is known from work in cortical cells that activation of mGlu3 receptors on astrocytes rather than mGlu2 receptors on neurons is needed for protection against NMDA-induced excitotoxicity (Corti et al. 2007), and it may be that this stimulates production of certain growth factors (e.g., GDNF, TGF-*beta*) (Battaglia et al. 2009; Caraci et al. 2009) which may be important in preventing apoptotic cell death (Almasieh et al. 2012). Although activation of Group III receptors has been suggested to be neuroprotective in the brain (Copani et al. 1995; Bruno et al. 2001; Ngomba et al. 2011), it is unclear whether this would be appropriate in the retina given the importance of the closely related mGlu6 (and mGlu8) receptor in visual processing (see above). In this respect it is noteworthy that mutations of the mGlu6 receptor encoding gene, GRM6, cause visual deficits including night blindness that are attributable to dysfunction of the ON pathway (Dryja et al. 2005; Zeitz et al. 2005, 2007). Thus, Group III mGlu receptors may not be suitable targets for retinal neuroprotection.

7 Conclusion

Glutamate receptors play a pivotal role in the normal signalling functions of the retina, as they do elsewhere in the nervous system, with a notable specialization in the function of mGlu6 in the ON pathway. Over-activation of the ionotropic receptors causes excitotoxic neuronal death, and this may be of relevance in the

pathophysiology of retinal diseases such as glaucoma. Neuroprotective strategies aimed at blocking these processes may be useful, but further work is required to translate experimental results to clinical usage. It may be the case that different mechanisms or combinations of mechanisms are applicable in different diseases/patients, or even at different stages of the same disease. Thus, neuroprotective strategies might need to be tailored to individual patients with appropriate monitoring of disease progression, and this should be attainable in the future (Cordeiro et al. 2010).

References

Allergan Inc. (2008). Press release on Memantine Trials, Fourth Quarter Operating Results, Jan 30 2008. Press Release, http://agn.client.shareholder.com/releasedetail.cfm?ReleaseID=290764

Almasieh, M., Wilson, A. M., Morquette, B., Cueva Vargas, J. L., & Di Polo, A. (2012). The molecular basis of retinal ganglion cell death in glaucoma. *Progress in Retinal and Eye Research, 31*, 152–181.

Baltmr, A., Duggan, J., Nizari, S., Salt, T. E., & Cordeiro, M. F. (2010). Neuroprotection in glaucoma – Is there a future role? *Experimental Eye Research, 91*, 554–566.

Battaglia, G., Molinaro, G., Riozzi, B., Storto, M., Busceti, C. L., Spinsanti, P., Bucci, D., Di Liberto, V., Mudo, G., Corti, C., Corsi, M., Nicoletti, F., Belluardo, N., & Bruno, V. (2009). Activation of mGlu3 receptors stimulates the production of GDNF in striatal neurons. *PloS One, 4*, e6591.

Beart, P. M., & O'Shea, R. D. (2007). Transporters for L-glutamate: An update on their molecular pharmacology and pathological involvement. *British Journal of Pharmacology, 150*, 5–17.

Beraudi, A., Bruno, V., Battaglia, G., Biagioni, F., Rampello, L., Nicoletti, F., & Poli, A. (2007). Pharmacological activation of mGlu2/3 metabotropic glutamate receptors protects retinal neurons against anoxic damage in the goldfish Carassius auratus. *Experimental Eye Research, 84*, 544–552.

Bond, A., O'Neill, M. J., Hicks, C. A., Monn, J. A., & Lodge, D. (1998). Neuroprotective effects of a systemically active group II metabotropic glutamate receptor agonist LY354740 in a gerbil model of global ischaemia. *Neuroreport, 9*, 1191–1193.

Bringmann, A., Pannicke, T., Biedermann, B., Francke, M., Iandiev, I., Grosche, J., Wiedemann, P., Albrecht, J., & Reichenbach, A. (2009). Role of retinal glial cells in neurotransmitter uptake and metabolism. *Neurochemistry International, 54*, 143–160.

Brooks, D. E., Garcia, G. A., Dreyer, E. B., Zurakowski, D., & Franco-Bourland, R. E. (1997). Vitreous body glutamate concentration in dogs with glaucoma. *American Journal of Veterinary Research, 58*, 864–867.

Bruno, V., Battaglia, G., Copani, A., D'Onofrio, M., Di Iorio, P., De Blasi, A., Melchiorri, D., Flor, P. J., & Nicoletti, F. (2001). Metabotropic glutamate receptor subtypes as targets for neuroprotective drugs. *Journal of Cerebral Blood Flow and Metabolism, 21*, 1013–1033.

Caraci, F., Battaglia, G., Bruno, V., Bosco, P., Carbonaro, V., Giuffrida, M. L., Drago, F., Sortino, M. A., Nicoletti, F., & Copani, A. (2009). TGF-beta1 pathway as a new target for neuroprotection in Alzheimer's disease. *CNS Neuroscience and Therapeutics*, *17*(4), 237–249.

Carter-Dawson, L., Crawford, M. L., Harwerth, R. S., Smith, E. L., 3rd, Feldman, R., Shen, F. F., Mitchell, C. K., & Whitetree, A. (2002). Vitreal glutamate concentration in monkeys with experimental glaucoma. *Investigative Ophthalmology & Visual Science, 43*, 2633–2637.

Casson, R. J. (2006). Possible role of excitotoxicity in the pathogenesis of glaucoma. *Clinical & Experimental Ophthalmology, 34*, 54–63.

Chaudhary, P., Ahmed, F., & Sharma, S. C. (1998). MK801-a neuroprotectant in rat hypertensive eyes. *Brain Research, 792*, 154–158.

Choi, D. W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron, 1*, 623–634.

Choi, D. W. (1992). Excitotoxic cell death. *Journal of Neurobiology, 23*, 1261–1276.

Copani, A., Bruno, V., Battaglia, G., Leanza, G., Pellitteri, R., Russo, A., Stanzani, S., & Nicoletti, F. (1995). Activation of metabotropic glutamate receptors protects cultured neurons against apoptosis induced by beta-amyloid peptide. *Molecular Pharmacology, 47*, 890–897.

Cordeiro, M. F., Guo, L., Coxon, K. M., Duggan, J., Nizari, S., Normando, E. M., Sensi, S. L., Sillito, A. M., Fitzke, F. W., Salt, T. E., & Moss, S. E. (2010). Imaging multiple phases of neurodegeneration:a novel approach to assessing cell death in vivo. *Cell Death and Disease, 1*, e3.

Corti, C., Battaglia, G., Molinaro, G., Riozzi, B., Pittaluga, A., Corsi, M., Mugnaini, M., Nicoletti, F., & Bruno, V. (2007). The use of knock-out mice unravels distinct roles for mGlu2 and mGlu3 metabotropic glutamate receptors in mechanisms of neurodegeneration/neuroprotection. *The Journal of Neuroscience, 27*, 8297–8308.

Curtis, D. R., & Watkins, J. C. (1963). Acidic amino acids with strong excitatory actions on mammalian neurones. *The Journal of Physiology, 166*, 1–14.

Dalton, R. (2001). Private investigations. *Nature, 411*, 129–130.

Danysz, W., & Parsons, C. G. (1998). Glycine and N-Methyl-D-Aspartate receptors: Physiological significance and possible therapeutic applications. *Pharmacological Reviews, 50*, 597–664.

Doble, A. (1999). The role of excitotoxicity in neurodegenerative disease: Implications for therapy. *Pharmacology & Therapeutics, 81*, 163–221.

Dreyer, E. B., Zurakowski, D., Schumer, R. A., Podos, S. M., & Lipton, S. A. (1996). Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. *Archives of Ophthalmology, 114*, 299–305.

Dryja, T. P., McGee, T. L., Berson, E. L., Fishman, G. A., Sandberg, M. A., Alexander, K. R., Derlacki, D. J., & Rajagopalan, A. S. (2005). Night blindness and abnormal cone electroretinogram ON responses in patients with mutations in the GRM6 gene encoding mGluR6. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 4884–4889.

Dyka, F. M., May, C. A., & Enz, R. (2004). Metabotropic glutamate receptors are differentially regulated under elevated intraocular pressure. *Journal of Neurochemistry, 90*, 190–202.

Fletcher, E. L., Hack, I., Brandstatter, J. H., & Wassle, H. (2000). Synaptic localization of NMDA receptor subunits in the rat retina. *The Journal of Comparative Neurology, 420*, 98–112.

Furuya, T., Pan, Z., & Kashiwagi, K. (2012). Role of retinal glial cell glutamate transporters in retinal ganglion cell survival following stimulation of NMDA receptor. *Current Eye Research, 37*, 170–178.

Fyk-Kolodziej, B., Qin, P., Dzhagaryan, A., & Pourcho, R. G. (2004). Differential cellular and subcellular distribution of glutamate transporters in the cat retina. *Visual Neuroscience, 21*, 551–565.

Greenfield, D. S., Girkin, C., & Kwon, Y. H. (2005). Memantine and progressive glaucoma. *Journal of Glaucoma, 14*, 84–86.

Guo, L., Salt, T. E., Maass, A., Luong, V., Moss, S. E., Fitzke, F. W., & Cordeiro, M. F. (2006). Assessment of neuroprotective effects of glutamate modulation on glaucoma-related retinal ganglion cell apoptosis in vivo. *Investigative Ophthalmology & Visual Science, 47*, 626–633.

Gustafson, E. C., Stevens, E. R., Wolosker, H., & Miller, R. F. (2007). Endogenous D-Serine contributes to NMDA-Receptor-Mediated light-evoked responses in the vertebrate retina. *Journal of Neurophysiology, 98*, 122–130.

Had-Aissouni, L. (2012). Toward a new role for plasma membrane sodium-dependent glutamate transporters of astrocytes: Maintenance of antioxidant defenses beyond extracellular glutamate clearance. *Amino Acids, 42*, 181–197.

Harada, T., Harada, C., Nakamura, K., Quah, H.-M. A., Okumura, A., Namekata, K., Saeki, T., Aihara, M., Yoshida, H., Mitani, A., & Tanaka, K. (2007). The potential role of glutamate

transporters in the pathogenesis of normal tension glaucoma. *The Journal of Clinical Investigation, 117*, 1763–1770.

Hare, W. A., WoldeMussie, E., Lai, R. K., Ton, H., Ruiz, G., Chun, T., & Wheeler, L. (2004). Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, I: Functional measures. *Investigative Ophthalmology & Visual Science, 45*, 2625–2639.

Hartveit, E., Brandstatter, J. H., Enz, R., & Wässle, H. (1995). Expression of the mRNA of seven metabotropic glutamate receptors (mGluR1 to 7) in the rat retina. An in situ hybridization study on tissue sections and isolated cells. *The European Journal of Neuroscience, 7*, 1472–1483.

Kalbaugh, T. L., Zhang, J., & Diamond, J. S. (2009). Coagonist release modulates NMDA receptor subtype contributions at synaptic inputs to retinal ganglion cells. *The Journal of Neuroscience, 29*, 1469–1479.

Kapin, M. A., Doshi, R., Scatton, B., DeSantis, L. M., & Chandler, M. L. (1999). Neuroprotective effects of eliprodil in retinal excitotoxicity and ischemia. *Investigative Ophthalmology and Visual Science, 40*, 1177–1182.

Kim, D. Y., Kim, S. H., Choi, H. B., C-k, M., & Gwag, B. J. (2001). High abundance of GluR1 mRNA and reduced Q/R editing of GluR2 mRNA in individual NADPH-Diaphorase neurons. *Molecular and Cellular Neuroscience, 17*, 1025–1033.

Kim, T. W., Kim, D. M., Park, K. H., & Kim, H. (2002). Neuroprotective effect of memantine in a rabbit model of optic nerve ischemia. *Korean Journal of Ophthalmology: KJO, 16*, 1–7.

Kobayashi, K. (1967). Studies on ERG, EOG and histological findings of the rabbit retinae treated with sodium L-glutamate. *Nihon Ganka Gakkai Zasshi, 71*, 1458–1465.

Koulen, P., & Brandstatter, J. H. (2002). Pre- and postsynaptic sites of action of mGluR8a in the mammalian retina. *Investigative Ophthalmology and Visual Science, 43*, 1933–1940.

Koulen, P., Malitschek, B., Kuhn, R., Wassle, H., & Brandstatter, J. H. (1996). Group II and Group III metabotropic glutamate receptors in the rat retina: Distributions and developmental expression patterns. *The European Journal of Neuroscience, 8*, 2177–2187.

Koulen, P., Kuhn, R., Wassle, H., & Brandstatter, J. H. (1997). Group I metabotropic glutamate receptors Mglur1 alpha and Mglur5a: Localization in both synaptic layers of the rat retina. *The Journal of Neuroscience, 17*, 2200–2211.

Kugler, P., & Beyer, A. (2003). Expression of glutamate transporters in human and rat retina and rat optic nerve. *Histochemistry and Cell Biology, 120*, 199–212.

Kwon, Y. H., Rickman, D. W., Baruah, S., Zimmerman, M. B., Kim, C. S., Boldt, H. C., Russell, S. R., & Hayreh, S. S. (2005). Vitreous and retinal amino acid concentrations in experimental central retinal artery occlusion in the primate. *Eye, 19*, 455–463.

Lebrun-Julien, F., Duplan, L., Pernet, V., Osswald, I., Sapieha, P., Bourgeois, P., Dickson, K., Bowie, D., Barker, P. A., & Di Polo, A. (2009). Excitotoxic death of retinal neurons in vivo occurs via a non-cell-autonomous mechanism. *The Journal of Neuroscience, 29*, 5536–5545.

Lee, A., Anderson, A. R., Barnett, N. L., Stevens, M. G., & Pow, D. V. (2012). Alternate splicing and expression of the glutamate transporter EAAT5 in the rat retina. *Gene, 506*, 283–288.

Lipton, S. A. (1993). Prospects for clinically tolerated NMDA antagonists: Open-channel blockers and alternative redox states of nitric oxide. *Trends in Neurosciences, 16*, 527–532.

Lipton, S. A. (2004). Paradigm shift in NMDA receptor antagonist drug development: Molecular mechanism of uncompetitive inhibition by memantine in the treatment of Alzheimer's disease and other neurologic disorders. *Journal of Alzheimer's Disease, 6*, S61–S74.

Lodge, D. (2009). The history of the pharmacology and cloning of ionotropic glutamate receptors and the development of idiosyncratic nomenclature. *Neuropharmacology, 56*, 6–21.

Low, C. M., & Wee, K. S. L. (2010). New Insights into the Not-So-New NR3 Subunits of N-Methyl-D-aspartate Receptor: Localization, structure, and function. *Molecular Pharmacology, 78*, 1–11.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Archives of Ophthalmology, 58*, 193–201.

Lukasiewicz, P. D. (2009). Retinal pharmacology: Inner retinal layers. In R. S. Larry (Ed.), *Encyclopedia of neuroscience* (pp. 255–261). Oxford: Academic Press.

Luo, T., Wu, W. H., & Chen, B. S. (2011). NMDA receptor signaling: Death or survival? *Frontiers in Biology, 6*, 468–476.

Meldrum, B. S. (2000). Glutamate as a neurotransmitter in the brain: Review of physiology and pathology. *The Journal of Nutrition, 130*, 1007S–1015S.

Morgans, C. W., Brown, R. L., & Duvoisin, R. M. (2010). TRPM1: The endpoint of the mGluR6 signal transduction cascade in retinal ON-bipolar cells. *BioEssays, 32*, 609–614.

Nakajima, Y., Iwakabe, H., Akazawa, C., Nawa, H., Shigemoto, R., Mizuno, N., & Nakanishi, S. (1993). Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *The Journal of Biological Chemistry, 268*, 11868–11873.

Nakanishi, N., Tu, S., Shin, Y., Cui, J., Kurokawa, T., Zhang, D., Chen, H. S., Tong, G., & Lipton, S. A. (2009). Neuroprotection by the NR3A subunit of the NMDA receptor. *The Journal of Neuroscience, 29*, 5260–5265.

Ngomba, R. T., Moyanova, S. G., Mastroiacovo, F., Kortenska, L. V., Mitreva, R. G., Fardone, E., Santolini, I., Sobrado, M., Battaglia, G., Bruno, V., & Nicoletti, F. (2011). Protective role for type 4 metabotropic glutamate receptors against ischemic brain damage. *Journal of Cerebral Blood Flow and Metabolism, 31*, 1107–1118.

Niswender, C. M., & Conn, P. J. (2010). Metabotropic glutamate receptors: Physiology, pharmacology, and disease. *Annual Review of Pharmacology, 50*, 295–322.

Osborne, N. N. (2009). Recent clinical findings with memantine should not mean that the idea of neuroprotection in glaucoma is abandoned. *Acta Ophthalmologica, 87*, 450–454.

Parsons, C. G., Danysz, W., & Quack, G. (1999). Memantine is a clinically well tolerated N-methyl–aspartate (NMDA) receptor antagonist–a review of preclinical data. *Neuropharmacology, 38*, 735–767.

Pignataro, L., Sitaramayya, A., Finnemann, S. C., & Sarthy, V. P. (2005). Nonsynaptic localization of the excitatory amino acid transporter 4 in photoreceptors. *Molecular and Cellular Neurosciences, 28*, 440–451.

Quraishi, S., Gayet, J., Morgans, C. W., & Duvoisin, R. M. (2007). Distribution of group-III metabotropic glutamate receptors in the retina. *The Journal of Comparative Neurology, 501*, 931–943.

Quraishi, S., Reed, B. T., Duvoisin, R. M., & Taylor, W. R. (2010). Selective activation of mGluR8 receptors modulates retinal ganglion cell light responses. *Neuroscience, 166*, 935–941.

Rauen, T., Taylor, W. R., Kuhlbrodt, K., & Wiessner, M. (1997). High-affinity glutamate transporters in the rat retina: A major role of the glial glutamate transporter GLAST-1 in transmitter clearance. *Cell and Tissue Research, 291*, 19–31.

Salt, T. E., & Cordeiro, M. F. (2006). Glutamate excitotoxicity in glaucoma: Throwing the baby out with the bathwater? *Eye, 20*, 730–731.

Schuettauf, F., Naskar, R., Vorwerk, C. K., Zurakowski, D., & Dreyer, E. B. (2000). Ganglion cell loss after optic nerve crush mediated through AMPA-kainate and NMDA receptors. *Investigative Ophthalmology and Visual Science, 41*, 4313–4316.

Shigeri, Y., Seal, R. P., & Shimamoto, K. (2004). Molecular pharmacology of glutamate transporters, EAATs and VGLUTs. *Brain Research Reviews, 45*, 250–265.

Slaughter, M. M. (2010). Glutamate receptors in retina. In A. D. Darlene (Ed.), *Encyclopedia of the eye* (pp. 222–226). Oxford: Academic Press.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., & Dingledine, R. (2010). Glutamate receptor ion channels: Structure, regulation, and function. *Pharmacological Reviews, 62*, 405–496.

Ward, M. M., Jobling, A. I., Puthussery, T., Foster, L. E., & Fletcher, E. L. (2004). Localization and expression of the glutamate transporter, excitatory amino acid transporter 4, within astrocytes of the rat retina. *Cell and Tissue Research, 315*, 305–310.

Wassle, H. (2004). Parallel processing in the mammalian retina. *Nature Reviews Neuroscience, 5*, 747–757.

Watkins, J. C., & Evans, R. H. (1981). Excitatory amino acid transmitters. *Annual Review of Pharmacology, 21*, 165–204.

Waxman, E. A., & Lynch, D. R. (2005). N-methyl-D-aspartate receptor subtypes: Multiple roles in excitotoxicity and neurological disease. *The Neuroscientist, 11*, 37–49.

Weinreb, R. N., & Khaw, P. T. (2004). Primary open-angle glaucoma. *Lancet, 363*, 1711–1720.

Wersinger, E., Schwab, Y., Sahel, J. A., Rendon, A., Pow, D. V., Picaud, S., & Roux, M. J. (2006). The glutamate transporter EAAT5 works as a presynaptic receptor in mouse rod bipolar cells. *Journal of Physiology (London), 577*, 221–234.

WoldeMussie, E., Yoles, E., Schwartz, M., Ruiz, G., & Wheeler, L. A. (2002). Neuroprotective effect of memantine in different retinal injury models in rats. *Journal of Glaucoma, 11*, 474–480.

Yucel, Y. H., Gupta, N., Zhang, Q., Mizisin, A. P., Kalichman, M. W., & Weinreb, R. N. (2006). Memantine protects neurons from shrinkage in the lateral geniculate nucleus in experimental glaucoma. *Archives of Ophthalmology, 124*, 217–225.

Zeitz, C., van Genderen, M., Neidhardt, J., Luhmann, U. F., Hoeben, F., Forster, U., Wycisk, K., Matyas, G., Hoyng, C. B., Riemslag, F., Meire, F., Cremers, F. P., & Berger, W. (2005). Mutations in GRM6 cause autosomal recessive congenital stationary night blindness with a distinctive scotopic 15-Hz flicker electroretinogram. *Investigative Ophthalmology & Visual Science, 46*, 4328–4335.

Zeitz, C., Forster, U., Neidhardt, J., Feil, S., Kalin, S., Leifert, D., Flor, P. J., & Berger, W. (2007). Night blindness-associated mutations in the ligand-binding, cysteine-rich, and intracellular domains of the metabotropic glutamate receptor 6 abolish protein trafficking. *Human Mutation, 28*, 771–780.

Zhang, J., & Diamond, J. S. (2009). Subunit- and pathway-specific localization of NMDA receptors and scaffolding proteins at ganglion cell synapses in rat retina. *The Journal of Neuroscience, 29*, 4274–4286.

Glutamate in the Pathogenesis of Gliomas

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Abstract

Recent studies have shown that glutamate may serve important roles in the pathobiology of primary brain tumors. Glutamate is produced and secreted from gliomas via specific glutamate transporters as a by-product of glutathione synthesis. Glutamate also plays a major role in the phenotype of malignant gliomas by several mechanisms. The consequential interaction of glutamate with peritumoral neuronal glutamate receptors leads to the development of seizures and excitotoxicity. The latter is thought to promote the expansion gliomas in the vacated surrounding tissue. Glutaminergic receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) which lack the GluR2 subunit can activate the AKT and MAPK pathway, to promote the invasion of glioma cells into normal brain tissue. Stimulation of glutaminergic receptors also leads to the activation of focal adhesion kinase, which regulates the proliferation and motility of malignant glioma cells. Given the extensive involvement of glutamate in the development of gliomas, it is likely that pharmacological therapies targeting glutaminergic receptors and glutamate transport may be useful to inhibit glutamate-mediated glioma growth.

Keywords

AMPA • Excitotoxicity • Gliomas • Glutamate • Glutathione

1 Introduction

Over the last decade, numerous studies have identified several unique factors that can promote the growth of malignant gliomas. These include a high abundance of vasculature, nutrients, and growth factors that promote the proliferation of tumor cells and specific white matter tracts that pave the way for invading cells to transverse across the brain. Similar to other cancers, the growth of malignant gliomas is dependent on important signalling pathways by activated tyrosine kinases and absence of important tumor suppressor protein such as p53. However, several clinical trials using therapy targeting traditional growth factor pathways such as epidermal growth factor receptor (EGFR), platelet derived growth factor receptor (PDGFR), mTOR, and protein kinase C (PKC) have been unsuccessful (Ohgaki and Kleihues 2009). This suggests that gliomas may possess yet unidentified characteristics which are likely to be of greater therapeutic value.

The excitotoxic neurotransmitter, glutamate, is of major importance in the development of gliomas since (1) it is a very abundant growth factor and (2) it acts as an

important motogen in the CNS (Noch and Khalili 2009). Glutamate is released by glioblastomas via system x_c^- to promote their malignant behavior triggering apoptosis of surrounding tissue and enhancing the invasive nature of gliomas (Ye et al. 1999; Kim et al. 2001). Current experience indicates that 80 % of glioblastomas recurrences occur within 2–3 cm of the perimeter of the original resection cavity (Kim et al. 2009). Therefore, reducing glutamate levels may be empirical for inhibiting the growth of these tumors (Ye and Sontheimer 1999a). Successful treatment of glioma cells may require an increased understanding of glutamate dynamics in the brain to develop novel approaches aimed at improving current ineffective therapies (Savaskan et al. 2011; Simon and von Lehe 2011).

2 Glutaminergic System in the CNS

Two broad categories of excitatory amino acid receptors have been identified in the brain: ionotropic receptors which contain an integral ion channel and metabotropic receptors which are coupled to G proteins (Lalo et al. 2011). The NMDA receptor of which there are also subtypes is one of the three types of ionotropic receptors, the other two being α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and the kainate receptor (Ekici et al. 2012; Fuchs et al. 2012). Glutamate is the primary endogenous excitatory neurotransmitter in the CNS which will activate these receptors. All neurons in the CNS appear to be sensitive to NMDA-mediated toxicity (Savaskan et al. 2011; Simon and von Lehe 2011).

Of the ionotropic receptor channels, the NMDA receptor is unusual in that it is gated by both ligands and voltage (Singh and Kaur 2009). Glutamate is unable to activate the NMDA receptor at normal resting membrane potential (−70 mV) due to the blockage of the ion channel by Mg^{2+} ions. Mg^{2+} is only removed when the target cell is partially depolarized (e.g., by additional synaptic inputs) to around −30 mV to −20 mV, allowing agonist induced currents to occur. Once activated, the NMDA receptor is permeable to Ca^{2+}, Na^+, and K^+ ions. Ca^{2+} entry is thought to activate Ca^{2+}-dependent secondary messenger cascade which contributes to certain forms of long-lasting synaptic modification (Frandsen et al. 1989; Mattson et al. 1991). It is thought that sustained increases in intracellular free Ca^{2+} due to excessive NMDA receptor activation may be central to neuronal cell death in several neurodegenerative diseases of the brain.

Glutamate uptake is the major route of degradation of glutamate from the extracellular space, making it an important function for nonmalignant astrocytes. The uptake of glutamate into astrocytes occurs predominantly by two Na^+-dependent excitatory amino acid transporters EAAT1 or EAAT2, otherwise known as Glt-1 and Glast. These transporters are highly localized in the astrocytic membrane (Allritz et al. 2010; Unger et al. 2012). Upon uptake into astrocytes, glutamate is converted to the glutamine by the catalytic activity of glutami synthetase (GS). Glutamine is then released and retaken into neurons where it is hydrolyzed by glutaminases to regenerate glutamate which is stored in synaptic vesicles (Chretien et al. 2002).

3 Glutamate Release from Gliomas

In neuronal cells, glutamate is generally released from specific synaptic vesicles in a Ca^{2+}-dependent pathway. However, in astrocytes, glutamate is released via a number of different mechanisms including vesicular release (Montana et al. 2006; Ponzio et al. 2006), reverse operation of Na^{+}-dependent glutamate transporters (Courtney et al. 1990), swelling of activated anion channels (Kimelberg et al. 1990a, b, c; Kimelberg and Kettenmann 1990), and through specific hemichannels (Ye et al. 2003). Recent studies have failed to associate these pathways as the mechanism of glutamate release in gliomas. These studies suggest that glutamate release in malignant gliomas may occur predominantly via the Na^{+}-independent system x_c^- cysteine glutamate exchanger (Ye and Sontheimer 1999b) (Fig. 1). This important amino acid transporter fosters the release of glutamate from glioma cells concomitantly with the uptake of cysteine necessary for the production of the endogenous antioxidant, glutathione (GSH) (Sato et al. 1999). In primary cells, GSH, which contains the highly reactive –SH group, maintains the normal cellular redox status by binding with reactive oxygen species and nitrosylative radicals. In gliomas, GSH provides a mechanism of protection for the malignant cells by enhancing the cell's redox status and stimulating excitotoxicity in the peritumoral brain (Kandil et al. 2010). Several analogs of the transported amino acids have been shown to disrupt system x_c^- and impair glutamate release from glioma in vivo (Patel et al. 2004). Additionally, inhibition of system x_c^- using the inhibitor, L-alpha-aminoadipate, leads to more than 50 % reduction in intracellular GSH levels in C6 gliomas, suggesting that the majority of GSH formed in these cells is due to system x_c^--mediated uptake of glutamate. Reduced GSH levels due to blockade of system x_c^- can trigger apoptosis of glioma cells via activation of the JNK and p38 MAPK pathways (Kandil et al. 2010).

Apart from its role in the production of GSH in glioma cells, glutamate can also be converted to α-ketoglutaraldehyde via the catalytic activity of glutamate dehydrogenase (GDH), which is highly expressed in glioma cells (Yang et al. 2009). Glutamate also serves as an important intermediate for energy production in the tricarboxylic cycle (TCA) due to its metabolism of the essential substrate α-ketoglutaraldehyde (Yang et al. 2009). Therefore, inhibition of glutamate release from gliomas provides a novel target to reduce the survival of malignant glioma cells by depleting important sources of energy for these glycolysis-impaired cells and reducing their redox defense status.

As previously mentioned, EAAT1–5 have been implicated in glutamate release in both astrocytes and neurons. However, these transporters appear to be dysfunctional, or not expressed in most gliomas (de Groot et al. 2005). One study showed that EAAT2 is highly expressed in low-grade astroglial tumors, but at significantly lower levels in high-grade glioblastoma tumors. Interestingly, the expression of EAAT2 can regulate glioma cell viability. For instance, the presence of EAAT2 in high-grade glioblastoma tumors makes these cells highly vulnerable to cytotoxicity

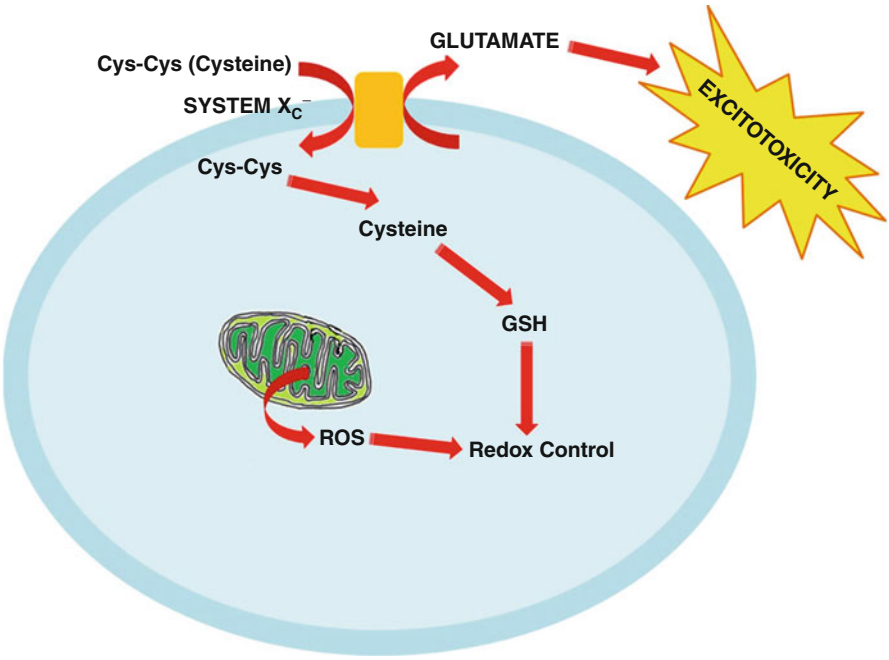

Fig. 1 Glutamate is released from glioma cells via system x_c^- involving the uptake of cysteine necessary for the production of the endogenous antioxidant glutathione (GSH)

via an apoptotic mechanism, and increased EAAT2 expression *ex vivo* can reduce tumorigenicity (de Groot et al. 2005).

Astrocyte elevated gene-1 (AEG-1) is an oncogene that is overexpressed in several tumor cells, including glioblastoma tumors, and is involved in glioma progression (Kang et al. 2005; Lee et al. 2011). AEG-1 has been shown to increase in astrocytes progressing into malignant gliomas, parallel to a decrease in EAAT2 expression (Hu et al. 2009; Baumann and Dorsey 2011; Noch et al. 2011). Recently, Lee et al (2011) showed that AEG-1 repressed EAAT2 expression at the transcriptional level, causing a reduction in glutamate uptake by glial cells, culminating in neuronal cell death (Lee et al. 2011). These findings have also been reiterated in samples of glioma cells obtained from glioma patients indicating a decline in NeuN expression correlating with increased AEG-1 expression (Lee et al. 2011).

4 Effect of Released Glutamate on Neighboring Brain Cells

In the brain, neurons, astrocytes, and microglial and vascular cells are located in close proximity to gliomas, all of which are equally responsive to the released glutamate by a number of different mechanisms.

4.1 Neuron

As earlier discussed, increased levels of glutamate in the extracellular spaces can lead to neurodegeneration by promoting glutamate excitotoxicity. This represents an alternative mechanism for the promotion and expansion of tumoral growth in the adjacent brain tissue. Excitotoxicity is a biochemical process that occurs in response to overactivation of neuronal NMDA receptors in the peritumoral brain, leading to hyperexcitability and seizures which occur frequently in conjunction with gliomas (Moots et al. 1995). Ye et al. (1999) showed that glioma cells release excitotoxic concentrations of glutamate which can induce widespread excitotoxicity in surrounding neurons as well as cellular edema (Ye and Sontheimer 1999a). The loss of these neuronal cells can be prevented by treating neurons with the NMDA receptor antagonist, MK-801/D(-)-2-amino-5-phosphonopentanoic acid, or inhibition of glutamate release from system x_c^- both in vitro and in tumor-bearing mice (Chung et al. 2005).

4.2 Astrocyte

Unlike neurons and oligodendrocytes, astrocytes are less vulnerable to millimolar concentrations of glutamate (Oka et al. 1993). Astrocytes typically respond to increased neuronal glutamate release by the rapid uptake of glutamate into the cytoplasm using the EAAT1 and EAAT2 transporters. It is likely that the function of these excitatory amino acid transporters may be impaired in astrocytes in the presence of gliomas, or they may lose their ability to catabolize glutamate to the nontoxic metabolite glutamine. Studies have shown that reactive gliosis, which is common during excitotoxicity, induces a loss in GS activity (Ortinski et al. 2010). Such loss may enhance the vulnerability of neurons to glutamate-mediated excitotoxicity and impair the normal homeostatic role of astrocytes in the brain. As well, reduced GS activity can lower GABAergic signalling by disrupting glutamate-glutamine cycling (Ortinski et al. 2010).

Astrocytes also express glutaminergic receptors. NMDA and AMPA ionotropic receptors are functionally expressed in astrocytes (Lee et al. 2010). Overactivation of the NMDA receptor can trigger glial cell death, whereas AMPA activation can alter astrocyte morphology, promote glutamate release from astrocytes, and induce deleterious changes in gene expression and normal cellular function (Weber et al. 2001). Metabotropic glutaminergic receptors may also be altered in response to changes in extracellular levels of glutamate and can modulate the expression of glutamate transporters (Aronica et al. 2003).

4.3 Glioma

Ionotropic NMDA, AMPA, and metabotropic glutaminergic receptors are all expressed in glioma cells in cell line cultures and patient-derived glioblastoma

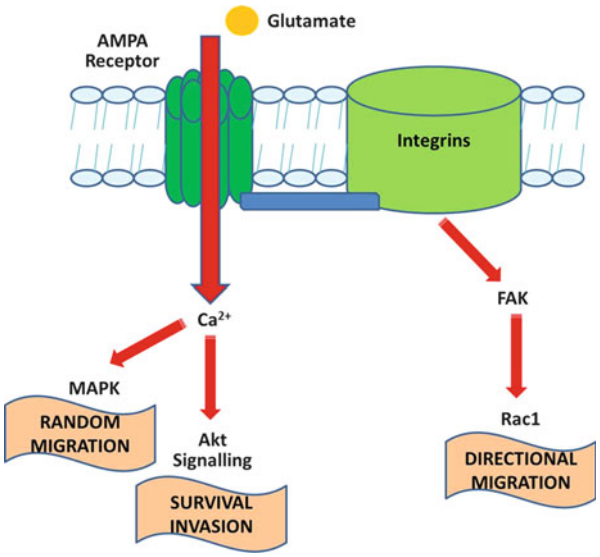

Fig. 2 Autocrine/paracrine-mediated activation of the AMPA receptor by glutamate promotes cell survival and cellular motility

specimens. The NMDA subunits NR2A, NR2B, and NR2C are expressed in several glioma cell lines (Stepulak et al. 2009). Additional studies have demonstrated antitumoral effects with NMDA receptor antagonists (Rzeski et al. 2001). Moreover, kainite receptors have also recently been discovered in several glioma cell lines cultures (Albasanz et al. 1997; Liu et al. 1997).

The AMPA receptor containing the subunits GluR1, GluR3, and GluR4 has been shown to be highly expressed in high-grade gliomas (Ishiuchi et al. 2007). The GluR2 subunit, which is lacking in most gliomas, modulates Ca^{2+} permeability. If an unedited form of the GluR2 subunit is present, Ca^{2+} permeable receptors are established. However, the presence of an edited form of the GluR2 subunit makes the AMPA receptor impermeable to Ca^{2+} and is the case at the synapses. Hence, the absence of the GluR2 subunit in gliomas induces the influx of Ca^{2+} through the AMPA receptor (Ishiuchi et al. 2007). Furthermore, one study showed that loss of GRIA2, the gene coding for GluR2 expression, leads to a poor prognosis in glioblastomas (Colman et al. 2010). Taken together, this suggests that the AMPA receptor is crucial for the development of glioblastomas. Increased Ca^{2+} appears to be an important mediator of glioma cell motility and tumor invasion.

Continued release of glutamate can lead to activation of AMPA receptor in the glioma and surrounding cells. Autocrine and paracrine activation of the AMPA receptor has been demonstrated in vivo (Lyons et al. 2007) (Fig. 2). Overexpression of the GluR1 subunit in the AMPA receptor can activate FAK-dependent signalling pathway independent of the AMPA receptor activation (Piao et al. 2009). Although the mechanism associated with FAK activation remains unclear, it may be associated with FERM protein or integrin activity. Activation of the AMPA receptor due to glutamate enhances cellular detachment via Rac1 and stimulates transwell migration in vitro and promotes tumor invasion in vivo (Piao et al. 2009).

Metabotropic receptors, including mGluR3 and mGluR5, have been described in several low- and high-grade glioma cell lines (Caruso et al. 1997; Condorelli et al. 1997). However, characterization of these receptors in human glioma samples remains to be accomplished.

5 Targeting the Glutaminergic System as Antitumoral Agents

Given the importance of glutamate in the biology of glioma growth and invasion, current agents which modulate the glutaminergic system may represent potential therapeutic agents. If modulators of the glutaminergic system can limit tumor invasion into the peritumoral brain, these drugs may represent novel therapies which can inhibit glioma infiltration in humans. Several clinical trials have been performed and are summarized here.

5.1 Antagonism of the AMPA Receptor

It is well established that glutamate released from gliomas can activate glutaminergic receptors on the same or neighboring cell. However, these drugs are limited due to severe side effects when translated into the clinic (Lipton 2004). Talampanel is a noncompetitive antagonist of the AMPA receptor that can penetrate the brain and demonstrated a favorable outcome in preclinical studies (Calabrese et al. 2007). The drug has demonstrated an excellent safety profile in a single-arm Phase II study in 32 patients with recurrent glioblastomas or anaplastic gliomas as a monotherapy (Iwamoto et al. 2010). In another larger Phase II study, talampanel was given in newly diagnosed glioblastoma patients in combination with radiation therapy and temozolomide (Grossman et al. 2009). The data collected so far indicates a favorable survival rate with no added toxicity when used in combination. However, additional randomized placebo-controlled trials are necessary to adequately establish the efficacy of this new drug compared with radiation and standard treatment regimes.

5.2 Antagonism of Other Glutaminergic Receptors

Other glutamate receptor antagonists may also be of clinical benefit for the treatment of patients with gliomas. Memantine, a noncompetitive NMDA ion channel blocker, is currently approved for the treatment of mild to moderate Alzheimer's disease (AD). Interestingly, preclinical studies have reported reduced glioma proliferation following treatment with memantine (Rzeski et al. 2001). Apart from its antitumoral activity, memantine may also attenuate excitotoxicity in neuronal cells due to increased glutamate in the extracellular space. This may also be important in inhibiting metabolic pathways utilized by malignant glioma cells.

Additionally, metabotropic glutamate receptors may also represent a useful target to attenuate NMDA receptor-mediated excitotoxicity and reduce neurological dysfunction, seizures, and increased tumor invasion. LY341495, a selective Group II metabotropic glutamate receptor antagonist, has been shown to reduce glioma proliferation, and clinical trials are under way (Arcella et al. 2005).

5.3 Blockade of System x_c^- in Gliomas

As previously discussed, the system x_c^- represents the main mechanism of glutamate release from gliomas. This unique transporter can be inhibited using sulfasalazine and FDA-approved drug that can significantly slow down tumor growth in mice (Chung et al. 2005). A Phase I study of this agent in 9 heavily pretreated patients with poor performance was terminated by the investigator due to poor efficacy outcomes.

5.4 Inhibition of Glutamate Reuptake

Enhancing glutamate uptake in glial cells represents an alternative means to prevent glutamate accumulation in peritumoral tissue and reduce glioma invasion in surrounding brain tissue. This notion has been demonstrated in vitro and in preclinical studies where ectopic expression of EAAT2 in gliomas limits their growth both in vitro and in vivo. However, this concept is yet to be translated to the clinic (Robe et al. 2004).

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References

Albasanz, J. L., Ros, M., et al. (1997). Characterization of metabotropic glutamate receptors in rat C6 glioma cells. *European Journal of Pharmacology, 326*(1), 85–91.

Allritz, C., Bette, S., et al. (2010). Comparative structural and functional analysis of the GLT-1/EAAT-2 promoter from man and rat. *Journal of Neuroscience Research, 88*(6), 1234–1241.

Arcella, A., Carpinelli, G., et al. (2005). Pharmacological blockade of group II metabotropic glutamate receptors reduces the growth of glioma cells in vivo. *Neuro-Oncology, 7*(3), 236–245.

Aronica, E., Gorter, J. A., et al. (2003). Expression and functional role of mGluR3 and mGluR5 in human astrocytes and glioma cells: Opposite regulation of glutamate transporter proteins. *European Journal of Neuroscience, 17*(10), 2106–2118.

Baumann, B. C., & Dorsey, J. F. (2011). Astrocyte-elevated gene-1 (AEG-1): Glioblastoma's helping hand during times of hypoxia and glucose deprivation? *Cancer Biology & Therapy, 11*(1), 40–42.

Calabrese, C., Poppleton, H., et al. (2007). A perivascular niche for brain tumor stem cells. *Cancer Cell, 11*(1), 69–82.

Caruso, A., Ciccarelli, R., et al. (1997). Opposite influence of the metabotropic glutamate receptor subtypes mGlu3 and -5 on astrocyte proliferation in culture. *Journal of Neurochemistry, 69*, S234–S234.

Chretien, F., Vallat-Decouvelaere, A. V., et al. (2002). Expression of excitatory amino acid transporter-2 (EAAT-2) and glutamine synthetase (GS) in brain macrophages and microglia of SIVmac251-infected macaques. *Neuropathology and Applied Neurobiology, 28*(5), 410–417.

Chung, W. J., Lyons, S. A., et al. (2005). Inhibition of cystine uptake disrupts the growth of primary brain tumors. *Journal of Neuroscience, 25*(31), 7101–7110.

Colman, H., Zhang, L., et al. (2010). A multigene predictor of outcome in glioblastoma. *Neuro-Oncology, 12*(1), 49–57.

Condorelli, D. F., DellAlbani, P., et al. (1997). Metabotropic glutamate receptor expression in cultured rat astrocytes and human gliomas. *Neurochemical Research, 22*(9), 1127–1133.

Courtney, M. J., Lambert, J. J., et al. (1990). The interactions between plasma-membrane depolarization and glutamate receptor activation in the regulation of cytoplasmic free calcium in cultured cerebellar granule cells. *Journal of Neuroscience, 10*(12), 3873–3879.

de Groot, J. F., Liu, T. J., et al. (2005). The excitatory amino acid transporter-2 induces apoptosis and decreases glioma growth in vitro and in vivo. *Cancer Research, 65*(5), 1934–1940.

Ekici, M., Keim, A., et al. (2012). Chromatin structure and expression of the AMPA receptor subunit Glur2 in human glioma cells: Major regulatory role of REST and Sp1. *Journal of Cellular Biochemistry, 113*(2), 528–543.

Frandsen, A., Drejer, J., et al. (1989). Direct evidence that excitotoxicity in cultured neurons is mediated via N-methyl-D-aspartate (NMDA) as well as non-NMDA receptors. *Journal of Neurochemistry, 53*(1), 297–299.

Fuchs, S. A., Peeters-Scholte, C. M., et al. (2012). Increased concentrations of both NMDA receptor co-agonists D-serine and glycine in global ischemia: A potential novel treatment target for perinatal asphyxia. *Amino Acids, 43*(1), 355–363.

Grossman, S. A., Ye, X. B., et al. (2009). Talampanel with standard radiation and Temozolomide in patients with newly diagnosed glioblastoma: A multicenter phase II trial. *Journal of Clinical Oncology, 27*(25), 4155–4161.

Hu, G., Wei, Y., et al. (2009). The multifaceted role of MTDH/AEG-1 in cancer progression. *Clinical Cancer Research, 15*(18), 5615–5620.

Ishiuchi, S., Yoshida, Y., et al. (2007). Ca^{2+}-permeable AMPA receptors regulate growth of human glioblastoma via Akt activation. *Journal of Neuroscience, 27*(30), 7987–8001.

Iwamoto, F. M., Kreisl, T. N., et al. (2010). Phase 2 trial of Talampanel, a glutamate receptor inhibitor, for adults with recurrent malignant gliomas. *Cancer, 116*(7), 1776–1782.

Kandil, S., Brennan, L., et al. (2010). Glutathione depletion causes a JNK and p38(MAPK)-mediated increase in expression of cystathionine-gamma-lyase and upregulation of the transsulfuration pathway in C6 glioma cells. *Neurochemistry International, 56*(4), 611–619.

Kang, D. C., Su, Z. Z., et al. (2005). Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. *Gene, 353*(1), 8–15.

Kim, J. Y., Kanai, Y., et al. (2001). Human cystine/glutamate transporter: cDNA cloning and upregulation by oxidative stress in glioma cells. *Biochimica et Biophysica Acta, 1512*(2), 335–344.

Kim, D. S., Na, D. G., et al. (2009). Distinguishing tumefactive demyelinating lesions from glioma or central nervous system lymphoma: Added value of unenhanced CT compared with conventional contrast-enhanced MR imaging. *Radiology, 251*(2), 467–475.

Kimelberg, H. K., & Kettenmann, H. (1990). Swelling-induced changes in electrophysiological properties of cultured Astrocytes and Oligodendrocytes.1. Effects on membrane-potentials, input impedance and cell-cell coupling. *Brain Research, 529*(1–2), 255–261.

Kimelberg, H. K., Goderie, S., et al. (1990a). Swelling-induced membrane-transport changes in Astrocytes. *Advances in Neural Regeneration Research, 60*, 199–214.

Kimelberg, H. K., Goderie, S. K., et al. (1990b). Volume changes of Astrocytes invitro as a model for pathological astrocytic swelling. *Differentiation and Functions of Glial Cells, 55*, 335–348.

Kimelberg, H. K., Goderie, S. K., et al. (1990c). Swelling-induced release of glutamate, Aspartate, and Taurine from Astrocyte cultures. *Journal of Neuroscience, 10*(5), 1583–1591.

Lalo, U., Pankratov, Y., et al. (2011). Ionotropic receptors in neuronal-astroglial signalling: What is the role of "excitable" molecules in non-excitable cells. *Biochimica et Biophysica Acta, 1813*(5), 992–1002.

Lee, M. C., Ting, K. K., et al. (2010). Characterisation of the expression of NMDA Receptors in human Astrocytes. *PloS One, 5*(11).

Lee, S. G., Kim, K., et al. (2011). Oncogene AEG-1 promotes glioma-induced neurodegeneration by increasing glutamate excitotoxicity. *Cancer Research, 71*(20), 6514–6523.

Lipton, S. A. (2004). Paradigm shift in NMDA receptor antagonist drug development: Molecular mechanism of uncompetitive inhibition by memantine in the treatment of Alzheimer's disease and other neurologic disorders. *Journal of Alzheimer's Disease, 6*(6), S61–S74.

Liu, Q. Y., Schaffner, A. E., et al. (1997). Astrocytes regulate amino acid receptor current densities in embryonic rat hippocampal neurons. *Journal of Neurobiology, 33*(6), 848–864.

Lyons, S. A., Chung, W. J., et al. (2007). Autocrine glutamate signaling promotes glioma cell invasion. *Cancer Research, 67*(19), 9463–9471.

Mattson, M. P., Wang, H., et al. (1991). Developmental expression, compartmentalization, and possible role in excitotoxicity of a putative NMDA receptor protein in cultured hippocampal neurons. *Brain Research, 565*(1), 94–108.

Montana, V., Malarkey, E. B., et al. (2006). Vesicular transmitter release from Astrocytes. *Glia, 54*(7), 700–715.

Moots, P. L., Maciunas, R. J., et al. (1995). The course of seizure disorders in patients with malignant gliomas. *Archives of Neurology, 52*(7), 717–724.

Noch, E., & Khalili, K. (2009). Molecular mechanisms of necrosis in glioblastoma: The role of glutamate excitotoxicity. *Cancer Biology & Therapy, 8*(19), 1791–1797.

Noch, E., Bookland, M., et al. (2011). Astrocyte-elevated gene-1 (AEG-1) induction by hypoxia and glucose deprivation in glioblastoma. *Cancer Biology & Therapy, 11*(1), 32–39.

Ohgaki, H., & Kleihues, P. (2009). Genetic alterations and signaling pathways in the evolution of gliomas. *Cancer Science, 100*(12), 2235–2241.

Oka, A., Belliveau, M. J., et al. (1993). Vulnerability of Oligodendroglia to glutamate – pharmacology, mechanisms, and prevention. *Journal of Neuroscience, 13*(4), 1441–1453.

Ortinski, P. I., Dong, J. H., et al. (2010). Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nature Neuroscience, 13*(5), 584–U593.

Patel, S. A., Warren, B. A., et al. (2004). Differentiation of substrate and non-substrate inhibitors of transport system x(c)(-): An obligate exchanger of L-glutamate and L-cystine. *Neuropharmacology, 46*(2), 273–284.

Piao, Y. J., Lu, L., et al. (2009). AMPA receptors promote perivascular glioma invasion via beta 1 integrin-dependent adhesion to the extracellular matrix. *Neuro-Oncology, 11*(3), 260–273.

Ponzio, T. A., Ni, Y., et al. (2006). Vesicular glutamate transporter expression in supraoptic neurones suggests a glutamatergic phenotype. *Journal of Neuroendocrinology, 18*(4), 253–265.

Robe, P. A., Bentires-Alj, M., et al. (2004). In vitro and in vivo activity of the nuclear factor-kappa B inhibitor sulfasalazine in human glioblastomas. *Clinical Cancer Research, 10*(16), 5595–5603.

Rzeski, W., Turski, L., et al. (2001). Glutamate antagonists limit tumor growth. *Proceedings of the National Academy of Sciences of the United States of America, 98*(11), 6372–6377.

Sato, H., Tamba, M., et al. (1999). Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. *Journal of Biological Chemistry, 274*(17), 11455–11458.

Savaskan, N. E., Seufert, S., et al. (2011). Dissection of mitogenic and neurodegenerative actions of cystine and glutamate in malignant gliomas. *Oncogene, 30*(1), 43–53.

Simon, M., & von Lehe, M. (2011). Glioma-related seizures: Glutamate is the key. *Nature Medicine, 17*(10), 1190–1191.

Singh, J., & Kaur, G. (2009). Transcriptional regulation of PSA-NCAM expression by NMDA receptor activation in RA-differentiated C6 glioma cultures. *Brain Research Bulletin, 79*(3–4), 157–168.

Stepulak, A., Luksch, H., et al. (2009). Expression of glutamate receptor subunits in human cancers. *Histochemistry and Cell Biology, 132*(4), 435–445.

Unger, T., Lakowa, N., et al. (2012). Transcriptional regulation of the GLAST/EAAT-1 gene in rat and man. *Cellular and Molecular Neurobiology, 32*(4), 539–547.

Weber, M., Dietrich, D., et al. (2001). 6-Hydroxykynurenic acid and kynurenic acid differently antagonise AMPA and NMDA receptors in hippocampal neurones. *Journal of Neurochemistry, 77*(4), 1108–1115.

Yang, C. D., Sudderth, J., et al. (2009). Glioblastoma cells require glutamate dehydrogenase to survive impairments of glucose metabolism or Akt signaling. *Cancer Research, 69*(20), 7986–7993.

Ye, Z. C., & Sontheimer, H. (1999a). Glioma cells release excitotoxic concentrations of glutamate. *Cancer Research, 59*(17), 4383–4391.

Ye, Z. C., & Sontheimer, H. (1999b). Metabotropic glutamate receptor agonists reduce glutamate release from cultured astrocytes. *Glia, 25*(3), 270–281.

Ye, Z. C., Rothstein, J. D., et al. (1999). Compromised glutamate transport in human glioma cells: Reduction-mislocalization of sodium-dependent glutamate transporters and enhanced activity of cystine-glutamate exchange. *Journal of Neuroscience, 19*(24), 10767–10777.

Ye, Z. C., Wyeth, M. S., et al. (2003). Functional hemichannels in astrocytes: A novel mechanism of glutamate release. *Journal of Neuroscience, 23*(9), 3588–3596.

Glutamate Neurotoxicity Related to Energy Failure

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Contents

Abstract

Glutamate is the major excitatory neurotransmitter mediating its effects via a plethora of receptors being either ionotropic or metabotropic. Each of these two mechanistically different receptor subtypes can be subdivided into distinctly different subclasses based on different pharmacological properties. Under physiological conditions the glutamatergic neurotransmission is instrumental for a large number of basic neurochemical functions such as learning and memory. Therefore, pharmacological manipulations of glutamatergic neurotransmission

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are associated with severe side effects. One particular pathophysiological area in which pharmacological intervention in glutamatergic neurotransmission has been of considerable interest is related to energy failure such as that observed during stroke leading to an ischemic condition. This has been shown to be associated with a large overflow of glutamate into the extracellular space of the brain which leads to over activation of glutamate receptors resulting in massive neuronal degeneration normally referred to as excitotoxicity. The reason for this overflow of glutamate is the fact that efficient removal of glutamate from the extrasynaptic area is mediated by a number of highly efficient, high-affinity glutamate transporters, the majority of which is located on astrocytes ensheathing the synapses. As the transporters are functionally coupled to the Na^+/K^+-ATPase, energy failure leading to reduced levels of ATP renders the transporters functionally inadequate resulting in efflux of glutamate from the cytoplasmic pool of glutamate. The energy substrates in the brain are under normal conditions limited to glucose and lactate but also glycogen which is selectively located in the astrocytes can play an important role both under physiological and pathophysiological conditions. These aspects are discussed in detail and evidence is presented pointing towards a hitherto neglected role of glycogen in the maintenance of glutamatergic activity during physiological conditions.

List of Abbreviations

AAT	Aspartate aminotransferase
AMPA	(*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid
EAAT-1	Excitatory amino acid transporter-1
EAAT-2	Excitatory amino acid transporter-2
GABA	Gamma-amino butyric acid
GDH	Glutamate dehydrogenase
GLAST	Glutamate-aspartate transporter
GLT-1	Glutamate transporter-1
GS	Glutamine synthetase
KA	Kainic acid
LDH	Lactate dehydrogenase
NMDA	N-methyl-D-aspartic acid
PAG	Phosphate-activated glutaminase
PC	Pyruvate carboxylase
TCA	Tricarboxylic acid

1 Introduction

Energy failure in the brain may be the result of stroke or global ischemia, both of which will lead to a drastic reduction of the availability of glucose and oxygen (Dugan and Kim-Han 2006). This condition is normally referred to as

oxygen-glucose deprivation (OGD) when used in in vitro systems such as cultured cells (e.g., Bonde et al. 2003). This condition is associated with degeneration of neurons in the brain as well as in tissue culture systems. It was shown in the pioneering work of Lucas and Newhouse (1957) that exposure of the central nervous system to excess amounts of glutamate will lead to neurodegeneration. Subsequently it was demonstrated that ischemia results in overflow of glutamate to the extracellular space in the brain (Benveniste et al. 1984). Based on this, it was suggested that glutamate would be likely to play a central role in the mechanisms leading to neurodegeneration induced by ischemia. The term excitotoxicity was coined by Olney et al. (1971) based on the observation that glutamate is not only an excitatory neurotransmitter (Curtis et al. 1959) but also acts as a neurotoxin.

The present review will deal with a delineation of the role of glutamate receptors in the neurotoxic actions of glutamate as well as a discussion of mechanisms involving glutamate transporters responsible for the overflow of glutamate during energy failure. This will include a discussion of glutamate metabolism as well as the role of glycogen, glucose, and lactate as energy substrates in relation to glutamate neurotransmission. Additionally, it will be discussed to what extent inhibitory neurotransmission mediated by GABA could play a role as a regulatory mechanism.

2 Glutamate Receptors

2.1 Ionotropic Receptors

Based on pharmacological characterization and subsequent cloning, the ionotropic glutamate receptors are subdivided into three major classes, i.e., N-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl) propionic acid (AMPA), and kainic acid (KA) receptors (Braüner-Osborne et al. 2000). As each of these subgroups consists of a number of subunits that can be assembled in different combinations, the total number of possible receptors is much higher and these different receptors have pharmacologically and functionally distinct properties (Braüner-Osborne et al. 2000). This has turned out to be very important in attempts to design strategies to ameliorate glutamate-mediated neurodegeneration (Schousboe and Frandsen 1995; Ikonomidou and Turski 1995; Braüner-Osborne et al. 2000).

2.2 Metabotropic Glutamate Receptors

Subsequent to the demonstration that exposure of nervous tissue preparations to glutamate resulted in stimulation of inositol phospholipid metabolism (Sladeczek et al. 1985; Nicoletti et al. 1986a, b), cloning studies have identified eight subtypes of metabotropic glutamate receptors (see Braüner-Osborne et al. 2000). These are further subdivided into three subgroups consisting of group I acting on

phospholipase C leading to an increase in inositol phosphates and groups II and III that are coupled to adenylate cyclase which is inhibited leading to a decrease in the cellular content of cyclic AMP (see Braüner-Osborne et al. 2000). Due to the fact that this group of receptors plays a fundamental role in the regulation of a number of important brain functions, an enormous effort has been made to make glutamate analogs of restricted conformation acting as agonists and antagonists on the different subtypes and a comprehensive list of such compounds is provided by Bräuner-Osborne et al. (2000).

3 Glutamate Transporters

Subsequent to its release as neurotransmitter and interaction with the receptors, glutamate must be inactivated. This process is mediated via high-affinity transporters, the capacity of which is more pronounced in astrocytes compared to neurons (Schousboe 1981; Gegeslashvili and Schousboe 1997; Danbolt 2001; Schousboe et al. 2012). This notion is based on studies of the kinetics of glutamate uptake into cultured neurons and astrocytes as well as detailed immunocytochemical localization studies using specific antibodies prepared from peptide libraries generated from the cloned transporters (see Schousboe et al. 2012 and references therein). The following sections will provide a short description of the two major astroglial transporters GLAST (EAAT-1) and GLT-1 (EAAT-2).

3.1 GLAST and GLT-1

A glutamate transporter was co-purified with the enzyme UDP galactose: ceramide galactosyltransferase (Schulte and Stoffel 1995) and this procedure was used to clone the glutamate-aspartate transporter named GLAST (Storck et al. 1992). At the same time another glutamate transporter was purified using a synaptosomal membrane preparation from rat brain (Danbolt et al. 1990). This enabled the production of a specific antibody which was subsequently used to clone this glutamate transporter named GLT-1 as it turned out to be different from GLAST (Pines et al. 1992). In spite of the fact that synaptosomal membranes had served as the starting material in the purification procedure, it became clear from immunohistochemical studies at the light and electron microscopic level that GLT-1 is almost exclusively localized in astroglial cells (Danbolt et al. 1992; Levy et al. 1993; Rothstein et al. 1994; Chaudhry et al. 1995). While GLAST is also essentially only expressed in astrocytes (Chaudhry et al. 1995; Lehre et al. 1995; Schmitt et al. 1997), the regional expression pattern of the two transporters shows a differential distribution with higher expression of GLT-1 in cortical areas and higher levels of expression of GLAST in cerebellum compared to cortex (Lehre et al. 1995).

3.2 Energy Requirements of Glutamate Transport

Detailed studies by Levy et al. (1998) of the stoichiometry of the ionic dependence of glutamate transport mediated by GLT-1 have shown that this requires co-transport of three sodium ions and one proton and countertransport of one potassium ion. This movement of ions needs to be reversed by the concerted action of the Na^+/K^+-ATPase and the Na^+/H^+ exchanger. As the latter process is also dependent on the ATPase, a total of four sodium ions need to be transported which consumes 1.3 mol of ATP. Interestingly, it has been reported recently that a tight coupling appears to exist between the glutamate transporters and the sodium-potassium ATPase (Cholet et al. 2002; Rose et al. 2009; Genda et al. 2011; Bauer et al. 2012) which would make the processes highly efficient.

It should be considered that the neurotransmission process consists of not only inactivation of glutamate by the astrocytic transporters but also of the vesicular packaging of glutamate prior to its release as a transmitter. This process involves the vesicular H^+-ATPase (Maycox et al. 1988) which likely has the same ATP stoichiometry as the mitochondrial F-type ATPase coupled to ATP production. Hence, vesicular filling is associated with approximately one third mol of ATP per mol of glutamate transported into the vesicles.

4 Glutamate Metabolism

4.1 Astrocytes

Glutamate transported into the astrocytes subsequent to release from vesicles in the presynaptic nerve endings will be metabolized either by the astrocyte specific enzyme glutamine synthetase (GS) or by oxidation in mitochondria after conversion to α-ketoglutarate catalyzed by either glutamate dehydrogenase (GDH) or aspartate aminotransferase (AAT) as described in detail elsewhere (e.g., Sonnewald et al. 1997; Schousboe et al. 2012). There is evidence to suggest that the metabolic pathways involved in the oxidation of glutamate are dependent on the prevailing glutamate concentration in the astrocyte (Sonnewald et al. 1997). Thus, the GDH catalyzed conversion to α-ketoglutarate prevails over the transamination catalyzed by AAT at elevated glutamate concentrations. This impacts on the energy homeostasis of glutamate metabolism since the GDH reaction produces NADH which subsequently gives rise to ATP production by oxidative phosphorylation (see McKenna et al. 2012), whereas the transamination reaction does not provide energy in the form of ATP. Regardless of the reaction responsible for the conversion of glutamate to α-ketoglutarate, subsequent metabolism of this tricarboxylic acid (TCA) cycle intermediate to carbon dioxide via pyruvate recycling (see Waagepetersen et al. 2009) provides 24 mol of ATP. Hence, glutamate can be regarded as an excellent energy substrate in the brain and its metabolism can to some extent replace that of glucose in astrocytes (Hertz et al. 2000).

4.2 Neurons

In both glutamatergic and GABAergic neurons, glutamate is formed by deamination of glutamine catalyzed by phosphate-activated glutaminase (PAG). Glutamine must be provided by surrounding astrocytes as neurons are incapable of performing a net synthesis of glutamine from glucose as they do not express the enzymes pyruvate carboxylase (PC) and GS (see Schousboe et al. 2012), both of which are astrocyte specific (Norenberg and Martinez-Hernandez 1979; Yu et al. 1983). In glutamatergic neurons this newly formed glutamate can be taken up into vesicles for transmitter use or it may be used as an energy substrate as explained above (Sect. 4.1). In GABAergic neurons it is either converted to transmitter GABA by decarboxylation or used as energy substrate (see Schousboe et al. 2012).

5 Consequences of Energy Failure in Glutamatergic Neurotransmission

5.1 Glycogen, Glucose, and Lactate as Energy Substrates

The brain is the most energy-demanding organ accounting for about 20 % of the resting metabolic rate albeit amounting to only 2 % of the body weight (see McKenna et al. 2012). The majority of the energy consumption (75 %) is related to signaling processes, whereas the remaining energy consumption is associated with basic cellular activities (McKenna et al. 2012). In the adult brain glucose is the main obligatory energy substrate but alternative substrates such as ketone bodies and lactate can be utilized. The use of ketone bodies will be prominent during prolonged fasting (McKenna et al. 2012). Interestingly, during strenuous exercise leading to lactate production in the muscles and high lactate plasma levels, lactate can be taken up into the brain (Quistorff et al. 2008). Glucose is metabolized in the glycolytic metabolic pathway leading to production of pyruvate which can either be oxidatively metabolized in the mitochondria to CO_2 and water or it can be reduced to lactate catalyzed by lactate dehydrogenase (LDH) in which case lactate is the end product of glucose metabolism. It has been proposed that in astrocytes glucose is preferentially converted to lactate which is subsequently transferred to the neurons where it is converted to pyruvate via the LDH reaction and further oxidatively metabolized (Pellerin and Magistretti 1994). This process, referred to as the astrocyte-neuron lactate shuttle, has subsequently been extensively discussed and it seems clear that astrocytes have a significant oxidative metabolism which on a volume basis is comparable to that in neurons (Hertz et al. 2007). It is important to note that the combination of glycolysis and oxidative metabolism of pyruvate requires the presence of the malate-aspartate shuttle (or an equivalent mechanism) which can secure exchange between the cytosol and the mitochondria of the redox couple NAD^+/NADH as the mitochondrial membrane is impermeable to NAD^+ and NADH (McKenna et al. 2012). In this context it should be noted that astrocytes are equipped with all enzymes as well as mitochondria necessary to carry out the

metabolic processes involved in oxidative metabolism of glucose to CO_2 and water (Lovatt et al. 2007). It is also interesting to note that lactate is unable to fully replace glucose as an energy substrate for neurons during enhanced activity as that seen during exposure to depolarizing signals in the form of glutamate or elevated potassium (Bak et al. 2006; 2012).

During recent years brain glycogen has attracted attention although its concentration is quite low compared to that in other organs such as liver and muscle. However, its concentration exceeds that of free glucose, but during glucose deprivation this amount of glycogen would, if metabolized by the concerted action of glycogenolysis and glycolysis, only provide enough energy to maintain brain function for a few minutes (McKenna et al. 2012). The availability of specific pharmacological tools to inhibit the key enzyme in glycogenolysis, glycogen phosphorylase, has facilitated studies aimed at elucidating the functional role of glycogen (Obel et al. 2012). Such studies have provided evidence that glycogen turnover involving the glycogen shunt, i.e., the conversion of glucose-6-phosphate to glycogen and its reappearance as glucose-1-phosphate and thus glucose-6-phosphate (see Schousboe et al. 2010; Kreft et al. 2012), is of fundamental importance for the maintenance of a fully functional glutamatergic neurotransmission system (Schousboe et al. 2007, 2010; Obel et al. 2012; Parpura et al. 2012). Since the functional integrity of this system is a prerequisite to avoid excitotoxic neuronal death, glycogen metabolism is obviously also important in relation to neurotoxic damage (Obel et al. 2012).

5.2 Energy Failure and Glutamatergic Neurotransmission

As pointed out above (Sect.3.2), glutamate transport is energy dependent due to the requirement of an intact transmembrane sodium gradient. In addition to this, the process responsible for packaging of transmitter glutamate into synaptic vesicles is ATP dependent since it utilizes the proton gradient generated by the action of the H^+-ATPase. It is interesting that the ATP required in both of these processes may originate from the glycolytic metabolic pathway rather than the TCA-coupled oxidative phosphorylation pathway (Ikemoto et al. 2003; Schousboe et al. 2011). As pointed out above, glycogen metabolism and turnover seems to play an important functional role in this context.

Regardless of the metabolic processes required for ATP production, it is clear that failure of generation of ATP will result in a reduced glutamatergic transmission, i.e., vesicular release will be decreased (Schousboe and Waagepetersen 2005). Since the GABA-mediated inhibitory neurotransmission is based on the same principles as the glutamatergic system, GABA neurotransmission will be reduced as well. A possible functional interrelationship regarding glutamatergic and GABAergic neurotransmission will be provided below (see Sect. 6). As pointed out above the high-affinity glutamate transporters are energy dependent and as a consequence of this, energy failure will result in reversal of the transporters and an overflow of glutamate from the metabolic, cytosolic pool into the extracellular

space (see Schousboe and Waagepetersen 2005). This, in turn, will lead to an overstimulation of glutamate receptors resulting in excitotoxic neurodegeneration (see Sect. 5.3).

5.3 Energy Failure and Excitotoxicity

The demonstration almost 30 years ago by Benveniste et al. (1984) using the microdialysis technique that ischemia leads to an excessive overflow of glutamate and aspartate in rat hippocampus provided compelling evidence that ischemia-induced energy failure leading to pronounced neurodegeneration was very likely related to an excitotoxic mechanism as described by Olney and Ho (1970). Numerous studies using pharmacological tools to delineate the role of the different types of glutamate receptors (see Sect. 2) performed in cultured neurons as well as in the intact brain in vivo during the years subsequent to the study by Benveniste et al. (1984) have demonstrated that overstimulation of these receptors is responsible for the neurodegeneration associated with brain energy failure (see Choi and Rothman 1990; Schousboe and Frandsen 1995).

In accordance with the fact that glutamate transporters are energy dependent, energy failure has been shown to result in reversal of the glutamate transporters (Nicholls and Attwell 1990; Phillis et al. 2000; Bonde et al. 2003). This therefore is the most likely explanation why ischemia leads to overflow of glutamate and aspartate as mentioned above.

The molecular mechanisms associated with stimulation of glutamate receptors subsequently leading to neuronal degeneration are extremely complex involving disturbances of Ca^{2+} homeostasis, free radical formation, and NO generation, the concerted action of which results in activation of proteases, DNA degradation, and disruption of the cytoskeleton (see, e.g., Dugan and Kim-Han 2006). A detailed discussion of this is beyond the scope of the present review.

6 Possible Interaction Between Excitatory and Inhibitory Neurotransmission

There is evidence to suggest that the expression level of $GABA_A$ receptors may have an impact on glutamatergic, excitatory activity and excitotoxicity is exacerbated by reduced GABA receptor activity (Schwartz-Bloom and Sah 2001). Additionally, the metabotropic $GABA_B$ receptors are involved in the regulation of glutamate release and hence excitatory transmission (Nicoll 2004). In this context it is interesting that cerebral ischemia and oxygen-glucose deprivation, both of which will result in energy failure, result in a decrease in the cell surface expression of $GABA_A$ receptors (Alicke and Schwartz-Bloom 1995; Mielke and Wang 2005) which as stated above contributes to ischemic brain damage (Schwartz-Bloom and Sah 2001).

This interrelationship between glutamatergic and GABAergic neurotransmission is of importance not only for neurodegeneration induced by energy failure but also for generation of seizures and epileptic episodes. A recent review provides a detailed discussion of the significance of the machinery for synthesis, release, transport, and metabolism of glutamate and GABA as targets for development of drugs for seizure control (Rowley et al. 2012). This may be interesting in relation to neurodegeneration as well.

7 Conclusion

The present short review has provided a discussion of the correlation between energy metabolism and the homeostatic mechanisms regulating excitatory, glutamatergic activity. It is clear that energy failure will inevitably result in excessive glutamate overflow which, in turn, will produce neurodegeneration. Three decades of intensive pharmacological research has provided a wealth of information about strategies to ameliorate such neuronal damage but little has so far been achieved regarding development of clinically efficient drugs to prevent ischemia-induced neurodegeneration.

References

Alicke, B., & Schwartz-Bloom, R. D. (1995). Rapid down-regulation of $GABA_A$ receptors in the gerbil hippocampus following transient cerebral ischemia. *Journal of Neurochemistry, 65*, 2808–2811.

Bak, L. K., Schousboe, A., Sonnewald, U., & Waagepetersen, H. S. (2006). Glucose is necessary to maintain neurotransmitter homeostasis during synaptic activity in cultured glutamatergic neurons. *Journal of Cerebral Blood Flow and Metabolism, 26*, 1285–1297.

Bak, L. K., Obel, L. F., Walls, A. B., Schousboe, A., Faek, S. A. A., Jajo, F. S., & Waagepetersen, H. S. (2012). Novel model of neuronal bioenergetics: Postsynaptic utilization of glucose but not lactate correlates positively with Ca^{2+} signaling in cultured mouse glutamatergic neurons. *ASN Neuro, 4*, 151–160.

Bauer, D. E., Jackson, J. G., Genda, E. N., Montoya, M. M., Yudkoff, M., & Robinson, M. B. (2012). The glutamate transporter, GLAST, participates in a macromoleculAr complex that supports glutamate metabolism. *Neurochemistry International, 61*, 566–574.

Benveniste, H., Drejer, J., Schousboe, A., & Diemer, N. H. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *Journal of Neurochemistry, 43*, 1369–1374.

Bonde, C., Sarup, A., Schousboe, A., Gegelashvili, G., Zimmer, J., & Noraberg, J. (2003). Neurotoxic and neuroprotective effects of the glutamate transporter inhibitor DL-threo-beta-benzyloxyaspartate (DL-TBOA) during physiological and ischemia-like conditions. *Neurochemistry International, 43*, 371–380.

Braüner-Osborne, H., Egebjerg, J., Nielsen, E. Ø., Madsen, U., & Krogsgaard-Larsen, P. (2000). Ligands for glutamate receptors: Design and therapeutic prospects. *Journal of Medicinal Chemistry, 43*, 2609–2645.

Chaudhry, F. A., Lehre, K. P., Campagne, M. V., Ottersen, O. P., Danbolt, N. C., & Storm-Mathisen, J. (1995). Glutamate transporters in glial plasma membranes: Highly differentiated

localizations revealed by qualitative ultrastructural immunocytochemistry. *Neuron, 15*, 711–720.

Choi, D. W., & Rothman, S. M. (1990). The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annual Review of Neuroscience, 13*, 171–182.

Cholet, N., Pellerin, L., Magistretti, P. J., & Hamel, E. (2002). Similar perisynaptic glial localization for the Na^+, K^+ - ATPase α_2 subunit and the glutamate transporters GLAST and GLT-1 in the rat somatosensory cortex. *Cerebral Cortex, 12*, 515–525.

Curtis, D. R., Phillis, J. W., & Watkins, J. C. (1959). Chemical excitation of spinal neurons. *Nature, 183*, 611–612.

Danbolt, N. C. (2001). Glutamate uptake. *Progress in Neurobiology, 65*, 1–105.

Danbolt, N. C., Pines, G., & Kanner, B. I. (1990). Purification and reconstitution of the sodium- and potassium-coupled glutamate transport glycoprotein from rat brain. *Biochemistry, 29*, 6734–6740.

Danbolt, N. C., Storm-Mathisen, J., & Kanner, B. I. (1992). An [Na^+ + K^+]coupled L-glutamate transporter purified from rat brain is located in glial processes. *Neuroscience, 51*, 295–310.

Dugan, L. L., & Kim-Han, J. S. (2006). Hypoxic-ischemic brain injury and oxidative stress. In G. J. Siegel, R. W. Albers, S. T. Brady, & D. L. Price (Eds.), *Basic neurochemistry-molecular, cellular, and medical aspects* (pp. 559–572). New York: Elsevier-Academic Press.

Gegeslashvili, G., & Schousboe, A. (1997). High affinity glutamate transporters: Regulation of expression and activity. *Molecular Pharmacology, 52*, 6–15.

Genda, E. N., Jackson, J. G., Sheldon, A. L., Locke, S. F., Greco, T. M., O'Donnell, J. C., Spruce, L. A., Xiao, R., Guo, W., Putt, M., Seeholzer, S., Ischiropoulos, H., & Robinson, M. B. (2011). Co-compartmentalization of the astroglial glutamate transporter, GLT-1, with glycolytic enzymes and mitochondria. *Journal of Neuroscience, 31*, 18275–18288.

Hertz, L., Yu, A. C. H., Kala, G., & Schousboe, A. (2000). Neuronal-astrocytic and cytosolic-mitochondrial metabolite trafficking during brain activation, hyperammonemia and energy deprivation. *Neurochemistry International, 37*, 83–102.

Hertz, L., Peng, L., & Dienel, G. A. (2007). Energy metabolism in astrocytes: High rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *Journal of Cerebral Blood Flow and Metabolism, 27*, 219–249.

Ikemoto, A., Bole, D. G., & Ueda, Y. (2003). Glycolysis and glutamate accumulation into synaptic vesicles. Role of glyceraldehyde phosphate dehydrogenase and 3-phosphoglycerate kinase. *Journal of Biological Chemistry, 278*, 5929–5940.

Ikonomidou, C., & Turski, L. (1995). Glutamate in neurodegenerative disorders. In T. W. Stone (Ed.), *CNS neurotransmitters and neuromodulators: Glutamate* (pp. 253–272). New York: CRC Press.

Kreft, M., Bak, L. K., Waagepetersen, H. S., & Schousboe, A. (2012). Aspects of astrocyte energy metabolism, amino acid neurotransmitter homeostasis and metabolic compartmentation. *ASN Neuro, 4*, 187–199.

Lehre, K. P., Levy, L. M., Ottersen, O. P., Storm-Mathisen, J., & Danbolt, N. C. (1995). Differential expression of two glial glutamate transporters in the rat brain: Quantitative and immunocytochemical observations. *Journal of Neuroscience, 15*, 1835–1853.

Levy, L. M., Lehre, K. P., Rolstad, B., & Danbolt, N. C. (1993). A monoclonal antibody raised against an [Na(+) + K(+)]coupled L-glutamate transporter purified from rat brain confirms glial cell localization. *FEBS Letters, 317*, 79–84.

Levy, L. M., Warr, O., & Attwell, D. (1998). Stoichiometry of the glial glutamate transporter GLT-1 expressed inducibly in a Chinese hamster ovary cell line selected for low endogenous Na+ -dependent glutamate uptake. *Journal of Neuroscience, 18*, 9620–9628.

Lovatt, D., Sonnewald, U., Waagepetersen, H. S., Schousboe, A., He, W., Lin, J. H., Han, X., Takano, T., Wang, S., Sim, F. J., Goldman, S. A., & Nedergaard, M. (2007). The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. *Journal of Neuroscience, 27*, 12255–12266.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium-L-glutamate on the inner layers of retina. *AMA Archives of Ophthalmology, 58*, 193–201.

Maycox, P. R., Deckwerth, T., Hell, J. W., & Jahn, R. (1988). Glutamate uptake by brain synaptic vesicles. *Journal of Biological Chemistry, 263*, 15423–15428.

McKenna, M. C., Dienel, G. A., Sonnewald, U., Waagepetersen, H. S., & Schousboe, A. (2012). Energy metabolism of the brain. In G. J. Siegel, R. W. Albers, S. T. Brady, & D. L. Price (Eds.), *Basic neurochemistry-molecular, cellular, and medical aspects* (pp. 200–231). New York: Elsevier-Academic Press.

Mielke, J. G., & Wang, Y. T. (2005). Insulin exerts neuroprotection by counteracting the decrease in cell-surface $GABA_A$ receptors following oxygen-glucose deprivation in cultured cortical neurons. *Journal of Neurochemistry, 92*, 103–113.

Nicholls, D., & Attwell, D. (1990). The release and uptake of excitatory amino acids. *Trends in Pharmacological Sciences, 11*, 462–468.

Nicoletti, F., Meek, J. L., Ladoralo, M. J., Chuang, D. M., Roth, B. L., & Costa, E. (1986a). Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus. *Journal of Neurochemistry, 46*, 40–46.

Nicoletti, F., Iadorola, M., Wroblewski, J. T., & Costa, E. (1986b). Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: developmental changes and interaction with α_1-adrenoceptors. *Proceedings of the National Academy of Sciences, 83*, 1931–1935.

Nicoll, R. A. (2004). My close encounter with $GABA_B$ receptors. *Biochemical Pharmacology, 68*, 1667–1674.

Norenberg, M. D., & Martinez-Hernandez, A. (1979). Fine structural localization of glutamine synthetase in astrocytes of rat brain. *Brain Research, 161*, 303–310.

Obel, L. F., Müller, M. S., Walls, A. B., Sickmann, H. M., Bak, L. K., Waagepetersen, H. S., & Schousboe, A. (2012). Brain glycogen-new perspectives on its metabolic function and regulation at the cellular level. *Frontiers Neuroenergetics*. doi:10.3389/fnene.2012.00003.

Olney, J. W., & Ho, O. L. (1970). Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature, 227*, 609–610.

Olney, J. W., Ho, O. L., & Rhee, V. (1971). Cytotoxic effects of acidic and sulphur –containing amino acids on the infant mouse central nervous system. *Experimental Brain Research, 14*, 61–76.

Parpura, V., Heneka, M. T., Montana, V., Oliet, S. H., Schousboe, A., Haydon, P. G., Stout, R. F., Jr., Spray, D. C., Reichenbach, A., Pannicke, T., Pekny, M., Pekna, M., Zorec, R., & Verkhratsky, A. (2012). Glial cells in (patho)physiology. *Journal of Neurochemistry, 121*, 4–27.

Pellerin, L., & Magistretti, P. J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc. Natl. Acad. Sci. 91*, 10625–10629.

Phillis, J. W., Ren, J., & O'Regan, M. H. (2000). Transporter reversal as a mechanism of glutamate release from the ischemic rat cerebral cortex: studies with DL-threo-beta-benzyloxyaspartate. *Brain Research, 868*, 105–112.

Pines, G., Danbolt, N. C., Bjøråas, M., Zhang, Y., Benhadan, A., Eide, L., Koepsell, H., Storm-Mathisen, J., Seeberg, E., & Kanner, B. I. (1992). Cloning and expression of a rat brain L-glutamate transporter. *Nature, 360*, 464–467.

Quistorff, B., Secher, N. H., & Van Lieshout, J. J. (2008). Lactate fuels the human brain during exercise. *The FASEB Journal, 22*, 3443–3449.

Rose, E. M., Koo, J. C. P., Antflick, J. E., Ahmed, S. M., Angers, S., & Hampson, D. R. (2009). Glutamate transporter coupling to Na,K-ATPase. *Journal of Neuroscience, 29*, 8143–8155.

Rothstein, J. D., Martin, L., Levey, A. I., Dykes-Hoberg, M., Jin, L., Wu, D., Nash, N. & Kuncl, R. W. (1994). Localization of neuronal and glial glutamate transporters. *Neuron, 13*, 713–725.

Rowley, N. M., Madsen, K. K., Schousboe, A. & White, H. S. (2012). Glutamate and GABA synthesis, release, transport and metabolism as targets for seizure control. *Neurochem. Int. 61*, 546–558.

Schmitt, A., Asan, E., Puschel, B., & Kugler, P. (1997). Cellular and regional distribution of the glutamate transporter GLAST in the CNS of rats: nonradioactive in situ hybridization and comparative immunocytochemistry. *Journal of Neuroscience, 17*, 1–10.

Schousboe, A. (1981). Transport and metabolism of glutamate and GABA in neurons and glial cells. *International Review of Neurobiology, 22*, 1–45.

Schousboe, A., & Frandsen, Å. (1995). Glutamate receptors and neurotoxicity. In T. W. Stone (Ed.), *CNS neurotransmitters and neuromodulators: Glutamate* (pp. 239–251). New York: CRC Press.

Schousboe, A., & Waagepetersen, H. S. (2005). Role of astrocytes in glutamate homeostasis: Implications for excitotoxicity. *Neurotoxicity Research, 8*, 221–225.

Schousboe, A., Bak, L. K., Sickmann, H. M., Sonnewald, U., & Waagepetersen, H. S. (2007). Energy substrates to support glutamatergic and GABAergic synaptic function: Role of glycogen, glucose and lactate. *Neurotoxicity Research, 12*, 263–268.

Schousboe, A., Sickmann, H. M., Walls, A. B., Bak, L. K., & Waagepetersen, H. S. (2010). Functional importance of the astrocytic glycogen-shunt and glycolysis for maintenance of an intact intra/extracellular glutamate gradient. *Neurotoxicity Research, 18*, 94–99.

Schousboe, A., Sickmann, H. M., Bak, L. K., Schousboe, I., Jajo, F. S., Faek, S. A., & Waagepetersen, H. S. (2011). Neuron-glia interactions in glutamatergic neurotransmission: Roles of oxidative and glycolytic adenosine triphosphate as energy source. *Journal of Neuroscience Research, 89*, 1926–1934.

Schousboe, A., Bak, L. K., Madsen, K. K., & Waagepetersen, H. S. (2012). Amino acid neurotransmitter synthesis and removal. In H. Kettenmann & R. R. Ransom (Eds.), *Neuroglia* (3rd ed., pp. 443–456). Oxford: Oxford University Press.

Schulte, S., & Stoffel, W. (1995). UDP galactose:ceramide galactosyltransferase and glutamate aspartate transporter-copurification, separation and characterization of the two glycoproteins. *European Journal of Biochemistry, 233*, 947–953.

Schwartz-Bloom, R. D., & Sah, R. (2001). Gamma-Amino butyric acid (A) neurotransmission and cerebral ischemia. *Journal of Neurochemistry, 77*, 353–371.

Sladeczek, F., Pin, J. P., Recasens, M., Bockaert, J., & Weiss, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurons. *Nature, 317*, 717–719.

Sonnewald, U., Westergaard, N., & Schousboe, A. (1997). Glutamate transport and metabolism in astrocytes. *Glia, 21*, 56–63.

Storck, T., Schulte, S., Hofmann, K., & Stoffel, W. (1992). Structure, expression, and functional analysis of a Na^+-dependent glutamate/aspartate transporter from rat brain. *Proceedings of National Academy of Sciences USA, 89*, 10955–10959.

Waagepetersen, H. S., Sonnewald, U., & Schousboe, A. (2009). Energy and amino acid neurotransmitter metabolism in astrocytes. In V. Parpura & P. G. Haydon (Eds.), *Astrocytes in (Patho)physiology of the Nervous System* (pp. 177–199). Boston: Springer.

Yu, A. C. H., Drejer, J., Hertz, L., & Schousboe, A. (1983). Pyruvate carboxylase activity in primary cultures of astrocytes and neurons. *Journal of Neurochemistry, 41*, 1484–1487.

Glutamatergic Receptors in Parkinson's Disease

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Abstract

Extracellular concentrations of the excitatory glutamate are maintained at relatively low levels to avoid an excessive activation of glutamate receptors (GluRs) that can trigger a cell death. This neuronal loss induced by hyperexcitation of GluRs has been associated with a wide range of acute and chronic neurological disorders such as Parkinson's disease (PD). In fact, altered glutamatergic neurotransmission and metabolical dysfunction seems to be crucial in the pathophysiology of PD. Moreover, degeneration of dopamine nigral neurons of the substantia nigra *pars compacta* (SNpc) provokes striatal dopaminergic denervation and a cascade of functional modifications in the activity of basal ganglia nuclei including excitatory glutamate transmission. Glutamate receptors are divided into two distinct groups, ionotropic and metabotropic receptors. The ionotropic receptors (iGluRs) are further subdivided into three groups: (i) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), (ii) kainate, and (iii) *N*-methyl-D-aspartate (NMDA) receptor channels. The metabotropic receptors (mGluRs) are coupled to GTP-binding proteins (G proteins) and regulate the production of intracellular messengers. In this chapter, we review recent progress in the research of GluRs with special emphasis on the molecular diversity of the mGluR system and its implications in the physiopathology of PD. Finally, based on these evidences, we highlight possible therapeutic strategies that might be important to slow down the progression of PD as well as to modulate levodopa-induced dyskinesias.

Keywords

AMPA • GluRs • Glutamate • Neurotoxicity • NMDA • Parkinson's disease • Substantia Nigra

1 Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor symptoms including tremor and bradykinesia (Dauer and Przedborski 2003; Schapira 2009). The primary pathophysiology underlying PD is the degeneration of dopaminergic neurons in the substantia nigra *pars compacta* (SNpc) (Jellinger 2002). Loss of these neurons causes pathological changes in neurotransmission in the basal ganglia circuits (Carlsson et al. 1957). The ability of ionotropic and metabotropic glutamate receptors to modulate neurotransmission throughout the basal ganglia suggests that these receptors may be targets for reversing the effects of altered neurotransmission in PD (Blandini and Armentero 2012; Leaver et al. 2008). Studies in animal models suggest that modulating the activity of these receptors may alleviate the primary motor symptoms of PD as well as the side effects induced by dopamine replacement therapy (Gardoni et al. 2012; Konitsiotis et al. 2000). Moreover, glutamate receptor ligands may slow disease progression by delaying progressive dopamine neuron degeneration

(Fell et al. 2009; Johnson et al. 2009). Antagonists of N-methyl-D-aspartate (NMDA) receptors have shown promise in reversing motor symptoms, levodopa-induced dyskinesias, and neurodegeneration in preclinical PD models (Blandini and Armentero 2012; Johnson et al. 2009). The effects of drugs targeting α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) receptors are more complex; while antagonists of these receptors exhibit utility in the treatment of levodopa-induced dyskinesias, AMPA receptor potentiates show promise for neuroprotection (Johnson et al. 2009; Kobylecki et al. 2010). Pharmacological modulation of metabotropic glutamate receptors (mGluRs) may hold even more promise for PD treatment due to the ability of mGluRs to fine-tune neurotransmission. Antagonists of mGluR5, as well as activators of group II mGluRs and mGluR4, have shown promise in several animal models of PD (Johnson et al. 2009). As these drugs reverse motor deficits in addition to providing protection against neurodegeneration, glutamate receptors therefore represent exciting targets for the development of novel pharmacological therapies for PD. However, several approaches have been undertaken concluding in few results remarkable for humans. Nonselective blockade induces unacceptable psychiatric and cognitive adverse effects, while selective blockade is still under investigation with little benefit to date (Fantin et al. 2008; Hallett and Standaert 2004). The arduousness of finding an appropriate pharmacological therapy targeting NMDA receptors prompted much interest on metabotropic glutamate receptors (mGluRs) as novel targets for the management of PD.

2 Glutamate Receptor Subtypes

2.1 Ionotropic Glutamate Receptors

There are three major types of ionotropic glutamate receptors (iGluRs), which are named after the agonists that were originally identified to activate them selectively, and are, thus, called NMDA, AMPA, and 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (kainate) receptors (Fonnum 1984; Kostandy 2012). Native receptors of all of these families are likely heteromeric assemblies comprising more than one type of subunit (Kew and Kemp 2005). Although the average overall amino acid identity of ionotropic glutamate receptor subunits across the three families is only in the 20–30 % range, they share common structural features, which clearly place them into a single large superfamily. The transmembrane topology of the glutamate receptor family differs from the classic four transmembrane domain model of the nicotinic acetylcholine receptor, which has extracellular amino- and carboxy-termini and a large intracellular loop between transmembrane domains 3 and 4 (Traynelis et al. 2010). Ionotropic glutamate receptor subunits possess an extracellular amino-terminal domain, which exhibits homology to the metabotropic glutamate receptor (mGluR) bilobed agonist-binding domain, followed by a first transmembrane domain, and then a pore-forming membrane-residing domain that does not cross the membrane but forms a reentrant loop entering from and exiting to the cytoplasm (Fonnum 1984). The second and third

transmembrane domains are linked by a large extracellular loop, and the third transmembrane domain is followed by an intracellular carboxy-terminus (Dingledine et al. 1999; Mayer and Armstrong 2004). The crystal structure of the iGluR ligand-binding domains, which comprise polypeptides in both the amino-terminus (S1 domain) and the extracellular loop between transmembrane domains 3 and 4 (S2 domain), has confirmed this topology model for the ionotropic glutamate receptor family (Armstrong et al. 1998; Mayer and Armstrong 2004). Several studies have shown an implication of NMDA and AMPA receptors in PD alterations but not kainate receptors, so we haven't focused on them in this chapter.

2.1.1 NMDA Receptors

N-methyl-D-aspartate (NMDA) glutamate receptors are excitatory receptors widely involved in the basal ganglia circuitry and therefore constitute a promising target for the development of new drugs to prevent or treat PD (Hallett and Standaert 2004). NMDA receptors are ligand-gated ion channels composed of multiple subunits, which have distinct cellular and regional patterns of expression. NMDA receptors are widely found in all of the structures implicated in basal ganglia circuits, although there is a different composition of its units depending on the physiology of the circuit. For example, NMDA receptors present in the striatum, which mainly are located postsynaptically on striatal neurons, are crucial for dopamine-glutamate interactions since the abundance, structure, and function of these striatal receptors are modified by dopamine depletion and they could be further varied by pharmacological treatments used in PD (Hallett et al. 2005). In animal models, NMDA receptor antagonists are effective antiparkinsonian agents and can reduce the complications of chronic dopaminergic therapy (wearing off and dyskinesias) (Del Dotto et al. 2001). Use of these agents in humans has been limited because of the adverse effects associated with nonselective blockade of NMDA receptor function, but the development of more potent and selective pharmaceuticals holds the promise of important new therapeutic approach for PD.

2.1.2 AMPA Receptors

AMPA receptors are mainly implicated in most of the excitatory neurotransmission in the brains of mammals and also taking part in synaptic plasticity processes thought to form the base of memory and learning and the organization of neural networks during embryogenesis (O'Neill and Witkin 2007). It has been shown, using molecular cloning techniques, that the AMPA receptor family has a group of four different subunits named GluR1–4 being native AMPA receptors most likely tetramers produced by the assembly of one or more of these subunits generating homomeric or heteromeric receptors (Santos et al. 2009). Experimental data indicate that the modulation of AMPA receptors, correlated with an increased hippocampal activity, could have a crucial role in the therapeutic treatment of cognitive deficits (Chappell et al. 2007). Moreover, clinical studies have indicated that AMPA receptor modulators enhance cognitive function not only in elderly subjects but even in neurological and psychiatric patients (Lees 2000). The pathway in which AMPA receptors is implicated

is still known, but it has been postulated, using in vitro and in vivo studies, that AMPA receptors could induce the activation of a protein tyrosine kinase, Lyn, which would recruit the mitogen-activated protein kinase (MAPK) signaling pathway increasing the expression of BDNF (Hayashi et al. 1999; Zhang et al. 2010). In conclusion, several selective and systemically active AMPA receptor potentiators have been reported which main functions are to modulate glutamatergic transmission, to enhance synaptic transmission and long-term potentiation, and to increase neurotrophin expression (O'Neill et al. 2004). All these functions situate AMPA receptor potentiators as a new potential class of drugs effective for treating cognitive impairments and depression and stimulating neuroprotective effects in PD.

2.2 Metabotropic Receptors

Glutamate receptors are a group of G protein-coupled receptors (GPCRs), which are differentially localized throughout the basal ganglia and could be a good therapeutic target for PD treatment (Waxman et al. 2007). Glutamate receptors regulate excitatory and inhibitory synaptic transmission acting on synaptic mechanisms (Waxman and Lynch 2005). Structurally, we can difference eight subtypes of mGluRs, divided into three groups according to their sequence homology and G protein-coupling specificity (Conn et al. 2005; Conn and Pin 1997; Pin and Acher 2002; Pin and Duvoisin 1995). Group I mGluRs include mGluR1 and mGlu5, both of them activate phospholipase and protein kinase C and raise intracellular calcium levels. Group II mGluRs consist of two mGluR subtypes (GluR2 and GluR3) which mainly inhibit adenylyl cyclase. Group III mGluRs include mGluR4, mGluR6, mGluR7, and mGluR8, which structurally are located presynaptically, and functionally act causing a reduction in neurotransmitter release (Waxman et al. 2007). mGluRs are the most relevant glutamate receptors implicated in therapeutic strategies for PD treatment at present and will be widely reviewed in this work.

3 Distribution of Glutamatergic Receptors and Excitotoxicity

In spite of its ubiquitous role as an excitatory neurotransmitter in the intermediary metabolism in the brain, glutamate can be highly toxic to neurons, a phenomenon known as "excitotoxicity." This neuronal death is a result of an excessive stimulation of the NMDA receptor and the subsequent massive influx of extracellular Ca^{+2}. This increase in cytoplasmic Ca^{+2} activates a number of Ca^{+2}-dependent enzymes implicated in the catabolism of phospholipids, proteins, nucleic acids, and the nitric oxide synthase activity. All this events lead to a global breakdown of cellular homeostasis including membrane rupture, cytoskeletal alterations, and nitric and oxide free-radical production (Koutsilieri and Riederer 2007) (Fig. 1). During the last decade numerous reviews have addressed the role of glutamate receptors and related synaptic mechanisms in preclinical research on neurodegenerative diseases

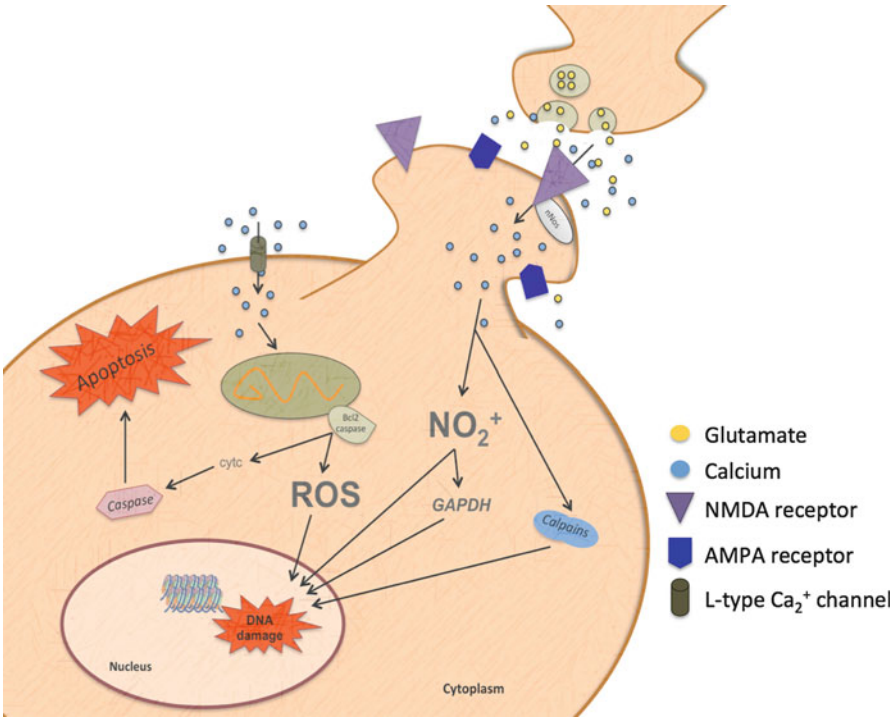

Fig. 1 Excitotoxicity of glutamate release and its implication in synaptic plasticity. One of the major functions of glutamate receptors, both metabotropic and ionotropic receptors, appears to be the modulation of synaptic plasticity. Overstimulation of NMDA receptor increases Ca^{2+} influx into the cell (neuron) which leads to the breakdown of cellular homeostasis, the lost of mitochondria function, and the release of nitric oxide and other free radicals

(Johnson et al. 2009; Lau and Tymianski 2010; Luscher and Huber 2010; Ondrejcak et al. 2010; Palop and Mucke 2010a, b; Randall et al. 2010).

3.1 Ionotropic Receptors

3.1.1 NMDA Receptors

Studies designed to determine NMDA receptor distribution have shown that these receptors are found throughout the brain but predominantly within the forebrain (Bean et al. 2006; Beckerman et al. 2012; Collingridge et al. 2013; Monaghan et al. 1998) (Fig. 2). Furthermore, quantitative comparisons of the distribution of NMDA receptors determined by the binding sites of the different ligands have suggested the presence of multiple pharmacologically distinct types of NMDA receptors (Monaghan and Jane 2009). Following the molecular cloning of NMDA receptor subunits, the distribution of each subunit was examined using in situ hybridization, histochemistry, and biochemical analysis using subunit-specific antibodies

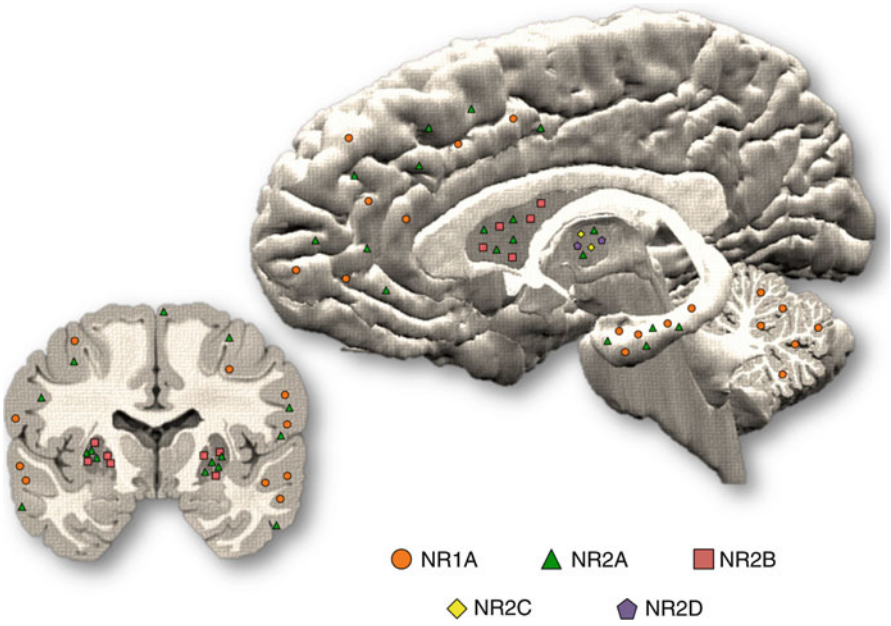

Fig. 2 Distribution of ionotropic glutamate receptors in the human brain. Using the binding sites of different ligands, the ubiquitous distribution of ionotropic glutamate receptors in the nervous system especially in the forebrain has been determined

(Dunah et al. 1999; Yamada and Nabeshima 1998). The NR1A mRNA is distributed widely in the brain but more so in the cerebral cortex, hippocampus, and cerebellum (Ehlers et al. 1995; Hara and Pickel 2008). The NR2A transcript is selectively present in the forebrain with a high-level expression in the cerebral cortex, hippocampus, septum, caudate-putamen, and olfactory bulb (Ling et al. 2012). The NR2B mRNA is expressed predominantly in the granule cell layer of the cerebellum, with weak expression in the olfactory bulb and thalamus (Jarzylo and Man 2012; Kaufmann et al. 2012). In sum, while NR2A and NR2B transcripts are found in a subset of hippocampal neurons, most likely interneurons (Kuppenbender et al. 2000), low levels of the NR2D transcript are detected in the thalamus, brain stem, and olfactory bulb, being predominant in the supraoptic nucleus (Doherty and Sladek 2011).

The location of NMDARs is also related with its signaling: (i) intrasynaptic NMDAR location is involved in neuronal survival, and (ii) extrasynaptic NMDARs have a crucial role in cell-death pathways (Papadia et al. 2005). Among other neurological alterations, NMDA receptor dysfunction may be a primary factor in the aetiology of schizophrenia, where NMDA receptor antagonists induce cortico-limbic degeneration and induct psychotic states, thus hastening a neurotoxicity and neuronal degeneration, which can be hampered using dopaminergic antagonists (Dawson et al. 2012).

3.1.2 AMPA Receptors

It has been shown that AMPA receptors in glutamatergic synapses mediate the transmission of low frequency and are involved in the expression of long-term potentiation (LTP) and long-term depression (LTD) processes, considered the cellular correlates of memory formation (Bliss and Collingridge 1993). At early time points of human embryogenesis, most of excitatory synapses are thought to be postsynaptically "silent," since their functional receptors are NMDA receptors but not AMPA ones (Daw et al. 2007; Isaac et al. 1995; Li and Zhuo 1998; Liao et al. 1995; Scott et al. 2007). Such selective pattern of synaptic activation has been termed "silent synapses" because the NMDA pore is largely hampered at resting membrane potentials (Isaac et al. 1995; Nowak et al. 1984; Scott et al. 2007). Following protocols that generate LTP, however, these functionally inhibited synapses rapidly become AMPA receptor responsive (Isaac et al. 1995; Liao et al. 1995). One possible mechanism to explain this observation is a synaptic transfer of a pool of preassembled AMPA receptors from an intracellular compartment to the postsynaptic membrane. Then, this intimate relation between AMPA and NMDA receptor is the basis of the search of alternative pathways to prevent the activation derived from an excessive NMDA excitotoxicity: NMDA antagonists, as AMPA-type receptors, have been tested and have demonstrated beneficial effects on the damage to the white substance covering the axons of neurons (Park et al. 2003). Its efficacy in preclinical models of stroke is greater than some NMDA receptors' antagonists, but some of these compounds exhibit problems of solubility or adverse effects, primarily by inhibiting the fast excitatory synaptic transmission (Clarkson et al. 2011). Excitotoxicity mediated by the alterations of AMPA receptor has been implicated in the selective vulnerability of motor neurons in amyotrophic lateral sclerosis (ALS) (Corona and Tapia 2007), in the induction of neurological deficits including schizophrenia (Tsai and Coyle 2002), and it could also underlie the cognitive deficits related with neurodegenerative diseases such as Alzheimer's disease (Kamenetz et al. 2003).

3.2 Metabotropic Receptors

Metabotropic receptors (mGluRs) are expressed throughout the CNS (Fig. 3). However, specific subtypes are expressed in restricted areas, such as the hippocampus where preferential expression of the receptor subtypes $mGluR1_b$, mGluR2/3, $mGluR4_a$, and mGluR5 has been demonstrated (Blumcke et al. 1996). mGluR3 is expressed widely in the CNS (Makoff et al. 1996a, b). mGluR4 expresses different subtypes in the thalamus, hypothalamus, and caudate nucleus, and mGluR6 is expressed in the retina (Vardi et al. 2000) (Fig. 2).

3.2.1 Group I mGluRs

Both mGlu1 and mGlu5 are most often located postsynaptically, where they are related to neuronal depolarization and excitability (Lujan et al. 1997). Nevertheless, in the midbrain, mGluR1 induces hyperpolarization of dopamine neurons

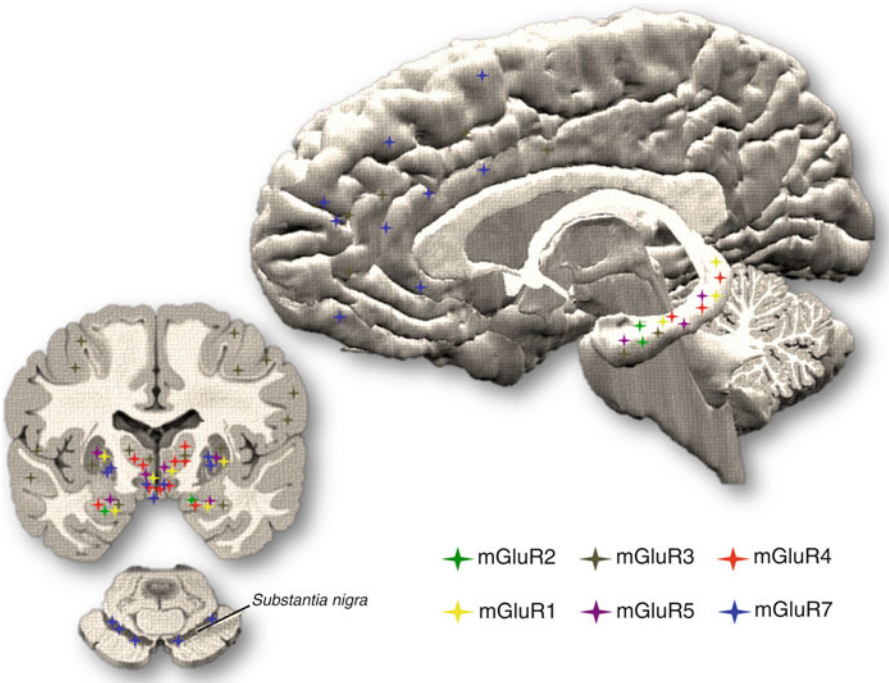

Fig. 3 Distribution of metabotropic glutamate receptors in the human brain. Metabotropic receptors are ubiquitously localized throughout the CNS, although its different subtypes are specifically expressed in the hippocampus, thalamus, and striatum

(Katayama et al. 2003). Both receptors are primarily coupled to Gq/G11 proteins (Masu et al. 1991) and have been implicated in some human disorders such as inherited ataxia and cerebellar disorders, associated with an altered expression of mGlu1 receptors in Purkinje cells (Catania et al. 2001; Conti et al. 2006; Sachs et al. 2007). However, although these data suggest that the mGluR1 could be implicated in inherited cerebellar disorders, no inductive alterations on this receptor have been determined in infant idiopathic early-onset ataxia patients (Rossi et al. 2010). Moreover, it has also been shown that mGlu1 receptor antagonists can protect hippocampal neurons against "postischemic" degeneration induced by oxygen-glucose deprivation (Pellegrini-Giampietro 2003). Recent studies have also proposed the blockade of mGlu1 receptors as a novel treatment for schizophrenia, but it has to be corroborated in the next years (Nicoletti et al. 2011).

On the other hand it has been postulated that mGlu5 has a synergic role with NMDA receptors, both acting in the control of the neuronal activity of prefrontal cortex and also being implicated in cognitive dysfunction associated with schizophrenia, being a potential treatment for schizophrenic syndromes (Conn et al. 2009).

3.2.2 Group II mGluRs

Group II mGluRs, mGlu2/3 receptors, are principally present in astrocytes surrounding the neuronal soma and synapses. They also have a presynaptic distribution inhibiting neurotransmitter release and leading to neuronal resting (Liu et al. 1998). These receptors are presynaptically localized on subthalamic nucleus terminals, and their activation inhibits excitatory transmission at subthalamic synapses resulting in a reduced excitatory drive through the indirect pathway (Murray et al. 2002).

Although both receptors, mGlu2 and mGluR3, are mostly expressed in the CNS, they have differences in their location, so while mGluR2 is uniquely in the preterminal region of the axons, far from the active zone of the neurotransmitter release (Lujan et al. 1997; Ohishi et al. 1993a; Tamaru et al. 2001), mGlu3 receptor is expressed not only in presynaptic and postsynaptic sites but also in glial cells (Ohishi et al. 1993b; Tamaru et al. 2001). Functionally, both receptors are implicated in the same human disorders. Both dysfunctions in the mGlu2/3 receptors are correlated with the following: (i) anxiety, both receptors regulate the synaptic transmission and plasticity in the amygdala (Lin et al. 2005; Wang and Gean 1999); (ii) schizophrenia, the hyperglutamatergic theory of schizophrenia has been corroborated using glutamate agonist in experimental animal models, and mGluR2/3 receptor agonists have been developed as antipsychotic drugs (Aghajanian and Marek 1999); and (iii) depression, the systemic treatment with a selective mGlu2/3 receptor antagonist has beneficial effects on this mental disorder (Feyissa et al. 2010). Apart from these effects described, mGluR2/3 receptors have a special significance in neurodegenerative diseases, as the inhibition of mGlu2 receptors decreases excitotoxic death, and mGlu3 receptors are also implicated in neuroprotective pathways (Corti et al. 2007).

3.2.3 Group III mGluRs

Group III mGluRs are identified in microglia and astrocytes as well as in presynaptic membrane like mGluR7 receptor (Geurts et al. 2005; Niswender and Conn 2010). This specific subcellular distribution is not still but modified during the development of the CNS and may play a key role for its proper progressive organization: (i) mGluR7 and mGluR8 are presynaptically located in the striatum, the globus pallidus, and in the substantia nigra pars reticulata (SNpr), where their activation inhibits GABA as well as glutamate transmission (Kosinski et al. 1999), and (ii) mGluR4 appears to be more selectively located in striatopallidal synapses and inhibits synaptic activity at this level (Valenti et al. 2003). In relation with this variety on location, glutamate also presents a changeable affinity for these receptors since it shows a high affinity to mGluR4 and mGluR8 but a very low one to mGluR7 (Schoepp et al. 1999). Apart from their implication in Parkinson's disease which would be further intensely described, these receptors have shown a significant relevance in human disorders such as (i) anxiety: it has demonstrated the negative modulation of both mGlu8 and mGlu4 receptors in the induced anxiety in rodents (Nicoletti et al. 2011), but mGlu7 has an uncertain role in this disease (Lavreysen and Dautzenberg 2008); and (ii) neuroinflammation and immune misbalanced: mGlu4 receptor has been recently implicated in the crosstalk between

the nervous and immune system, where the activation of this receptor induces the differentiation of antigen specific T cells into T regulatory ones (Fallarino et al. 2010). With regard to mGlu6 expression is not as much ubiquitous in the CNS as the other group III receptors, and physiologically it is almost exclusively implicated in the visual region since it is localized in the dendrites of bipolar cells of the retina acting on the visual physiology in darkness and being part of human visual pathologies (Nawy 2000; Xu et al. 2012).

4 Implication of Glutamate Receptors in Parkinson's Disease

4.1 NMDA Receptors as a Therapy for Parkinson's Disease

Under physiological conditions there is a neurotransmitter balance in striatum between the activation of striatal neurons through NMDA receptors and inhibition by the D2 receptors (Di Chiara et al. 1994). In PD, the depletion of nigrostriatal dopamine results in disinhibition of the striatal neurons and therefore in a relative glutamatergic overactivity (Greenamyre 1993). Moreover, the dopaminergic deficit results in enhanced activity of the STN which projects to the output nuclei of the basal ganglia rendering them overactive (Rodriguez et al. 1998). These known regulations classify PD as "a secondary glutamate overactivity syndrome" (Riederer et al. 2001) (Fig. 4). Changes in abundance of NMDA subunits in striatum have been described in dopamine-denervated rats as well as in MPTP-treated parkinsonian monkeys (Betarbet et al. 2004; Hallett et al. 2005). In the 6-OHDA-lesioned rat model, dopamine depletion results in alterations in abundance of NR1 and NR2B subunits in striatal synaptosomal membrane fractions, while NR2A is unchanged (Hallett and Standaert 2004). This was shown to be due to a selective reduction of complexes composed of NR1/NR2B without any change in receptors composed of NR1/NR2A. Chronic levodopa treatment of 6-OHDA-lesioned rats restores the abundance of NR1, NR2A, and NR2B subunits in homogenate and synaptosomal membrane fractions (Hallett and Standaert 2004). Similar redistribution occurs in the MPTP-lesioned macaque where the dopamine depletion induced by MPTP intoxications resulted in a marked decrease in striatal NR2B subunits, while the upregulation of NR2A expression in synaptosomal membranes may play a pivotal role in levodopa-induced dyskinesias (Hallett et al. 2005).

A large number of studies have examined the efficacy of nonselective NMDA antagonists in animal models of PD showing that NMDA receptor blockade attenuates parkinsonian findings and increases dopaminergic therapy (Lange et al. 1997; Marin et al. 2000). For humans, amantadine has been the mostly studied drug in clinical trials, showing antidyskinetic properties in PD patients with motor complications as well as an increased lifespan (Hubsher et al. 2012). A related compound, memantine, which is approved for treatment of dementia, did not seem to share the efficacy of amantadine in PD (Merello et al. 1999; Varanese et al. 2010). Finally, dextromethorphan, an antitussive, reduces dyskinesias in PD patients, but patients show somnolence (Chen 2011; Jimenez et al. 1999).

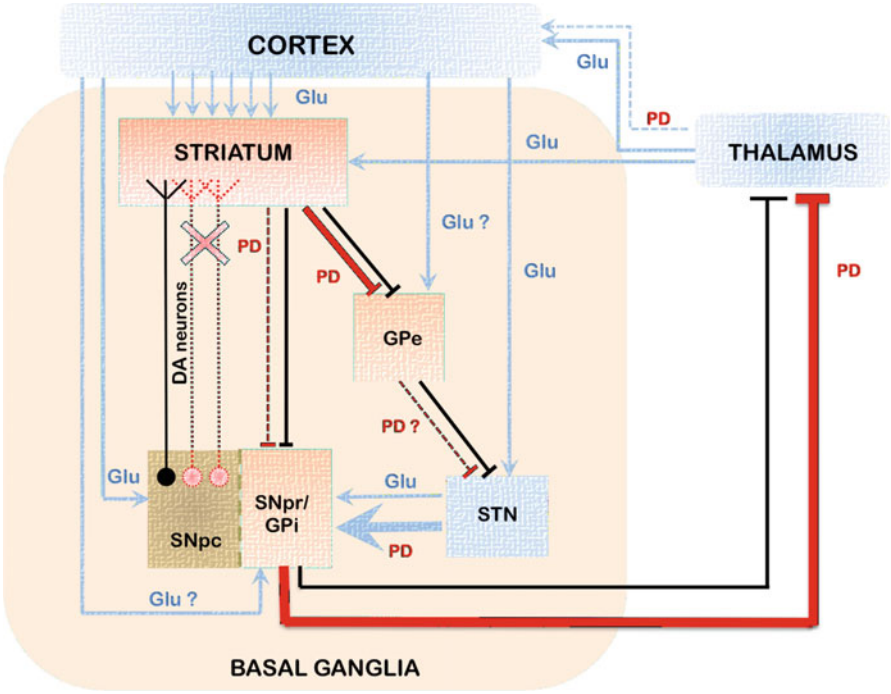

Fig. 4 Glutamatergic pathways involved in Parkinson's disease. In Parkinson's disease the striatal dopaminergic deficit results in an enhanced activity of the subthalamic nucleus which projects to the output nuclei of the basal ganglia rendering them overactive

Nowadays, based on the experimental evidence on the significance of the NMDA receptor subtypes and the limitations in the beneficial effects of the current therapy, future studies should be focused on selective NMDA antagonists in order to achieve powerful antiparkinsonian effects without adverse effects in cognition. This would facilitate cell preservation during excitotoxic processes while not causing complete inhibition of the receptor and therefore allowing physiological neurotransmission.

4.2 AMPA Receptors as a Therapy for Parkinson's Disease

It is difficult to define an antiparkinsonian treatment that targets AMPA receptors since they have dual effects, while AMPAR antagonists provide symptomatic benefits, AMPAR potentiators induces neuroprotection (Johnson et al. 2009). The role of AMPA receptors in dyskinesias has been well described: using animal models such as MPTP-lesioned nonhuman primates and 6-OHDA-lesioned rats, it has been demonstrated that pharmacological blockade of AMPARs decreases levodopa-induced dyskinesias (LIDs) and possibly enhances the antiparkinsonian

effect of levodopa (Bibbiani et al. 2005; Kobylecki et al. 2010; Konitsiotis et al. 2000). On the other hand, AMPAR agonists administered either alone or in combination with levodopa can induce dyskinesias (Konitsiotis et al. 2000). Moreover, using animal models it has been shown that the blockade of AMPA receptors could improve LIDs (Kobylecki et al. 2011; Silverdale et al. 2005). The antidyskinetic effect induced by AMPA receptor blockade derives of its action at the cellular level, as it has been demonstrated that AMPAR antagonists reduce the striatal upregulation of met-enkephalin gene expression induced by DA depletion and lately exacerbated by chronic levodopa administration (Perier et al. 2002). All these data suggest that the pathophysiological adaptations of medium spiny neurons caused by DA depletion and chronic levodopa treatment are mediated by the stimulation of AMPA receptors (Sgambato-Faure and Cenci 2012).

4.3 mGluRs as a Therapy for Parkinson's Disease

Several studies have shown that the pharmacological modulation of mGluRs can ameliorate motor abnormalities in experimental models of PD. There is also evidence of a neuroprotective effect against glutamatergic toxicity, to date in a lesser degree as with iGluRs. To avoid an excessive extension of the text and facilitate its comprehension, we will exclude the full-extension names of the molecules used in the different studies.

4.3.1 Group I mGluRs Studies

Inhibition of group I mGluRs (mGluR1 and mGluR5) seems to be promising in PD therapy (Fig. 5). The role of group I mGluRs leading to excitotoxicity and their antagonist protective properties have been proved in non-PD models of neurodegeneration (Rao et al. 2000). There is high experimental evidence of the contribution of group I mGluRs to posttraumatic or postischemic neuronal excitotoxicity. Selective mGluR5 antagonists such as MPEP or the more selective MTEP are known neuroprotective agents. Whether this effect is mediated by mGluR5 or by off-target inhibition of postsynaptic NMDAR is controversial (Lea et al. 2005). In a striatal model of excitotoxicity, MPEP did show a neuroprotective effect, but the direct blockade of NMDA receptors appeared not to be involved (Popoli et al. 2004). The role of mGluR1 ligands in excitotoxicity is not still clear. Whereas the antagonists have shown neuroprotective activity (Faden et al. 2001), the agonists may facilitate either neurotoxicity or neuroprotection (Allen et al. 2000; Nicoletti et al. 1999). EMQMCM, a novel, highly selective uncompetitive antagonist of mGlu1 receptors, has demonstrated significant effects against kainate-induced excitotoxicity in both in vitro (in the cortical and hippocampal neuron) and in vivo animal models (Smialowska et al. 2012). In the particular case of basal ganglia, prevalent distribution of mGluR5 in the striatum and in the limbic system is well documented: both subtypes of group I mGluR are postsynaptically expressed by medium spiny neurons (Tallaksen-Greene et al. 1998) as well as by all interneurons, including cholinergic cells. As mGluRs do not cause any effect on the

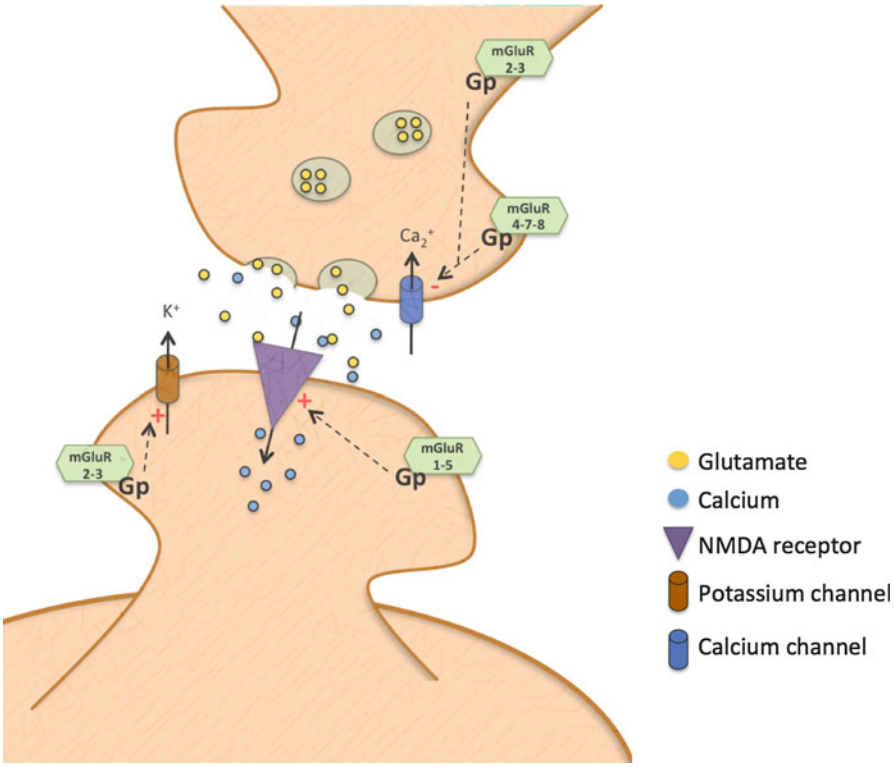

Fig. 5 Pre- and postsynaptic location of different metabotropic glutamate receptors. Among the glutamate receptors, metabotropic glutamate receptors are mostly implicated in Parkinson's disease. mGluR2 and mGluR3 are both pre- and postsynaptically located; however, mGluR4, mGluR7, and mGluR8 are located at the presynaptic level and mGluR1–mGluR5 in the postsynaptic neuron

membrane properties of the spiny neurons, its action should be indirect. Instead, they enhance the cationic currents gated by NMDA receptors (Pisani et al. 2001). Postsynaptic location of $mGluR1_a$ and mGluR5 in the subthalamic nucleus has been demonstrated showing an excitatory effect (Awad et al. 2000). PET scan of the striatum of MPTP parkinsonian monkeys showed an increased binding of mGluR5, and this upregulation of mGluR5 might play a role in disease progression in PD (Sanchez-Pernaute et al. 2008). More specifically, the blockade of mGluR5 was shown to alleviate akinesia in both 6-OHDA- and haloperidol-treated animals (Breysse et al. 2002; Ossowska et al. 2001). In addition, a study showed that mGluR5 knockout mice are less sensitive to MPTP toxicity, and mGluR5 antagonists are effective in reducing the MPTP-induced nigrostriatal damage in wild-type mice (Battaglia et al. 2003). Additionally, chronic and systemic treatment with MPEP, a selective mGluR5 antagonist, has not only anxiolytic-like effects but also neuroprotection as diminished dopaminergic cell loss in the SNpc in 6-OHDA-treated mice and rats (Chen et al. 2011).

4.3.2 Group II mGluRs Studies

Group II and III mGluRs modulate the corticostriatal input which is the major excitatory drive to the basal ganglia (Fig. 5). Activation of presynaptic group II and III mGluRs inhibits glutamatergic transmission in the striatum (Rouse et al. 2000). Moreover, all three groups of mGluRs have shown to depress transmission at glutamatergic synapses in the SNpc modulating the dopaminergic pathway from the SNpc to the striatum (Wigmore and Lacey 1998). Activation of presynaptic mGluRs in the nigrostriatal synapse increases dopamine release in the direct pathway (Rouse et al. 2000). In fact, intranigral or intraventricular injection of the group II mGluRs agonist DCG-IV reduces akinesia in the reserpine-treated rat model of PD. The selective group II mGluRs agonist LY379268 was assessed to provide both functional relief and neuroprotection in the same rodent model of PD (Dawson et al. 2000). In MPTP-lesioned mice, prior treatment with LY379268 reduced the extent of nigrostriatal degeneration. Since now on, electrophysiological studies suggest that group II mGluRs are presynaptically located on STN terminals in the SNpr and the activation of these receptors selectively reduces transmission at excitatory STN synapses. Therefore, LY354740, a highly selective agonist of group II mGluRs, inhibits synaptic excitation of the SNpr (Bradley et al. 2000). However, these results were dependent on the dose and regimen of MPTP administration: (i) they were only observed under conditions in which MPTP produced a massive degeneration of nigral dopaminergic neurons, and there was no effect with lower doses of MPTP; (ii) while systemic administration of LY379268 failed to reverse akinesia or to produce typical antiparkinsonian rotational behavior, intraventricular administration provided a dose-dependent increase in locomotor activity in these rats. Moreover, the group II mGluRs antagonist, LY341495, blocked the protective effect of the LY379268 and amplified the damage produced by MPTP, even with low exposure (Battaglia et al. 2003).

4.3.3 Group III mGluRs Studies

The largest evidence of clinical and pathological antiparkinsonian properties belongs to group III mGluRs. Research with general group III mGluR agonists has been developed. In this instance, intracerebral treatment with the L-SOP and L-AP4 (group III mGluR agonists) significantly reduced the akinesia induced by reserpine in rats (MacInnes et al. 2004). Injections were performed in the SNpr, GP, and intraventricularly. But the main studies have focused on specific group III mGluR subtypes.

Group III mGluRs, mGlu4R, and mGlu7R are expressed in synaptic terminals of the striatum, while there is no evidence about the presence of mGluR8. The two group III mGluR agonists ACPT-I and L-AP4 have shown similar potencies on mGluR4 and mGluR8 and lower affinity for mGluR7. No consistent effects on other mGluRs have been demonstrated. Pallidal injections of each glutamate receptor agonist have antiparkinsonian actions in haloperidol- and 6-OHDA-treated rats (Lopez et al. 2007), but the infusion of the same molecules into the SNpr had opposite effects in controls and 6-OHDA-treated rats, inducing catalepsy. Nevertheless, this akinetic effect is probably mediated by mGluR8 activation and not by

mGluR4. More recently, a new group III orthosteric agonist has been identified: LSP1-3081 activates selectively and dose dependently all group III receptors transiently expressed in HEK293 cells (its potency is equivalent to that of L-AP4). LSP1-3081 was found to display the highest potency on mGlu4 receptor, followed by mGlu8, and very low on mGlu7. It is also devoid of nonspecific agonist or antagonist activity on group I or II mGluRs, and on targets other than mGluRs, it has less nonspecific effect than L-AP4 (Cuomo et al. 2009). The same authors proved that LSP1-3081 and L-AP4 depressed corticostriatal glutamatergic transmission by modulating presynaptic group III mGluRs. Both compounds also inhibited GABA input to striatal MSNs. Presumably, a reduction of glutamatergic activity on subthalamo-pallidal synapses and GABA transmission in the striatopallidal pathway are also implicated in antiparkinsonian and anti-excitotoxicity properties of group III mGluRs activation. In this sense, presynaptic activation of group III mGluRs has been shown to inhibit GABA release in the GP (Matsui and Kita 2003; Valenti et al. 2003), SNpc (Valenti et al. 2005), and SNpr (Wittmann et al. 2001). The effect of L-AP4 on striatopallidal GABAergic transmission was absent in mGluR4 KO mice (Valenti et al. 2003). These data indicate that activation of mGluR4 could decrease (or modulate) the increased inhibition of the GP that has been suggested in PD models. In the same study, direct ventricular injections of L-AP4 produced robust antiparkinsonian effect in both acute (haloperidol-induced catalepsy and reserpine-induced akinesia) and chronic 6-OHDA PD models. Taking into account the low affinity for mGluR7 of the above-described group III mGluR agonists and the fact that mGluR8 has a low distribution in the striatum, mGluR4 is probably the principal vehicle to start the response to group III mGluRs agonists. To confirm the previous hypothesis, the selective mGluR8 agonist (S)-DCPG was tested to inhibit glutamatergic signaling (Cuomo et al. 2009). However, no effects were found. The same tests were performed with L-AP4 and LSP1-3081 in mGluR4 KO mice. There was no modification of postsynaptic currents in the slices of these mice. Meanwhile, in the wild type, LSP1-3081 significantly inhibited the amplitude of postsynaptic currents. Finally, the same researchers demonstrated that intrastriatal L-AP4 injection improves akinesia in 6-OHDA-lesioned rats. The former studies emphasize the importance of subtype-selective group III mGluR agonists as antiparkinsonian drugs. However, the high conservation of the glutamate-binding site makes it difficult to develop highly selective orthosteric ligands for individual mGluR subtypes. To improve specificity, ligands that interact at sites other than the orthosteric one have been developed. These are called positive allosteric modulators (PAMs). In mice histological studies, previous systemic injection of the selective PAM of mGluR4, PHCCC, protects nigrostriatal neurons against MPTP toxicity. In knockout mice for mGlu4R, there was no benefit from PHCCC. Direct GP infusion showed the same results as systemic treatment (Battaglia et al. 2006). Unfortunately, PHCCC has low potency and poor aqueous solubility, and it is also an mGluR1 antagonist with similar potency as mGluR4 (Annoura et al. 1999). Thus, it is not useful for PD.

Using high-throughput screening (HTS), multiple novel PAMs of mGluR4 were identified. The lead from this cluster of structurally related compounds was

VU0155041. Among its particularities, it exhibits submicromolar potency; it is highly selective for mGluR4 relative to other mGluRs and other targets including NMDAR and has an improved aqueous solubility. The same researches demonstrated efficacy of VU0155041 in two rodent models of PD: haloperidol-induced catalepsy and reserpine-induced akinesia (Niswender et al. 2008). A few additional novel chemical mGluR4 PAMs have been introduced later on and have shown some antiparkinsonian activity in both haloperidol-induced catalepsy and reserpine-induced akinesia models (Hopkins et al. 2009). One of them, VU0364770, has exhibited efficacy in reversing haloperidol-induced catalepsy, forelimb asymmetry, and attention deficits in 6-OHDA-lesioned rats. When coadministered with an adenosine 2A receptor antagonist or with levodopa, it also showed potentiation of their efficacy (Jones et al. 2012). The latest could mean a levodopa-sparing role of the compound. In conclusion, mGluR4 positive allosteric modulation is a promising area of research to bring about symptomatic as well as potentially disease-modifying treatment.

Another potential specific target for the management of PD is mGluR7s. While mGluR4 is highly expressed in the striatum and the GP, it is little or not expressed in SN where mGluR7 is predominant (Corti et al. 2002). mGluR7s have also high expression in the striatum and very low pallidal expression. In the striatum, mGluR7s are localized presynaptically on the corticostriatal glutamatergic synapses, where they are found to decrease the glutamatergic tone and attenuate transmission in cholinergic interneurons. AMN082 is an mGluR7 selective PAM (Mitsukawa et al. 2005). Intrastriatal injection of this compound reverses haloperidol- and 6-OHDA-induced parkinsonism in rats and haloperidol-induced Parkinsonism in mice. This effect was absent in mGluR7 knockout mice, demonstrating that it is mediated by mGluR7 (Greco et al. 2010). The anti-cataleptic property of the compound was evident only in narrow doses, causing motor impairment at higher doses. AMN082, at high doses, decreases locomotor activity of mGluR7 knockout and wild-type mice, demonstrating that this negative effect is not mGluR7 related (Palucha et al. 2007; Salling et al. 2008).

Apart from the striatum and GP, the SN is another key location for group III mGluRs. Injection of L-SOP or L-AP4 into the SNpr reversed akinesia in the reserpine-treated rat model, while infusion of L-AP4 in the SNpc prevented the akinesia and the nigrostriatal degeneration in the 6-OHDA-lesioned rat. These responses were each inhibited by pretreatment with the group III mGluRs antagonist CPPG, confirming they were mediated by activation of group III mGluRs (Austin et al. 2010). This effect seems to be mediated by presynaptic inhibition of glutamatergic input from the STN. A similar study explored selective targeting of mGluR4, mGluR7, and mGluR8 in the SNpr. Activation of mGluR4 (with PHCCC in combination with a subthreshold concentration of L-AP4) or mGluR7 (with AMN082 also in combination with a subthreshold concentration of L-AP4) reduced the release of the glutamate analogue, [^{3}H]-D-aspartate, from SNpr tissue prisms in vitro. In contrast, the mGluR8 selective agonist, (S)-3,4-DCPG, failed to provoke the same inhibition. Provided that glutamatergic afferents from the STN comprise the major excitatory input into the SNpr, mGluR4, and mGluR7,

activation may be effective for restricting glutamate release in this location (Broadstock et al. 2012). Reversal of reserpine-induced akinesia was produced following direct injection of PHCCC or AMN082 into the SNpr. Both of them acted in the absence of a subthreshold dose of L-AP4. The effects of both PHCCC and AMN082 were inhibited by CPPG, the selective group III mGluRs antagonist, confirming that these responses are mediated through group III mGluRs. Contrary to what it should be expected, injection of (S)-3,4-DCPG into the SNpr produced a dose-dependent reversal of akinesia. However, pretreatment with CPPG did not inhibit this effect, suggesting it was driven through other mechanisms independent of group III mGluRs.

Group III mGluRs have also a neuroprotective effect by preventing excitotoxicity induced by iGluRs. In this instance, excess of extracellular glutamate activates presynaptic group III mGluRs, this inhibits glutamate release, and then the activation of postsynaptic NMDARs is diminished (Vera and Tapia 2012).

5 Conclusion

In spite of the innumerable preclinical studies conducted in experimental animal models of PD associated with LIDs, such as rodents and nonhuman primates, some of which generated impressive results, the possibility to consistently attenuate or mitigate the development of LIDs in the clinical setting is still an unmet need. This is mostly due to the incomplete understanding of the neural and molecular substrates of this phenomenon, although numerous studies suggest a decisive role of glutamate receptors on this phenomenon. In fact, non-dopaminergic neurotransmitter receptors expressed by striatal neurons, such as specific subtypes of ionotropic (NMDA) and metabotropic (mGluR5) glutamate receptors, play a crucial role in the modulation of corticostriatal inputs, as well as in the modulation of the striatal output to downstream nuclei of the basal ganglia circuit. As consequence, in addition to the changes directly caused by PD, the involvement of these receptors triggers further modifications in basal ganglia functional organization, which set the ground for the functional alterations that underlie LIDs. Based on these concepts, drugs acting not only directly on glutamate receptors as NMDA and mGluR5 but even indirectly on adenosine A2A receptors have been proposed as antidyskinetic agents, showing auspicious effects. Considering that mGluR5 receptors contribute to these receptor mosaics, it is likely that new anti-LID pharmacological strategies will have to rely on compounds capable of acting, simultaneously, on all the components of these receptor mosaics, thereby combining the effects of adenosine and metabotropic receptor antagonists. However, the ubiquitous location of the different subtypes of glutamatergic receptors leaves open new ways of research in the field of PD.

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References

Aghajanian, G. K., & Marek, G. J. (1999). Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S.]. *Brain Research, 825* (1–2), 161–171.

Allen, J. W., Knoblach, S. M., & Faden, A. I. (2000). Activation of group I metabotropic glutamate receptors reduces neuronal apoptosis but increases necrotic cell death in vitro. [Research support, U.S. Gov't, non-P.H.S. research support, U.S. Gov't, P.H.S.]. *Cell Death and Differentiation, 7*(5), 470–476. doi:10.1038/sj.cdd.4400678.

Annoura, H., Nakanishi, K., Uesugi, M., Fukunaga, A., Miyajima, A., Tamura-Horikawa, Y., & Tamura, S. (1999). A novel class of Na+ and Ca^{2+} channel dual blockers with highly potent anti-ischemic effects. *Bioorganic & Medicinal Chemistry Letters, 9*(20), 2999–3002.

Armstrong, N., Sun, Y., Chen, G. Q., & Gouaux, E. (1998). Structure of a glutamate-receptor ligand-binding core in complex with kainate. [Research support, non-U.S. Gov't research support, U.S. Gov't, non-P.H.S. Research support, U.S. Gov't, P.H.S.]. *Nature, 395*(6705), 913–917. doi:10.1038/27692.

Austin, P. J., Betts, M. J., Broadstock, M., O'Neill, M. J., Mitchell, S. N., & Duty, S. (2010). Symptomatic and neuroprotective effects following activation of nigral group III metabotropic glutamate receptors in rodent models of Parkinson's disease. [Research support, non-U.S. Gov't]. *British Journal of Pharmacology, 160*(7), 1741–1753. doi:10.1111/j.1476-5381.2010.00820.x.

Awad, H., Hubert, G. W., Smith, Y., Levey, A. I., & Conn, P. J. (2000). Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. [In vitro research support, non-U.S. Gov't research support, U.S. Gov't, non-P.H.S. Research support, U.S. Gov't, P.H.S.]. *Journal of Neuroscience, 20*(21), 7871–7879.

Battaglia, G., Busceti, C. L., Molinaro, G., Biagioni, F., Traficante, A., Nicoletti, F., & Bruno, V. (2006). Pharmacological activation of mGlu4 metabotropic glutamate receptors reduces nigrostriatal degeneration in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. [Research support, non-U.S. Gov't]. *Journal of Neuroscience, 26*(27), 7222–7229. doi:10.1523/JNEUROSCI.1595-06.2006.

Battaglia, G., Busceti, C. L., Fabrizio, P., Francesca, B., Francesco, F., Antonio, P., Valeria, B., Stefano, R., & Ferdinando, N. (2003). Protective role of group-II metabotropic glutamate receptors against nigro-striatal degeneration induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. *Neuropharmacology, 45*(2), 155–166.

Bean, L., Zheng, H., Patel, K. P., & Monaghan, D. T. (2006). Regional variations in NMDA receptor downregulation in streptozotocin-diabetic rat brain. *Brain Research, 1115*(1), 217–222. doi:10.1016/j.brainres.2006.07.090.

Beckerman, M. A., Van Kempen, T. A., Justice, N. J., Milner, T. A., & Glass, M. J. (2012). Corticotropin-releasing factor in the mouse central nucleus of the amygdala: Ultrastructural distribution in NMDA-NR1 receptor subunit expressing neurons as well as projection neurons to the bed nucleus of the stria terminalis. *Experimental Neurology*. doi:10.1016/j.expneurol.2012.10.009.

Betarbet, R., Poisik, O., Sherer, T. B., & Greenamyre, J. T. (2004). Differential expression and ser897 phosphorylation of striatal N-methyl-D-aspartate receptor subunit NR1 in animal models of Parkinson's disease. [Research support, U.S. Gov't, P.H.S.]. *Experimental Neurology, 187*(1), 76–85. doi:10.1016/j.expneurol.2003.12.012.

Bibbiani, F., Oh, J. D., Kielaite, A., Collins, M. A., Smith, C., & Chase, T. N. (2005). Combined blockade of AMPA and NMDA glutamate receptors reduces levodopa-induced motor complications in animal models of PD. [Comparative study]. *Experimental Neurology, 196*(2), 422–429. doi:10.1016/j.expneurol.2005.08.017.

Blandini, F., & Armentero, M. T. (2012). New pharmacological avenues for the treatment of L-DOPA-induced dyskinesias in Parkinson's disease: Targeting glutamate and adenosine receptors. [Review]. *Expert Opinion on Investigational Drugs, 21*(2), 153–168. doi:10.1517/13543784.2012.651457.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. [Review]. *Nature, 361*(6407), 31–39. doi:10.1038/361031a0.

Blumcke, I., Behle, K., Malitschek, B., Kuhn, R., Knopfel, T., Wolf, H. K., & Wiestler, O. D. (1996). Immunohistochemical distribution of metabotropic glutamate receptor subtypes mGluR1b, mGluR2/3, mGluR4a and mGluR5 in human hippocampus. [Research support, non-U.S. Gov't]. *Brain Research, 736*(1–2), 217–226.

Bradley, S. R., Marino, M. J., Wittmann, M., Rouse, S. T., Awad, H., Levey, A. I., & Conn, P. J. (2000). Activation of group II metabotropic glutamate receptors inhibits synaptic excitation of the substantia nigra pars reticulata. [Research support, non-U.S. Gov't research support, U.S. Gov't, non-P.H.S. Research support, U.S. Gov't, P.H.S.]. *Journal of Neuroscience, 20*(9), 3085–3094.

Breysse, N., Baunez, C., Spooren, W., Gasparini, F., & Amalric, M. (2002). Chronic but not acute treatment with a metabotropic glutamate 5 receptor antagonist reverses the akinetic deficits in a rat model of parkinsonism. [Research support, non-U.S. Gov't]. *Journal of Neuroscience, 22*(13), 5669–5678. doi:20026513.

Broadstock, M., Austin, P. J., Betts, M. J., & Duty, S. (2012). Antiparkinsonian potential of targeting group III metabotropic glutamate receptor subtypes in the rodent substantia nigra pars reticulata. [Research support, non-U.S. Gov't]. *British Journal of Pharmacology, 165*(4b), 1034–1045. doi:10.1111/j.1476-5381.2011.01515.x.

Carlsson, A., Lindqvist, M., & Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature, 180*(4596), 1200.

Catania, M. V., Bellomo, M., Di Giorgi-Gerevini, V., Seminara, G., Giuffrida, R., Romeo, R., De Blasi, A., & Nicoletti, F. (2001). Endogenous activation of group-I metabotropic glutamate receptors is required for differentiation and survival of cerebellar Purkinje cells. [Research support, non-U.S. Gov't]. *Journal of Neuroscience, 21*(19), 7664–7673.

Chappell, A. S., Gonzales, C., Williams, J., Witte, M. M., Mohs, R. C., & Sperling, R. (2007). AMPA potentiator treatment of cognitive deficits in Alzheimer disease. [Randomized controlled trial research support, non-U.S. Gov't]. *Neurology, 68*(13), 1008–1012. doi:10.1212/01.wnl.0000260240.46070.7c.

Chen, J. J. (2011). Pharmacologic safety concerns in Parkinson's disease: Facts and insights. [Review]. *International Journal of Neuroscience, 121*(Suppl 2), 45–52. doi:10.3109/00207454.2011.620193.

Chen, L., Liu, J., Ali, U., Gui, Z. H., Hou, C., Fan, L. L., Wang, Y., & Wang, T. (2011). Chronic, systemic treatment with a metabotropic glutamate receptor 5 antagonist produces anxiolytic-like effects and reverses abnormal firing activity of projection neurons in the basolateral nucleus of the amygdala in rats with bilateral 6-OHDA lesions. [Research support, non-U.S. Gov't]. *The Brain Research Bulletin, 84*(3), 215–223. doi:10.1016/j.brainresbull.2011.01.005.

Clarkson, A. N., Overman, J. J., Zhong, S., Mueller, R., Lynch, G., & Carmichael, S. T. (2011). AMPA receptor-induced local brain-derived neurotrophic factor signaling mediates motor recovery after stroke. [Research support, non-U.S. Gov't]. *Journal of Neuroscience, 31*(10), 3766–3775. doi:10.1523/JNEUROSCI.5780-10.2011.

Collingridge, G. L., Volianskis, A., Bannister, N., France, G., Hanna, L., Mercier, M., Tidball, P., Fang, G., Irvine, M. W., Costa, B. M., Monaghan, D. T., Bortolotto, Z. A., Molnar, E., Lodge, D., & Jane, D. E. (2013). The NMDA receptor as a target for cognitive enhancement. *Neuropharmacology, 64*(1), 13–26. doi:.

Conn, P. J., Battaglia, G., Marino, M. J., & Nicoletti, F. (2005). Metabotropic glutamate receptors in the basal ganglia motor circuit. [Research support, N.I.H., extramural research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S. review]. *Nature Reviews Neuroscience, 6*(10), 787–798. doi:10.1038/nrn1763.

Conn, P. J., Lindsley, C. W., & Jones, C. K. (2009). Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. [Review]. *Trends in Pharmacological Sciences, 30*(1), 25–31. doi:10.1016/j.tips.2008.10.006.

Conn, P. J., & Pin, J. P. (1997). Pharmacology and functions of metabotropic glutamate receptors. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S. review]. *Annual Review of Pharmacology and Toxicology, 37*, 205–237. doi:10.1146/annurev.pharmtox.37.1.205.

Conti, V., Aghaie, A., Cilli, M., Martin, N., Caridi, G., Musante, L., Candiano, G., Castagna, M., Fairen, A., Ravazzolo, R., Guenet, J. L., & Puliti, A. (2006). crv4, a mouse model for human ataxia associated with kyphoscoliosis caused by an mRNA splicing mutation of the metabotropic glutamate receptor 1 (Grm1). [Research support, non-U.S. Gov't]. *International Journal of Molecular Medicine, 18*(4), 593–600.

Corona, J. C., & Tapia, R. (2007). Ca^{2+} −permeable AMPA receptors and intracellular Ca^{2+} determine motor neuron vulnerability in rat spinal cord in vivo. [Research support, non-U.S. Gov't]. *Neuropharmacology, 52*(5), 1219–1228. doi:10.1016/j.neuropharm.2006.12.008.

Corti, C., Aldegheri, L., Somogyi, P., & Ferraguti, F. (2002). Distribution and synaptic localisation of the metabotropic glutamate receptor 4 (mGluR4) in the rodent CNS. [Research support, non-U.S. Gov't]. *Neuroscience, 110*(3), 403–420.

Corti, C., Crepaldi, L., Mion, S., Roth, A. L., Xuereb, J. H., & Ferraguti, F. (2007). Altered dimerization of metabotropic glutamate receptor 3 in schizophrenia. *Biological Psychiatry, 62*(7), 747–755. doi:10.1016/j.biopsych.2006.12.005.

Dario, C., Giuseppina, M., Emanuela, B., Paola, P., Daniela, V., Graziella, M., Chelliah, S., Cyril, G., Nadia, O., Jean-Philippe, P., Francine, A., Antonio, P., Corinne, B., Christophe, M., Lydia Kerkerian-Le, G., & Paolo, G. (2009). Metabotropic glutamate receptor subtype 4 selectively modulates both glutamate and GABA transmission in the striatum: Implications for Parkinson's disease treatment. [Research support, non-U.S. Gov't]. *Journal of Neurochemistry, 109*(4), 1096–1105. doi:10.1111/j.1471-4159.2009.06036.x.

Dauer, W., & Przedborski, S. (2003). Parkinson's disease: Mechanisms and models. [Research support, non-U.S. Gov't research support, U.S. Gov't, non-P.H.S. Research support, U.S. Gov't, P.H.S. review]. *Neuron, 39*(6), 889–909.

Daw, M. I., Scott, H. L., & Isaac, J. T. (2007). Developmental synaptic plasticity at the thalamocortical input to barrel cortex: Mechanisms and roles. [Research support, N.I.H., intramural research support, non-U.S. Gov't review]. *Molecular and Cellular Neuroscience, 34*(4), 493–502. doi:10.1016/j.mcn.2007.01.001.

Dawson, L., Chadha, A., Megalou, M., & Duty, S. (2000). The group II metabotropic glutamate receptor agonist, DCG-IV, alleviates akinesia following intranigral or intraventricular administration in the reserpine-treated rat. [Research support, non-U.S. Gov't]. *British Journal of Pharmacology, 129*(3), 541–546. doi:10.1038/sj.bjp.0703105.

Dawson, N., Xiao, X., McDonald, M., Higham, D. J., Morris, B. J., & Pratt, J. A. (2012). Sustained NMDA receptor hypofunction induces compromised neural systems integration and schizophrenia-like alterations in functional brain networks. *Cerebral Cortex*. doi:10.1093/cercor/bhs322.

Del Dotto, P., Pavese, N., Gambaccini, G., Bernardini, S., Metman, L. V., Chase, T. N., & Bonuccelli, U. (2001). Intravenous amantadine improves levadopa-induced dyskinesias: An acute double-blind placebo-controlled study. [Clinical trial randomized controlled trial]. *Movement Disorders, 16*(3), 515–520.

Di Chiara, G., Morelli, M., & Consolo, S. (1994). Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/NMDA interactions. [Research support, non-U.S. Gov't review]. *Trends in Neuroscience, 17*(6), 228–233.

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S. review]. *Pharmacological Reviews, 51*(1), 7–61.

Doherty, F. C., & Sladek, C. D. (2011). NMDA receptor subunit expression in the supraoptic nucleus of adult rats: Dominance of NR2B and NR2D. [Research support, N.I.H., extramural]. *Brain Research, 1388*, 89–99. doi:10.1016/j.brainres.2011.03.015.

Dunah, A. W., Yasuda, R. P., Luo, J., Wang, Y., Prybylowski, K. L., & Wolfe, B. B. (1999). Biochemical studies of the structure and function of the N-methyl-D-aspartate subtype of glutamate receptors. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S. review]. *Molecular Neurobiology, 19*(2), 151–179. doi:10.1007/BF02743658.

Ehlers, M. D., Tingley, W. G., & Huganir, R. L. (1995). Regulated subcellular distribution of the NR1 subunit of the NMDA receptor. [Research support, non-U.S. Gov't]. *Science, 269*(5231), 1734–1737.

Faden, A. I., O'Leary, D. M., Fan, L., Bao, W., Mullins, P. G., & Movsesyan, V. A. (2001). Selective blockade of the mGluR1 receptor reduces traumatic neuronal injury in vitro and improve so outcome after brain trauma. [In vitro research support, U.S. Gov't, non-P.H.S. Research support, U.S. Gov't, P.H.S.]. *Experimental Neurology, 167*(2), 435–444. doi:10.1006/exnr.2000.7577.

Fallarino, F., Volpi, C., Fazio, F., Notartomaso, S., Vacca, C., Busceti, C., Bicciato, S., Battaglia, G., Bruno, V., Puccetti, P., Fioretti, M. C., Nicoletti, F., Grohmann, U., & Di Marco, R. (2010). Metabotropic glutamate receptor-4 modulates adaptive immunity and restrains neuroinflammation. [Research support, non-U.S. Gov't]. *Nature Medicine, 16*(8), 897–902. doi:10.1038/nm.2183.

Fantin, M., Auberson, Y. P., & Morari, M. (2008). Differential effect of NR2A and NR2B subunit selective NMDA receptor antagonists on striato-pallidal neurons: Relationship to motor response in the 6-hydroxydopamine model of parkinsonism. [Research support, non-U.S. Gov't]. *Journal of Neurochemistry, 106*(2), 957–968. doi:10.1111/j.1471-4159.2008.05439.x.

Fell, M. J., Perry, K. W., Falcone, J. F., Johnson, B. G., Barth, V. N., Rash, K. S., Lucaites, V. L., Threlkeld, P. G., Monn, J. A., McKinzie, D. L., Marek, G. J., Svensson, K. A., & Nelson, D. L. (2009). In vitro and in vivo evidence for a lack of interaction with dopamine D2 receptors by the metabotropic glutamate 2/3 receptor agonists 1S,2S,5R,6S-2-aminobicyclo[3.1.0]hexane-2,6-bicaroxylate monohydrate (LY354740) and (−)-2-oxa-4-aminobicyclo[3.1.0] Hexane-4,6-dicarboxylic acid (LY379268). [Research support, non-U.S. Gov't]. *Journal of Pharmacology and Experimental Therapeutics, 331*(3), 1126–1136. doi:10.1124/jpet.109.160598.

Feyissa, A. M., Woolverton, W. L., Miguel-Hidalgo, J. J., Wang, Z., Kyle, P. B., Hasler, G., Stockmeier, C. A., Iyo, A. H., & Karolewicz, B. (2010). Elevated level of metabotropic glutamate receptor 2/3 in the prefrontal cortex in major depression. [Research support, N.I.H., extramural research support, non-U.S. Gov't]. *Progress in Neuropsychopharmacology & Biological Psychiatry, 34*(2), 279–283. doi:10.1016/j.pnpbp.2009.11.018.

Flavio, K., Taisuke, T., Helen, H., Guy, S., David, B., Takeshi, I., Sangram, S., & Roberto, M. (2003). APP processing and synaptic function. *Neuron, 37*(6), 925–937.

Fonnum, F. (1984). Glutamate: A neurotransmitter in mammalian brain. [Review]. *Journal of Neurochemistry, 42*(1), 1–11.

Gardoni, F., Sgobio, C., Pendolino, V., Calabresi, P., Di Luca, M., & Picconi, B. (2012). Targeting NR2A-containing NMDA receptors reduces L-DOPA-induced dyskinesias. [Research support, non-U.S. Gov't]. *Neurobiology of Aging, 33*(9), 2138–2144. doi:10.1016/j.neurobiolaging.2011.06.019.

Geurts, J. J., Wolswijk, G., Bo, L., Redeker, S., Ramkema, M., Troost, D., & Aronica, E. (2005). Expression patterns of group III metabotropic glutamate receptors mGluR4 and mGluR8 in multiple sclerosis lesions. [Comparative study research support, non-U.S. Gov't]. *The Journal of Neuroimmunology, 158*(1–2), 182–190. doi:10.1016/j.jneuroim.2004.08.012.

Greco, B., Lopez, S., van der Putten, H., Flor, P. J., & Amalric, M. (2010). Metabotropic glutamate 7 receptor subtype modulates motor symptoms in rodent models of Parkinson's disease. [Research support, non-U.S. Gov't]. *Journal of Pharmacology and Experimental, 332*(3), 1064–1071. doi10.1124/jpet.109.162115.

Greenamyre, J. T. (1993). Glutamate-dopamine interactions in the basal ganglia: Relationship to Parkinson's disease. [Research support, non-U.S. Gov't research support,

U.S. Gov't, non-P.H.S.research support, U.S. Gov't, P.H.S.review]. *Journal of Neural Transmission. General Section, 91*(2–3), 255–269.

Hallett, P. J., Dunah, A. W., Ravenscroft, P., Zhou, S., Bezard, E., Crossman, A. R., Brotchie, J., & Standaert, D. G. (2005). Alterations of striatal NMDA receptor subunits associated with the development of dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. [Comparative study research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S.]. *Neuropharmacology, 48*(4), 503–516. doi:10.1016/j.neuropharm.2004.11.008.

Hallett, P. J., & Standaert, D. G. (2004). Rationale for and use of NMDA receptor antagonists in Parkinson's disease. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S. review]. *Pharmacology & Therapeutics, 102*(2), 155–174. doi:10.1016/j.pharmthera.2004.04.001.

Hara, Y., & Pickel, V. M. (2008). Preferential relocation of the N-methyl-D-aspartate receptor NR1 subunit in nucleus accumbens neurons that contain dopamine D1 receptors in rats showing an apomorphine-induced sensorimotor gating deficit. [Research support, N.I.H., extramural research support, non-U.S. Gov't]. *Neuroscience, 154*(3), 965–977. doi:10.1016/j.neuroscience.2008.04.009.

Hayashi, T., Umemori, H., Mishina, M., & Yamamoto, T. (1999). The AMPA receptor interacts with and signals through the protein tyrosine kinase Lyn. [Research support, non-U.S. Gov't]. *Nature, 397*(6714), 72–76. doi:10.1038/16269.

Hopkins, C. R., Lindsley, C. W., & Niswender, C. M. (2009). mGluR4-positive allosteric modulation as potential treatment for Parkinson's disease. [Research support, non-U.S. Gov't review]. *Future Medicinal Chemistry, 1*(3), 501–513. doi:10.4155/fmc.09.38.

Hubsher, G., Haider, M., & Okun, M. S. (2012). Amantadine: The journey from fighting flu to treating Parkinson disease. [Review]. *Neurology, 78*(14), 1096–1099. doi:10.1212/WNL.0b013e31824e8f0d.

Isaac, J. T., Nicoll, R. A., & Malenka, R. C. (1995). Evidence for silent synapses: Implications for the expression of LTP. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S.]. *Neuron, 15*(2), 427–434.

Jarzylo, L. A., & Man, H. Y. (2012). Parasynaptic NMDA receptor signaling couples neuronal glutamate transporter function to AMPA receptor synaptic distribution and stability. [Comparative study research support, N.I.H., extramural research support, non-U.S. Gov't]. *Journal of Neuroscience, 32*(7), 2552–2563. doi:10.1523/JNEUROSCI.3237-11.2012.

Jellinger, K. A. (2002). Recent developments in the pathology of Parkinson's disease. [Research support, non-U.S. Gov't review]. *Journal of Neural Transmission, Supplement* (62), 347–376.

Jimenez, A., Marin, C., Bonastre, M., & Tolosa, E. (1999). Narrow beneficial effect of dextromethorphan on levodopa-induced motor response alterations in an experimental model of parkinsonism. [Research support, non-U.S. Gov't]. *Brain Research, 839*(1), 190–193.

Johnson, K. A., Conn, P. J., & Niswender, C. M. (2009). Glutamate receptors as therapeutic targets for Parkinson's disease. [Research support, N.I.H., extramural research support, non-U.S. Gov't review]. *CNS Neurological Disorders-drug Targets, 8*(6), 475–491.

Jones, C. K., Bubser, M., Thompson, A. D., Dickerson, J. W., Turle-Lorenzo, N., Amalric, M., Blobaum, A. L., Bridges, T. M., Morrison, R. D., Jadhav, S., Engers, D. W., Italiano, K., Bode, J., Daniels, J. S., Lindsley, C. W., Hopkins, C. R., Conn, P. J., & Niswender, C. M. (2012). The metabotropic glutamate receptor 4-positive allosteric modulator VU0364770 produces efficacy alone and in combination with L-DOPA or an adenosine 2A antagonist in preclinical rodent models of Parkinson's disease. [Research support, N.I.H., extramural research support, non-U.S. Gov't]. *Journal of Pharmacology and Experimental Therapeutics, 340*(2), 404–421. doi:10.1124/jpet.111.187443.

Katayama, J., Akaike, N., & Nabekura, J. (2003). Characterization of pre-and post-synaptic metabotropic glutamate receptor-mediated inhibitory responses in substantia nigra dopamine neurons. *Neuroscience Research, 45*(1), 101–115.

Kaufmann, W. A., Matsui, K., Jeromin, A., Nerbonne, J. M., & Ferraguti, F. (2012). Kv4.2 potassium channels segregate to extrasynaptic domains and influence intrasynaptic NMDA receptor NR2B subunit expression. *Brain Structure & Function, 218*(5), 1115–1132. doi:10.1007/s00429-012-0450-1.

Kew, J. N., & Kemp, J. A. (2005). Ionotropic and metabotropic glutamate receptor structure and pharmacology. [Review]. *Psychopharmacology (Berl), 179*(1), 4–29. doi:10.1007/s00213-005-2200-z.

Kobylecki, C., Cenci, M. A., Crossman, A. R., & Ravenscroft, P. (2010). Calcium-permeable AMPA receptors are involved in the induction and expression of L-DOPA-induced dyskinesia in Parkinson's disease. [Research support, non-U.S. Gov't]. *Journal of Neurochemistry, 114*(2), 499–511. doi:10.1111/j.1471-4159.2010.06776.x.

Kobylecki, C., Hill, M. P., Crossman, A. R., & Ravenscroft, P. (2011). Synergistic antidyskinetic effects of topiramate and amantadine in animal models of Parkinson's disease. [Comparative study research support, non-U.S. Gov't]. *Movement Disorders, 26*(13), 2354–2363. doi:10.1002/mds.23867.

Konitsiotis, S., Blanchet, P. J., Verhagen, L., Lamers, E., & Chase, T. N. (2000). AMPA receptor blockade improves levodopa-induced dyskinesia in MPTP monkeys. [Comparative study research support, non-U.S. Gov't]. *Neurology, 54*(8), 1589–1595.

Kosinski, C. M., Risso Bradley, S., Conn, P. J., Levey, A. I., Landwehrmeyer, G. B., Penney, J. B., Jr., Young, A. B., & Standaert, D. G. (1999). Location of metabotropic glutamate receptor 7 mRNA and mGluR7a protein in the rat basal ganglia. [Research support, non-U.S. Gov't research support, U.S. Gov't, non-P.H.S. research support, U.S. Gov't, P.H.S.]. *The Journal of Comparative Neurology, 415*(2), 266–284.

Kostandy, B. B. (2012). The role of glutamate in neuronal ischemic injury: The role of spark in fire. [Review]. *Neurology of Science, 33*(2), 223–237. doi:10.1007/s10072-011-0828-5.

Koutsilieri, E., & Riederer, P. (2007). Excitotoxicity and new antiglutamatergic strategies in Parkinson's disease and Alzheimer's disease. [Review]. *Parkinsonism & Related Disorders , 13*(Suppl 3), S329–S331. doi:10.1016/S1353-8020(08)70025-7.

Kuppenbender, K. D., Standaert, D. G., Feuerstein, T. J., Penney, J. B., Jr., Young, A. B., & Landwehrmeyer, G. B. (2000). Expression of NMDA receptor subunit mRNAs in neurochemically identified projection and interneurons in the human striatum. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S.]. *The Journal of Comparative Neurology, 419*(4), 407–421.

Lange, K. W., Kornhuber, J., & Riederer, P. (1997). Dopamine/glutamate interactions in Parkinson's disease. [Research support, non-U.S. Gov't review]. *Neuroscience & Biobehavioral Reviews, 21*(4), 393–400.

Lau, A., & Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. [Review]. *Pflugers Archiv, 460*(2), 525–542. doi:10.1007/s00424-010-0809-1.

Lavreysen, H., & Dautzenberg, F. M. (2008). Therapeutic potential of group III metabotropic glutamate receptors. [Review]. *Current Medicinal Chemistry, 15*(7), 671–684.

Lea, P. M. T., Movsesyan, V. A., & Faden, A. I. (2005). Neuroprotective activity of the mGluR5 antagonists MPEP and MTEP against acute excitotoxicity differs and does not reflect actions at mGluR5 receptors. [Comparative study research support, N.I.H., extramural research support, U.S. Gov't, non-P.H.S. research support, U.S. Gov't, P.H.S.]. *British Journal of Pharmacology, 145*(4), 527–534. doi:10.1038/sj.bjp.0706219.

Leaver, K. R., Allbutt, H. N., Creber, N. J., Kassiou, M., & Henderson, J. M. (2008). Neuroprotective effects of a selective N-methyl-D-aspartate NR2B receptor antagonist in the 6-hydroxydopamine rat model of Parkinson's disease. [Comparative study research support, non-U.S. Gov't]. *Clinical and Experimental Pharmacology and Physiology, 35*(11), 1388–1394. doi:10.1111/j.1440-1681.2008.05046.x.

Lees, G. J. (2000). Pharmacology of AMPA/kainate receptor ligands and their therapeutic potential in neurological and psychiatric disorders. [Research support, non-U.S. Gov't review]. *Drugs, 59*(1), 33–78.

Li, P., & Zhuo, M. (1998). Silent glutamatergic synapses and nociception in mammalian spinal cord. [In vitro research support, U.S. Gov't, P.H.S.]. *Nature, 393*(6686), 695–698. doi:10.1038/31496.

Liao, D., Hessler, N. A., & Malinow, R. (1995). Activation of post-synaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. [In vitro research support, U.S. Gov't, P.H.S.]. *Nature, 375*(6530), 400–404. doi:10.1038/375400a0.

Lin, C. H., Lee, C. C., Huang, Y. C., Wang, S. J., & Gean, P. W. (2005). Activation of group II metabotropic glutamate receptors induces depotentiation in amygdala slices and reduces fear-potentiated startle in rats. [Comparative study in vitro research support, non-U.S. Gov't]. *Learning and Memory, 12*(2), 130–137. doi:10.1101/lm.85304.

Ling, W., Chang, L., Song, Y., Lu, T., Jiang, Y., Li, Y., & Wu, Y. (2012). Immunolocalization of NR1, NR2A, and PSD-95 in rat hippocampal subregions during postnatal development. [Research support, non-U.S. Gov't]. *Acta Histochemica, 114*(3), 285–295. doi:10.1016/j.acthis.2011.06.005.

Liu, X. B., Munoz, A., & Jones, E. G. (1998). Changes in subcellular location of metabotropic glutamate receptor subtypes during postnatal development of mouse thalamus. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S.]. *The Journal of Comparative Neurology, 395*(4), 450–465.

Lopez, S., Turle-Lorenzo, N., Acher, F., De Leonibus, E., Mele, A., & Amalric, M. (2007). Targeting group III metabotropic glutamate receptors produces complex behavioral effects in rodent models of Parkinson's disease. [Comparative study research support, non-U.S. Gov't]. *Journal of Neuroscience, 27*(25), 6701–6711. doi:10.1523/JNEUROSCI.0299-07.2007.

Lujan, R., Roberts, J. D., Shigemoto, R., Ohishi, H., & Somogyi, P. (1997). Differential plasma membrane distribution of metabotropic glutamate receptors mGluR1 alpha, mGluR2 and mGluR5, relative to neurotransmitter release sites. [Research support, non-U.S. Gov't]. *Journal of Chemical Neuroanatomy, 13*(4), 219–241.

Luscher, C., & Huber, K. M. (2010). Group 1 mGluR-dependent synaptic long-term depression: Mechanisms and implications for circuitry and disease. [Research support, N.I.H., extramural research support, non-U.S. Gov't review]. *Neuron, 65*(4), 445–459. doi:10.1016/j.neuron.2010.01.016.

MacInnes, N., Messenger, M. J., & Duty, S. (2004). Activation of group III metabotropic glutamate receptors in selected regions of the basal ganglia alleviates akinesia in the reserpine-treated rat. [Comparative study research support, non-U.S. Gov't]. *British Journal of Pharmacology, 141*(1), 15–22. doi:10.1038/sj.bjp.0705566.

Makoff, A., Lelchuk, R., Oxer, M., Harrington, K., & Emson, P. (1996a). Molecular characterization and location of human metabotropic glutamate receptor type 4. *Brain Research Molecular Brain Research, 37*(1–2), 239–248.

Makoff, A., Volpe, F., Lelchuk, R., Harrington, K., & Emson, P. (1996b). Molecular characterization and location of human metabotropic glutamate receptor type 3. [Comparative study]. *Brain Research Molecular Brain Research, 40*(1), 55–63.

Marin, C., Jimenez, A., Bonastre, M., Chase, T. N., & Tolosa, E. (2000). Non-NMDA receptor-mediated mechanisms are involved in levodopa-induced motor response alterations in Parkinsonian rats. [Research support, non-U.S. Gov't]. *Synapse, 36*(4), 267–274. doi:10.1002/(SICI)1098-2396(20000615)36:4<267::AID-SYN3>3.0.CO;2-Y.

Masu, M., Tanabe, Y., Tsuchida, K., Shigemoto, R., & Nakanishi, S. (1991). Sequence and expression of a metabotropic glutamate receptor. [Comparative study research support, non-U.S. Gov't]. *Nature, 349*(6312), 760–765. doi:10.1038/349760a0.

Matsui, T., & Kita, H. (2003). Activation of group III metabotropic glutamate receptors presynaptically reduces both GABAergic and glutamatergic transmission in the rat globus pallidus. [Comparative study in vitro research support, U.S. Gov't, P.H.S.]. *Neuroscience, 122*(3), 727–737.

Mayer, M. L., & Armstrong, N. (2004). Structure and function of glutamate receptor ion channels. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S. review]. *Annual Review of Physiology, 66*, 161–181. doi:10.1146/annurev.physiol.66.050802.084104.

Merello, M., Nouzeilles, M. I., Cammarota, A., & Leiguarda, R. (1999). Effect of memantine (NMDA antagonist) on Parkinson's disease: A double-blind crossover randomized study. [Clinical trial randomized controlled trial]. *Clinical Neuropharmacology, 22*(5), 273–276.

Mitsukawa, K., Yamamoto, R., Ofner, S., Nozulak, J., Pescott, O., Lukic, S., Stoehr, N., Mombereau, C., Kuhn, R., McAllister, K. H., van der Putten, H., Cryan, J. F., & Flor, P. J. (2005). A selective metabotropic glutamate receptor 7 agonist: activation of receptor signaling via an allosteric site modulates stress parameters in vivo. [Research support, N.I.H., extramural]. *Proceedings of the National Academy of Sciences of the United States of America, 102*(51), 18712–18717. doi:10.1073/pnas.0508063102.

Monaghan, D. T., Andaloro, V. J., & Skifter, D. A. (1998). Molecular determinants of NMDA receptor pharmacological diversity. [Review]. *Progress in Brain Research , 116*, 171–190.

Monaghan, D. T., & Jane, D. E. (2009). Pharmacology of NMDA receptors. In A. M. Van Dongen (Ed.), *Biology of the NMDA receptor*. Boca Raton: CRC Press.

Murray, T. K., Messenger, M. J., Ward, M. A., Woodhouse, S., Osborne, D. J., Duty, S., & O'Neill, M. J. (2002). Evaluation of the mGluR2/3 agonist LY379268 in rodent models of Parkinson's disease. *Pharmacology Biochemistry & Behavior, 73*(2), 455–466.

Nawy, S. (2000). Regulation of the on bipolar cell mGluR6 pathway by Ca^{2+}. [In vitro research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S.]. *Journal of Neuroscience, 20*(12), 4471–4479.

Nicoletti, F., Bockaert, J., Collingridge, G. L., Conn, P. J., Ferraguti, F., Schoepp, D. D., Wroblewski, J. T., & Pin, J. P. (2011). Metabotropic glutamate receptors: From the workbench to the bedside. [Review]. *Neuropharmacology, 60*(7–8), 1017–1041. doi:10.1016/j.neuropharm.2010.10.022.

Nicoletti, F., Bruno, V., Catania, M. V., Battaglia, G., Copani, A., Barbagallo, G., Ceña, V., Sanchez-Prieto, J., Spano, P. F., & Pizzi, M. (1999). Group-I metabotropic glutamate receptors: Hypotheses to explain their dual role in neurotoxicity and neuroprotection. [Research support, non-U.S. Gov't review]. *Neuropharmacology, 38*(10), 1477–1484.

Niswender, C. M., & Conn, P. J. (2010). Metabotropic glutamate receptors: Physiology, pharmacology, and disease. [Research support, N.I.H., extramural research support, non-U.S. Gov't review]. *The Annual Review of Pharmacology and Toxicology, 50*, 295–322. doi:10.1146/annurev.pharmtox.011008.145533.

Niswender, C. M., Johnson, K. A., Weaver, C. D., Jones, C. K., Xiang, Z., Luo, Q., Rodriguez, A. L., Marlo, J. E., de Paulis, T., Thompson, A. D., Days, E. L., Nalywajko, T., Austin, C. A., Williams, M. B., Ayala, J. E., Williams, R., Lindsley, C. W., & Conn, P. J. (2008). Discovery, characterization, and antiparkinsonian effect of novel positive allosteric modulators of metabotropic glutamate receptor 4. [In vitro research support, N.I.H., extramural research support, non-U.S. Gov't]. *Molecular Pharmacology, 74*(5), 1345–1358. doi:10.1124/mol.108.049551.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., & Prochiantz, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. [Research support, non-U.S. Gov't]. *Nature, 307*(5950), 462–465.

O'Neill, M. J., Bleakman, D., Zimmerman, D. M., & Nisenbaum, E. S. (2004). AMPA receptor potentiators for the treatment of CNS disorders. [Review]. *Current Drug Targets. CNS and Neurological Disorders, 3*(3), 181–194.

O'Neill, M. J., & Witkin, J. M. (2007). AMPA receptor potentiators: Application for depression and Parkinson's disease. [Review]. *Current Drug Targets, 8*(5), 603–620.

Ohishi, H., Shigemoto, R., Nakanishi, S., & Mizuno, N. (1993a). Distribution of the messenger RNA for a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat. [In vitro research support, non-U.S. Gov't]. *Neuroscience, 53*(4), 1009–1018.

Ohishi, H., Shigemoto, R., Nakanishi, S., & Mizuno, N. (1993b). Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: An in situ hybridization study. [Research support, non-U.S. Gov't]. *The Journal of Comparative Neurology, 335*(2), 252–266. doi:10.1002/cne.903350209.

Ondrejcak, T., Klyubin, I., Hu, N. W., Barry, A. E., Cullen, W. K., & Rowan, M. J. (2010). Alzheimer's disease amyloid beta-protein and synaptic function. [Research support, non-U.S. Gov't review]. *Neuromolecular Medicine, 12*(1), 13–26. doi:10.1007/s12017-009-8091-0.

Ossowska, K., Konieczny, J., Wolfarth, S., Wieronska, J., & Pilc, A. (2001). Blockade of the metabotropic glutamate receptor subtype 5 (mGluR5) produces antiparkinsonian-like effects in rats. [Research support, non-U.S. Gov't]. *Neuropharmacology, 41*(4), 413–420.

Palop, J. J., & Mucke, L. (2010a). Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: From synapses toward neural networks. [Research support, N.I.H., extramural research support, non-U.S. Gov't review]. *Nature Neuroscience, 13*(7), 812–818. doi:10.1038/nn.2583.

Palop, J. J., & Mucke, L. (2010b). Synaptic depression and aberrant excitatory network activity in Alzheimer's disease: Two faces of the same coin? [Research support, N.I.H., extramural research support, non-U.S. Gov't review]. *Neuromolecular Medicine, 12*(1), 48–55. doi:10.1007/s12017-009-8097-7.

Palucha, A., Klak, K., Branski, P., van der Putten, H., Flor, P. J., & Pilc, A. (2007). Activation of the mGlu7 receptor elicits antidepressant-like effects in mice. [Research support, non-U.S. Gov't]. *Psychopharmacology (Berl), 194*(4), 555–562. doi:10.1007/s00213-007-0856-2.

Papadia, S., Stevenson, P., Hardingham, N. R., Bading, H., & Hardingham, G. E. (2005). Nuclear Ca^{2+} and the cAMP response element-binding protein family mediate a late phase of activity-dependent neuroprotection. [Comparative study in vitro research support, non-U.S. Gov't]. *Journal of Neuroscience, 25*(17), 4279–4287. doi:10.1523/JNEUROSCI.5019-04.2005.

Park, E., Liu, Y., & Fehlings, M. G. (2003). Changes in glial cell white matter AMPA receptor expression after spinal cord injury and relationship to apoptotic cell death. [Research support, non-U.S. Gov't]. *Experimental Neurology, 182*(1), 35–48.

Pellegrini-Giampietro, D. E. (2003). The distinct role of mGlu1 receptors in post-ischemic neuronal death. [Review]. *Trends in Pharmacological Sciences, 24*(9), 461–470. doi:10.1016/S0165-6147(03)00231-1.

Perier, C., Tremblay, L., Feger, J., & Hirsch, E. C. (2002). Behavioral consequences of bicuculline injection in the subthalamic nucleus and the zona incerta in rat. [Research support, non-U.S. Gov't]. *Journal of Neuroscience, 22*(19), 8711–8719.

Pin, J. P., & Acher, F. (2002). The metabotropic glutamate receptors: Structure, activation mechanism and pharmacology. [Review]. *Current Drug Targets. CNS and Neurological Disorders, 1*(3), 297–317.

Pin, J. P., & Duvoisin, R. (1995). The metabotropic glutamate receptors: Structure and functions. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S. review]. *Neuropharmacology, 34*(1), 1–26.

Pisani, A., Bonsi, P., Centonze, D., Bernardi, G., & Calabresi, P. (2001). Functional coexpression of excitatory mGluR1 and mGluR5 on striatal cholinergic interneurons. [In vitro research support, non-U.S. Gov't]. *Neuropharmacology, 40*(3), 460–463.

Popoli, P., Pintor, A., Tebano, M. T., Frank, C., Pepponi, R., Nazzicone, V., Grieco, R., Pèzzola, A., Reggio, R., Minghetti, L., De Berardinis, M. A., Martire, A., Potenza, R. L., Domenici, M. R., & Massotti, M. (2004). Neuroprotective effects of the mGlu5R antagonist MPEP towards quinolinic acid-induced striatal toxicity: Involvement of pre- and post-synaptic mechanisms and lack of direct NMDA blocking activity. [Research support, non-U.S. Gov't]. *Journal of Neurochemistry, 89*(6), 1479–1489. doi:10.1111/j.1471-4159.2004.02448.x.

Randall, A. D., Witton, J., Booth, C., Hynes-Allen, A., & Brown, J. T. (2010). The functional neurophysiology of the amyloid precursor protein (APP) processing pathway. [Review]. *Neuropharmacology, 59*(4–5), 243–267. doi:10.1016/j.neuropharm.2010.02.011.

Rao, A. M., Hatcher, J. F., & Dempsey, R. J. (2000). Neuroprotection by group I metabotropic glutamate receptor antagonists in forebrain ischemia of gerbil. [Research support, non-U.S. Gov't]. *Neuroscience Letter, 293*(1), 1–4.

Riederer, P., Reichmann, H., Janetzky, B., Sian, J., Lesch, K. P., Lange, K. W., Double, K. L., Nagatsu, T., & Gerlach, M. (2001). Neural degeneration in Parkinson's disease. [Review]. *Advances in Neurology, 86*, 125–136.

Rodriguez, M. C., Obeso, J. A., & Olanow, C. W. (1998). Subthalamic nucleus-mediated excitotoxicity in Parkinson's disease: A target for neuroprotection. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S. review]. *Annals of Neurology, 44*(3 Suppl 1), S175–S188.

Rossi, P. I., Vaccari, C. M., Terracciano, A., Doria-Lamba, L., Facchinetti, S., Priolo, M., Ayuso, C., De Jorge, L., Gimelli, S., Santorelli, F. M., Ravazzolo, R., & Puliti, A. (2010). The metabotropic glutamate receptor 1, GRM1: Evaluation as a candidate gene for inherited forms of cerebellar ataxia. [Multicenter study research support, non-U.S. Gov't]. *Journal of Neurology, 257*(4), 598–602. doi:10.1007/s00415-009-5380-3.

Rouse, S. T., Marino, M. J., Bradley, S. R., Awad, H., Wittmann, M., & Conn, P. J. (2000). Distribution and roles of metabotropic glutamate receptors in the basal ganglia motor circuit: Implications for treatment of Parkinson's disease and related disorders. [Research support, non-U.S. Gov't research support, U.S. Gov't, non-P.H.S. research support, U.S. Gov't, P.H.S. review]. *Pharmacology & Therapeutics, 88*(3), 427–435.

Sachs, A. J., Schwendinger, J. K., Yang, A. W., Haider, N. B., & Nystuen, A. M. (2007). The mouse mutants recoil wobbler and nmf373 represent a series of Grm1 mutations. [Research support, N.I.H., extramural]. *Mammalian Genome, 18*(11), 749–756. doi:10.1007/s00335-007-9064-y.

Salling, M. C., Faccidomo, S., & Hodge, C. W. (2008). Nonselective suppression of operant ethanol and sucrose self-administration by the mGluR7 positive allosteric modulator AMN082. [Research support, N.I.H., extramural]. *Pharmacology Biochemistry & Behavior, 91*(1), 14–20. doi:10.1016/j.pbb.2008.06.006.

Sanchez-Pernaute, R., Wang, J. Q., Kuruppu, D., Cao, L., Tueckmantel, W., Kozikowski, A., Isacson, O., & Brownell, A. L. (2008). Enhanced binding of metabotropic glutamate receptor type 5 (mGluR5) PET tracers in the brain of parkinsonian primates. [Research support, N.I.H., extramural]. *Neuroimage, 42*(1), 248–251. doi:10.1016/j.neuroimage.2008.04.170.

Santos, S. D., Carvalho, A. L., Caldeira, M. V., & Duarte, C. B. (2009). Regulation of AMPA receptors and synaptic plasticity. [Research support, non-U.S. Gov't review]. *Neuroscience, 158*(1), 105–125. doi:10.1016/j.neuroscience.2008.02.037.

Schapira, A. H. (2009). Neurobiology and treatment of Parkinson's disease. [Review]. *Trends in Pharmacological Sciences, 30*(1), 41–47. doi:10.1016/j.tips.2008.10.005.

Schoepp, D. D., Jane, D. E., & Monn, J. A. (1999). Pharmacological agents acting at subtypes of metabotropic glutamate receptors. [Review]. *Neuropharmacology, 38*(10), 1431–1476.

Scott, H. L., Braud, S., Bannister, N. J., & Isaac, J. T. (2007). Synaptic strength at the thalamocortical input to layer IV neonatal barrel cortex is regulated by protein kinase C. [In vitro research support, N.I.H., intramural research support, non-U.S. Gov't]. *Neuropharmacology, 52*(1), 185–192. doi:10.1016/j.neuropharm.2006.06.016.

Sgambato-Faure, V., & Cenci, M. A. (2012). Glutamatergic mechanisms in the dyskinesias induced by pharmacological dopamine replacement and deep brain stimulation for the treatment of Parkinson's disease. [Review]. *Progress in Neurobiology, 96*(1), 69–86. doi:10.1016/j.pneurobio.2011.10.005.

Silverdale, M. A., Nicholson, S. L., Crossman, A. R., & Brotchie, J. M. (2005). Topiramate reduces levodopa-induced dyskinesia in the MPTP-lesioned marmoset model of Parkinson's disease. [Research support, non-U.S. Gov't]. *Movement Disorders, 20*(4), 403–409. doi:10.1002/mds.20345.

Smialowska, M., Golembiowska, K., Kajta, M., Zieba, B., Dziubina, A., & Domin, H. (2012). Selective mGluR1 antagonist EMQMCM inhibits the kainate-induced excitotoxicity in primary neuronal cultures and in the rat hippocampus. [Research support, non-U.S. Gov't]. *Neurotoxicity Research, 21*(4), 379–392. doi:10.1007/s12640-011-9293-4.

Tallaksen-Greene, S. J., Kaatz, K. W., Romano, C., & Albin, R. L. (1998). Location of mGluR1a-like immunoreactivity and mGluR5-like immunoreactivity in identified

populations of striatal neurons. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S.]. *Brain Research, 780*(2), 210–217.

Tamaru, Y., Nomura, S., Mizuno, N., & Shigemoto, R. (2001). Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: Differential location relative to pre- and post-synaptic sites. [Research support, non-U.S. Gov't]. *Neuroscience, 106*(3), 481–503.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., & Dingledine, R. (2010). Glutamate receptor ion channels: Structure, regulation, and function. [Research support, N.I.H., extramural research support, non-U.S. Gov't review]. *Pharmacological Reviews, 62*(3), 405–496. doi:10.1124/pr.109.002451.

Tsai, G., & Coyle, J. T. (2002). Glutamatergic mechanisms in schizophrenia. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S. review]. *Annual Review of Pharmacology and Toxicology, 42*, 165–179. doi:10.1146/annurev.pharmtox.42.082701.160735.

Valenti, O., Mannaioni, G., Seabrook, G. R., Conn, P. J., & Marino, M. J. (2005). Group III metabotropic glutamate-receptor-mediated modulation of excitatory transmission in rodent substantia nigra pars compacta dopamine neurons. *Journal of Pharmacology and Experimental Therapeutics, 313*(3), 1296–1304. doi:10.1124/jpet.104.080481.

Valenti, O., Marino, M. J., Wittmann, M., Lis, E., DiLella, A. G., Kinney, G. G., & Conn, P. J. (2003). Group III metabotropic glutamate receptor-mediated modulation of the striatopallidal synapse. [In vitro research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S.]. *Journal of Neuroscience, 23*(18), 7218–7226.

Varanese, S., Howard, J., & Di Rocco, A. (2010). NMDA antagonist memantine improves levodopa-induced dyskinesias and "on-off" phenomena in Parkinson's disease. [Case reports Letter]. *Movement Disorders, 25*(4), 508–510. doi:10.1002/mds.22917.

Vardi, N., Duvoisin, R., Wu, G., & Sterling, P. (2000). Location of mGluR6 to dendrites of ON bipolar cells in primate retina. [Research support, U.S. Gov't, P.H.S.]. *The Journal of Comparative Neurology, 423*(3), 402–412.

Vera, G., & Tapia, R. (2012). Activation of group III metabotropic glutamate receptors by endogenous glutamate protects against glutamate-mediated excitotoxicity in the hippocampus in vivo. [Research support, non-U.S. Gov't]. *Journal of Neuroscience Research, 90*(5), 1055–1066. doi:10.1002/jnr.23006.

Wang, S. J., & Gean, P. W. (1999). Long-term depression of excitatory synaptic transmission in the rat amygdala. [In vitro research support, non-U.S. Gov't]. *Journal of Neuroscience, 19*(24), 10656–10663.

Waxman, E. A., Baconguis, I., Lynch, D. R., & Robinson, M. B. (2007). N-methyl-D-aspartate receptor-dependent regulation of the glutamate transporter excitatory amino acid carrier 1. [Research support, N.I.H., extramural]. *The Journal of Biological Chemistry, 282*(24), 17594–17607. doi:10.1074/jbc.M702278200.

Waxman, E. A., & Lynch, D. R. (2005). N-methyl-D-aspartate receptor subtype mediated bidirectional control of p38 mitogen-activated protein kinase. [Research support, N.I.H., extramural research support, U.S. Gov't, P.H.S.]. *The Journal of Biological Chemistry, 280*(32), 29322–29333. doi:10.1074/jbc.M502080200.

Wigmore, M. A., & Lacey, M. G. (1998). Metabotropic glutamate receptors depress glutamate-mediated synaptic input to rat midbrain dopamine neurones in vitro. [In vitro research support, non-U.S. Gov't]. *British Journal of Pharmacology, 123*(4), 667–674. doi:10.1038/sj.bjp.0701662.

Wittmann, M., Marino, M. J., Bradley, S. R., & Conn, P. J. (2001). Activation of group III mGluRs inhibits GABAergic and glutamatergic transmission in the substantia nigra pars reticulata. [Research support, non-U.S. Gov't research support, U.S. Gov't, P.H.S.]. *The Journal of Neurophysiology, 85*(5), 1960–1968.

Xu, Y., Dhingra, A., Fina, M. E., Koike, C., Furukawa, T., & Vardi, N. (2012). mGluR6 deletion renders the TRPM1 channel in retina inactive. [Research support, N.I.H., extramural research support, non-U.S. Gov't]. *The Journal of Neurophysiology, 107*(3), 948–957. doi:10.1152/jn.00933.2011.

Yamada, K., & Nabeshima, T. (1998). Changes in NMDA receptor/nitric oxide signaling pathway in the brain with aging. [Review]. *Microscopy Research and Technique, 43*(1), 68–74. doi:10.1002/(SICI)1097-0029(19981001)43:1<68::AID-JEMT10>3.0.CO;2-W.

Zhang, Q. G., Han, D., Hu, S. Q., Li, C., Yu, C. Z., Wang, R., & Zhang, G. Y. (2010). Positive modulation of AMPA receptors prevents downregulation of GluR2 expression and activates the Lyn-ERK1/2-CREB signaling in rat brain ischemia. [Research support, non-U.S. Gov't]. *Hippocampus, 20*(1), 65–77. doi:10.1002/hipo.20593.

Lead and Excitotoxicity

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Contents

Abstract

Lead (Pb^{2+}) is a known neurotoxicant, but the mechanism of its neurotoxicity is not clearly understood. Several biochemical alterations have been shown to be caused by Pb^{2+} exposure in mammalian brain, but none of these changes alone can explain the mechanism of Pb^{2+}-induced impairment of learning and memory. The most mechanistically relevant biochemical abnormalities that are directly involved in learning and memory are the excitotoxic effects caused by modulation of the *N*-methyl-D-aspartate-type glutamate receptors (NMDAR) in glutamatergic synapses. Pb^{2+} is known to affect not only the expression of the different subunits of the NMDARs but also the ontogenic developmental switch

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of the various NMDAR subunits that is essential for learning and memory. Overactivation of serine/threonine protein phosphatases (PPs) appears to be involved in these synaptic changes. PPs may not only affect the functions of the various subunits of the NMDAR directly by modulating the phosphorylation state of these subunits but may also affect their downstream function by modulation of the phosphorylation state of the downstream effectors like the cyclic AMP response element binding protein (CREB) and other proteins involved in this process. There is a great need to put these isolated pieces of information together and workout the exact pathway(s) that are disturbed by Pb^{2+}.

Keywords
Excitotoxicity • Hippocampus • Lead • Learning and memory • Neurotoxicity • NMDAR • Protein phosphatases

1 Introduction

Lead (Pb^{2+}) is a toxic heavy metal with no known physiological functions in the body. Because of its excessive use in industry, humans have been, and are constantly being, exposed to Pb^{2+}. Although Pb^{2+} poisoning in both its acute and chronic forms has gradually declined with a reduction in the use of leaded gasoline over the last few decades and with increasing surveillance of industrial and domestic exposure, it has not disappeared (Dorea 2004; Bellingr and Bellinger 2006). Pb^{2+} gets into the body through food and water, environmental pollution, agricultural technology, and food processing. Absorption and retention in the body depends on age, chemical environment of the gastrointestinal tract, and nutritional status of the individual. Generally conditions that favor calcium absorption also favor Pb^{2+} absorption and retention. The total body amount does not affect absorption, as there is no feedback mechanism for its absorption (DeMichele 1984).

Pb^{2+} toxicity affects several organ systems including the nervous, hemopoietic, renal, reproductive, endocrine, and skeletal systems (Goyer 1995; Borja-Aburto et al. 1999). The severity of these toxic effects depends on the duration of exposure, dose, and the developmental stage of the subjects. Children are particularly at increased risk of toxicity because of their frequent exposure and increased absorption and retention capacity. The hemopoietic, renal, and reproductive systems are affected at relatively high doses, whereas the central nervous system (CNS) is affected by low doses (Borja-Aburto et al. 1999).

Pb^{2+} has long been known as a neurotoxic metal but the underlying biochemical mechanism of the neurotoxic effects of Pb^{2+} is not well understood at present despite the large body of research done on this subject. Three very exhaustive reviews on the health effects of Pb toxicity and in particular on the neurotoxic effects of Pb^{2+} have been written in the past decade (Toscano and Guilarte 2005; White et al. 2007; Neal and Guilarte 2010). Of the many biochemical changes

studied, the most relevant and best studied area is the excitatory effect of Pb^{2+} at the glutamatergic synapses. This chapter is aimed to focus on the excitotoxic effects of Pb^{2+} and to augment these reviews by adding recent findings reported in the literature in the area of Pb^{2+} neurotoxicity.

2 Neurotoxicity of Lead

Pb^{2+} neurotoxicity is known since the late nineteenth century (reviewed by Toscano and Guilarte 2005). While high blood Pb^{2+} levels (>70 μg/dL) are known to cause overt encephalopathy (Chisolm 2001), low levels (as low as 7 μg/dL) in children are associated with neurobehavioral and endocrine alterations such as an increase in hyperactivity and distractibility, delayed puberty, and cognitive deficits in the form of IQ changes (Needleman et al. 1979; Bellinger 1995; Nevin 2000; Selevan et al. 2003). Studies have demonstrated a cognitive deficit of 0–5 points on the IQ scale for every 10 μg/dL increase in blood Pb level (Bellinger 1995). It has been estimated by the USEPA that a reduction of a single IQ point results in an added financial burden of US$ 8,346 per intoxicated child (Reviewed by Toscano and Guilarte 2005).

The current limit of concern for childhood Pb^{2+} intoxication set by the Center for Disease Control and Prevention (CDC) is 10 μg/dL. However, recent data suggest that CNS toxicity may be exerted by blood Pb levels of <10 μg/dL (Lanphear et al. 2000; Canfield et al. 2003), as the nervous system is especially sensitive to the effects of Pb. In a prospective study, a lifetime blood Pb^{2+} levels <10 μg/dL was shown to be associated with cognitive deficits in children at 3 and 5 years of age (Canfield et al. 2003). In experimental animal models, Pb^{2+}-exposure causes deficits in spatial learning and long-term potentiation (LTP) (Altmann et al. 1991; Kuhlmann et al. 1997; Gilbert and Mack 1998; Nihei et al. 2000). It has been suggested that there is a "developmental window" that spans from gestation through lactation, in which exposure to low levels of Pb^{2+} is able to cause long-lasting cognitive function deficits (Kuhlmann et al. 1997). This concept of developmental window has been demonstrated by the reports that children that were previously, but not currently, exposed to Pb^{2+} exhibited lasting neurobehavioral and cognitive deficits (Needleman et al. 1979).

The hippocampus plays a pivotal role in learning and memory processes, and it has been suggested that this structure is particularly affected by Pb^{2+} (Bielarczyk et al. 1996; Sharifi et al. 2002). The most troubling aspect of Pb^{2+} toxicity in children is that neurotoxicity caused by Pb^{2+} exposure is irreversible. Chelation therapy, which is the primary means of treating children with blood Pb^{2+} levels of >45 μg/dL, can reduce the body burden of Pb^{2+} but does not reverse the cognitive or behavioral deficits associated with Pb^{2+} exposure (US, CDC 2002; Rogan et al. 2001; Dietrich et al. 2004). This highlights the possibility that Pb^{2+} exposure induces long-lasting (or permanent) changes in the brain during a critical period of development in childhood (Neal and Guilarte 2010).

Many biochemical changes have been reported in the brain that may explain the mechanism of Pb^{2+} neurotoxicity. Some of the reported biochemical alterations

caused by Pb^{2+} exposure in the brain include: altered adenylyl cyclase activity and reduction in the heme-containing enzymes and lower energy metabolism in the developing brain (Clarkson 1987), interference with cell adhesion molecules (Silbergeld 1992), reduced activity of alkaline phosphatase (Antonio and Lert 2000), decreased expression (Nihei et al. 2001) and activity (Xu et al. 2005) of protein kinase C, decreased production of transthyretin and low availability of thyroid hormone to the developing CNS (Zhang et al. 1996), decreased levels of nitric oxide in the hippocampus (Sun et al. 2005), altered neurotransmitter activity in the hippocampus (Reddy et al. 2007; Wang et al. 2007), and altered protein phosphorylation and impairment of the glutamatergic synapse transmission (Neal and Guilarte 2010). None of these biochemical changes alone explains the learning and memory deficits caused by the low-dose Pb^{2+} exposure. There is a need to put these pieces together and workout the pathway(s) involved in learning and memory that are affected by Pb^{2+} exposure. The understanding of such pathway(s) is essential to devising any therapeutic/intervention strategies to combat Pb^{2+}-induced neurotoxicity.

3 Hippocampal Plasticity, NMDA Receptor, and Learning

Hippocampus is the main brain region involved in the acquisition and consolidation of higher brain function, particularly spatial learning and memory. The disruption of hippocampal function by a variety of methods produces deficits in such brain functions (Izquierdo 1993; McNamara and Skelton 1993). The major cellular mechanism within the hippocampus believed to be responsible for acquisition of new memories is LTP, which is a long-lasting increase in synaptic efficacy following brief periods of stimulation of specific synapses (Malenka and Nicoll 1999; Hashemzadeh-Gargari and Guilarte 1999; Nihei et al. 2000, 2001; Shimizu et al. 2000).

Some forms of LTP in the hippocampus, specifically those induced in Schaffer collateral-CA1 and perforant path-dentate gyrus synapses, are dependent upon *N*-methyl-D-aspartate-type glutamate receptor (NMDAR) activation (Madison et al. 1991; Malenka and Nicoll 1993, 1999; Teyler and DiScenna 1987; Zalutsky and Nicoll 1990). LTP requires presynaptic glutamate release and subsequent activation of the postsynaptic NMDAR (Collingridge and Bliss 1987; Massicotte and Baudry 1991; McNaughton 1993). Disruption of NMDAR function pharmacologically or by deletion of specific NMDAR subunits using gene knockout techniques are associated with disruption of hippocampal LTP and learning and memory (Gilbert and Mack 1990; Robinson and Reed 1992; Morris et al. 1986; Neal and Guilarte 2010).

4 NMDA Receptors and Their Structure

Glutamate receptors are classified into metabotropic and ionotropic subtypes according to whether they exist as G-protein-coupled receptor or as an ion channel.

Both these receptor types mediate the actions of glutamate. Metabotropic glutamate receptors (mGluRs) have been extensively studied in hippocampal physiology. mGluRs are composed of eight isoforms (mGluR1–8), which are classified into groups I–III. Of these mGluR5, which belongs to group I, is primarily postsynaptic and is coupled preferentially to Gq/11 and its downstream effectors. Recent studies have demonstrated that mGluR5 is involved in learning and memory. mGluR5 has been shown to be critically important for both hippocampal synaptic plasticity and hippocampus-based learning and memory (reviewed by Xu et al. 2009a, b). Inhibition of mGluR5 with specific antagonist MPEF impaired the acquisition and consolidation of hippocampus-dependent memory, whereas its activation by specific agonist showed the opposite results (Gasparini et al. 1999).

Ionotropic receptors are further classified based on their selective agonists as NMDA, AMPA, and Kainate receptors (Hassel and Dingledine 2006). These receptors bind with glutamate with different affinities. Of these, NMDAR are the most tightly regulated and the most extensively studied. Activation of the NMDAR plays a central role in brain development, learning, and memory as well as in neurodegenerative diseases (Collingridge and Lester 1989; Ozawa et al. 1998; Scheetz and Constantine-Paton 1994). These receptors are located primarily in the hippocampus but also in the cerebral cortex (Monaghan et al. 1983; Monyer et al. 1994; Moriyoshi et al. 1991) and play an essential role in hippocampus-mediated learning and memory (Morris et al. 1982, 1986).

NMDAR is a tetrameric complex assembled from the ubiquitous NR1 subunit which is an essential component of all NMDAR complexes along with various combinations of NR2 or NR3 subunits. NR1 subunit is a constitutional component of NMDA receptors and is widely expressed throughout the CNS at all ages, whereas NR2A and NR2B are functional components whose expression varies with the developmental stage of the animal and the different regions of the brain (Xu and Rajanna 2006). A single gene encodes NR1, but at least eight splice variants of NR1 subunits (NR1A to NR1H) have been found (Laurie and Seeburg 1994; Zukin and Bennett 1995). These splice variants of NR1 impart different pharmacological characteristics to the NMDAR (Durand et al. 1992). Alternatively spliced exon 5 at the N terminus (N-cassette) encodes for a 21 amino acid sequence. Splicing of exon 5 results in transcripts designated as lacking (NR1-a) or containing (NR1-b) the N-cassette (Zukin and Bennett 1995). Exons 21 and 22 encode for two C-terminus cassettes, C1 and C2, which code for 37 and 38 amino acid sequences, respectively. The individual splicing of the C1 or C2 cassette results in transcripts designated as NR1–2 andNR1–3. The presence or absence of both C-terminus cassettes results in NR1–1 and NR1–4 variants. Deletion of the C2 cassette alters the reading frame and results in the creation of an additional coding region of 22 amino acids, the C2' cassette (Toscano and Guilarte 2005).

Compared to NR1, NR2 and NR3 are more complex and mostly determine the function of NMDAR channel. NR2 subunits are encoded by four distinct genes (NR2A–NR2D), while NR3 subunits are encoded by two distinct genes (NR3A and NR3B). NR2 subunits play positive roles in gating the NMDAR channel, while NR3 subunits play negative roles by forming an unconventional channel

(Perez-Otano and Ehlers 2004). The molecular biology of these subunits, their deferential developmental and regional expression and their distinct intracellular protein associations and functions are thoroughly reviewed by Neal and Guilarte 2010. Together, the associations between NR1 splice variants with other subunits exhibit an exquisite degree of heterogeneity and specialization and play essential roles in synaptic activity (Neal et al. 2011).

NMDAR subunits play critical roles in hippocampal synaptic plasticity. Blockades of NMDAR containing either NR2A or NR2B subunits lead to a selective defect in either LTP or long-term depression (LTD), respectively (Liu et al. 2004). The differential expression of various NMDR subunits across the developmental time span of peak LTP and hippocampus-mediated learning, together with the impairment of learning due to targeted knockout of NMDAR subunits strongly support the essential role of NMDAR in synaptic plasticity and learning and memory (Neal and Guilarte 2010).

5 Activity-Dependent Expression of NMDA Receptor Subunit

The expression of synaptic NMDAR subunit is controlled not only by a genetically programmed development of excitatory circuitry but also by the level of activity present at the synapse. Both in vivo and vitro studies from neuronal cultures have provided evidence for the activity-dependent modifications in NMDAR subunit expression (Toscano and Guilarte 2005). The expression of NR2A subunit, but not NR2B, has been shown to be dependent upon calcium influx mediated by NMDAR and L-type calcium channels since pharmacological blockade of NMDAR and L-type calcium channels decreased NR2A subunit expression but had no effect on NR2B expression. This resulted in the expression of NMDAR complexes with higher proportion of NR2B subunits with the corresponding functional implications (Hoffmann et al. 2000). A reduction in presynaptic exocytosis produced similar results (Lindlbauer et al. 1998). In addition, activity-dependent changes of NMDAR subunits also occur at the postsynaptic density (PSD). Pharmacologically blocking of the sodium channels resulted in decreased PSD levels of NR2A but increased levels of NR1 and NR2B subunits, whereas, activating these channels had the opposite effects (Ehlers 2003).

6 Lead and Synaptic Transmission

It has been suggested that a number of neurotoxic effects of Pb^{2+} may be due to its interference with neurotransmitter systems, particularly those which involve cellular calcium homeostasis, and calcium-dependent enzymes (Guilarte 1997; Finkelstein et al. 1998; Savolainen et al. 1998a, b; Bressler et al. 1999; Goyer and Clarkson 2001; Nihei and Guilarte 2001; Gilbert and Lasley 2002). Establishment of neuronal circuitries in the developing central nervous system depends on the

pattern of electrical activity going through the synapses. At early stages of brain development, most neurons fire spontaneously, and this spontaneous electrical activity is believed to be required for axonal outgrowth, pruning of synaptic connections, and maturation of neuronal signaling properties (Moody 1998). Therefore, it can be inferred that Pb^{2+}-induced impairment of learning and memory in children is a result of altered synaptic activity in the brain, particularly in hippocampus which is involved in cognitive processing (Swanson et al. 1997).

The mechanism by which Pb^{2+} alters synaptic activity remains unknown. It has been proposed that Pb^{2+} affect synaptic activity by mimicking the activity of calcium (Ca^{2+}). The ability of Pb^{2+} to substitute for Ca^{2+} is one of the primary mechanisms proposed for Pb^{2+} toxicity in the brain. This Ca^{2+}-mimetic ability of Pb^{2+} has been reported to enhance not only spontaneous neurotransmitter release but also inhibit evoked neurotransmitter release due to impeding Ca^{2+} influx through voltage-sensitive Ca^{2+} channels (Minnema et al. 1988; Kober and Cooper 1976; Atchison and Narahashi 1984; Braga et al. 1999a; Peng et al. 2002; Xiao et al. 2006). Pb^{2+} is also known to bind with intracellular Ca^{2+}-binding proteins and may prevent the detection of Ca^{2+} signaling essential to neurotransmission (Bouton et al. 2001; Marchetti 2003). Ca^{2+}-regulated proteins that are known to be targeted by Pb^{2+} include protein kinases, protein phosphatases, voltage-gated Ca^{2+} channels (VGCC), and Ca^{2+}-binding proteins that regulate mobilization and docking of synaptic vesicles (Atchison 2003; Suszkiw 2004; Braga et al. 1999a, b).

Pb^{2+} is also known to affect the neurotransmission of glutamate, which is the most abundant excitatory neurotransmitter in the brain. Glutamate and its receptors have an important role in LTP and synaptic plasticity, which are fundamental processes involved in learning and memory (Malenka and Nicoll 1999). Chronic developmental Pb^{2+} exposure of rats as well as in vitro acute Pb^{2+} exposure of hippocampal cultures and slices have been reported to decrease the release of glutamate and γ-aminobutyric acid (GABA) (Lasley and Gilbert 2002; Braga et al. 1999a; Xiao et al. 2006).

7 Lead and LTP

The effects of Pb^{2+} on hippocampal LTP has been demonstrated since the early 1990s. Developmental Pb^{2+} exposure increases the threshold for hippocampal LTP induction, reduces the magnitude of potentiation, and accelerates its decay (Reviewed by Toscano and Guilarte 2005; Altmann et al. 1994; Gilbert and Mack 1998; Gilbert et al. 1999a, b). Animals exposed to Pb^{2+} from early life showed deficient excitatory postsynaptic potential (EPSP) as well as cellular (population spike) components of this field response, and these effects persisted to adulthood despite termination of Pb^{2+} exposure at weaning (Gilbert et al. 1996, 1999a). Similar effects of Pb^{2+} exposure on LTP were also reported in hippocampal slices from exposed animals (Altmann et al. 1993; Sui et al. 2000). These data indicate that Pb^{2+} exposure reduced LTP magnitude and thus impaired the efficacy of the cellular mechanisms that support learning in the hippocampus (White et al. 2007).

This Pb^{2+}-induced deficits in hippocampal LTP has been suggested to be associated with a reduction in Ca^{2+}-dependent glutamate release (Lasley and Gilbert 1996; Lasley et al. 2001). However, the induction of LTP in mossy fiber-CA3, which is dependent upon an increase in glutamate release, is not affected by Pb^{2+} exposure (Kawamura et al. 2004), while the induction of LTP at excitatory synapses that are not dependent on increased glutamate release but are NMDAR dependent (i.e., Schaffer collateral-CA1 LTP or perforant path-dentate gyrus LTP) is impaired in the Pb^{2+}-exposed brain. These results clearly indicate that Pb^{2+}-induced deficits in LTP are NMDAR-specific (Toscano and Guilarte 2005).

8 Lead, Calcium, and Glutamate Release

Pb^{2+} mimics Ca^{2+} and disrupts cellular calcium homeostasis as well as the role of Ca^{2+} as an important intracellular second messenger (Simons 1993). Voltage-sensitive calcium channels (VSCCs), which regulate Ca^{2+} influx, are essential for many neuronal processes such as neurotransmitter release (Kobayashi and Mori 1998). Pb^{2+} may affect neuronal functions in two ways; first by inhibiting the entry of Ca^{2+} into the cell, as Pb^{2+} is a very potent inhibitor of VSCCs (Audesirk 1993; Busselberg et al. 1993; Evans et al. 1991; Minnema et al. 1988). Second, Pb^{2+} itself may enter cells through Ca^{2+} channels and by mimicking Ca^{2+} may affect its functions (Reviewed by Loikkanen et al. 2003).

A dose-dependent reduction in glutamate release in animals developmentally exposed to Pb^{2+} has been reported (Gilbert and Lasley 2007). Chronic Pb^{2+} exposure beginning in utero or in the early postweaning period and continuing throughout life alters presynaptic release of glutamate in the rat hippocampus. However, this Pb^{2+}-induced glutamate release appears to be dose-dependent. At lower Pb levels synaptic release of glutamate was diminished and this effect was reversed at higher level of Pb^{2+} exposure (Lasley and Gilbert 2002). This biphasic dose response indicates the presence of more than one mechanisms of Pb^{2+} action. The diminished glutamate release at lower Pb^{2+} levels appears to be due to the blocking of VSCC by Pb^{2+}. The increased glutamate release at higher level of Pb^{2+} exposure suggests the diminution of the K^+-stimulated transmitter response (White et al. 2007). Similar findings were reported from acute exposure of cultured hippocampal neurons to Pb^{2+} (Braga et al. 1999a, b). Recent studies have revealed that the loss of proteins involved in vesicular release, namely, synaptophysin and synaptobrevin, is also involved in Pb^{2+}-induced modulation of transmitter release (Neal et al. 2010).

In addition, nicotinic modulation of synaptic transmission has also been implicated in Pb^{2+}-induced transmitter release (Nihei et al. 2000). Hippocampal neurons exposed to various concentrations of Pb^{2+} inhibited somatodendritic $\alpha_4\beta_2$ nAChRs and α_7 nAChRs and caused substantial inhibition of transmitter release. This effect was found to be due the direct interaction of Pb^{2+} with these receptors (Ishihara et al. 1995; Mike et al. 2000). It has also been hypothesized that Pb-induced inhibition of nicotinic cholinergic modulation of action potential-dependent

transmitter release is mediated by a PKC-dependent mechanism (Braga et al. 2004). It has been suggested that Pb^{2+} activates PKC which in turn phosphorylates nAChRs, proteins associated with the receptors, and/or proteins linking the receptors to the action potential-dependent transmitter release process. Direct phosphorylation of nAChRs by PKC has been shown to reduce receptor activity in sympathetic neurons (Downing and Role 1987). However, this hypothesis has been disputed by other investigators (Seguela et al. 1993; Moss et al. 1996; Fenster et al. 1999).

9 Lead and NMDA Receptor

Glutamate receptors, particularly, the NMDAR are one of the most important targets of Pb^{2+} (reviewed by Xu and Rajanna 2006). The observation that Pb^{2+} exposure during development affects NMDAR-dependent LTP (LTP dependent on increased glutamate release) but not NMDAR-independent LTP in hippocampus provided the first experimental evidence that Pb^{2+} targets NMDARs (Gutowski et al. 1998; Kawamura et al. 2004). Since then, several studies demonstrated that Pb^{2+} is a selective and potent noncompetitive inhibitor of the NMDAR (Toscano and Guilarte 2005; White et al. 2007; Neal and Guilarte 2010; Neal et al. 2011). Pb^{2+} in micromolar concentration causes a reversible inhibition of the current activated by glutamate through the NMDAR channel in cultured and acutely dissociated neurons and reduces access to the NMDA receptor channel in brain tissue homogenates (Marchetti 2003; Lasley and Gilbert 1999). Decreased NMDA-specific glutamate receptor binding has also been reported in the brain of Pb^{2+}-exposed rats (Rajanna et al. 1997). A possible mechanism for this Pb^{2+}-induced inhibition of the NMDAR is the binding of Pb^{2+} at the zinc (Zn^{2+}) regulatory (inhibitory) site of the NMDAR in a voltage-independent manner (Lasley and Gilbert 1999).

The effect of Pb^{2+} on NMDA-activated current is dependent on developmental stages and NR subunit types (Ishihara et al. 1995; Omelchenko et al. 1997; Ujihara and Albuquerque 1992). In addition to acting as an NMDAR antagonist, Pb^{2+} exposure also disrupts normal NMDAR ontogeny. Pb^{2+}-induced changes in NMDAR subunits during development may form the basis for Pb^{2+} effects on synaptic plasticity and cognitive function (Reviewed by Lau et al. 2002). Developmental Pb^{2+} exposure has been shown to cause alterations in NR1 splice variant expression, NR2 subunit ontogeny, and NMDAR-dependent signaling (Neal et al. 2011). Adult rats exposed to Pb^{2+} during development and postweaning into adolescence suffered marked reductions in gene expression of the NR1 subunit of the NMDAR in the hippocampus (Monyer et al. 1992). Chronic developmental Pb^{2+} exposure in animals is known to alter expression of NR1 splice variants (Guilarte and McGlothan 2003; Xy et al. 2002; Guilarte et al. 2000). The mechanism of these Pb^{2+}-induced deficits in NR1 is proposed to be the altered targeting and cell surface expression of NMDAR subunits to the synapse due to changes in NR1 splice variant expression. The lower levels of NR1 subunit mRNA expressed

in the Pb^{2+}-exposed hippocampus are principally due to decreased levels of the NR1-4 and NR1-2 splice variants. A unique characteristic of these splice variants is that they lack the C1 cassette and impart the highest cell surface expression, PKC potentiation, and calcium kinetics to NMDAR complexes (Neal et al. 2011).

NR2A and NR2B are abundantly expressed in the hippocampus and may be involved in mediating Pb^{2+}-toxicity (Guilarte and McGlothan 2003; Lau et al. 2002; Marchetti 2003; Nihei and Guilarte 1999, 2001; Nihei et al. 2000; Perez-Otano and Ehlers 2004; Toscano et al. 2002; Waters and Machaalani 2004). Chronic Pb^{2+} exposure alters the composition of the NR2 subunits of the NMDR in the rat brain (Nihei and Guilarte 1999). Specifically, developmental Pb^{2+} exposure in animals decreased expression of the NR2A subunit with no change or a small increase in NR2B subunit expression (Nihei et al. 2000; Nihei and Guilarte 1999; Zhang et al. 2002; Guilarte and McGlothan 1998; Toscano et al. 2002; Neal et al. 2011). Chronic Pb^{2+} exposure not only reduces NMDAR level but also prevents or delays the developmental switch of NR1/NR2B complex to NR1/NR2A complex with reduction of activity-dependent synaptic plasticity in the mature brain. During early development, NR2B-containing NMDARs predominate until a developmental switch occurs, resulting in the incorporation of the NR2A subunit (Monyer et al. 1994). It is suggested that this developmental switch from predominately NR2B-containing NMDARs to NR2A-containing NMDARs is delayed or impaired during Pb^{2+} exposure (Toscano et al. 2002; Toscano and Guilarte 2005).

These NR2 subunit-specific effects of lead could be explained by the differential sensitivities of different subunits to Pb^{2+}. Gavazzo et al. (2008) have shown that Pb^{2+} interacts at the Zn^{2+} regulatory site of NMDAR complexes containing the NR2A but not the NR2B subunit. The NR2 subunits have different Zn^{2+} binding sites. NR2A-NMDAR binds Zn^{2+} at a high affinity (nM affinity), while the NR2B-NMDAR binds Zn^{2+} with lower affinity (μM range) (Paoletti et al. 2000; Rachline et al. 2005). These findings have been corroborated by the observations that recombinant NR1/NR2A complexes are more sensitive to Pb^{2+} inhibition than NR1/NR2B complexes (Omelchenko et al. 1996). Since Pb^{2+} and Zn^{2+} have similar potencies in inhibiting the NMDAR (Guilarte et al. 1994, 1995), it is likely that changes observed with NR2A-NMDARs but not NR2B-NMDARs may be a result of preferential inhibition of Pb^{2+} for NR2A-NMDARs (Guilarte et al. 1995; Gavazzo et al. 2008).

Furthermore, different concentrations of Pb^{2+} have differential influence on NMDAR subunits, and different subunits of NMDAR display different sensitivities to Pb^{2+}. A dose-dependent reduction by Pb in NMDAR-NR1, NR2A, and NR2B protein levels has been reported (Lau et al. 2002). The rank order of sensitivity of different subunits to Pb inhibition has been reported as follows: NR1A-NR2B > NR1A-NR2A > NR1A-NR2C (Gavazzo et al. 2001) and NR1B-NR2A > NR1B-NR2C > NR1B-NR2D > NR1B-NR2AC (Omelchenko et al. 1997).

Region-specific effects of Pb^{2+} on the expression of NR1 and NR2B subunits have also been reported. In cortical neurons, expression of NR1 was unchanged but that of NR2B was significantly increased by Pb^{2+}. In contrast, expression of

both NR1 and NR2B was significantly decreased in hippocampal neurons. Thus, it is likely that the toxic effects of Pb^{2+} may cause differential damage to different types of memory that are mediated by cortical and hippocampal neurons, respectively (Lau et al. 2002). Furthermore, both regional and developmental differences in the hippocampal neurons have been reported in Pb^{2+}-exposed rats. For example, the expression of NR1-2a mRNA in Pb^{2+}-exposed rats was significantly increased in areas CA1, CA4, and dentate gyrus (DG) at postnatal days (PND) 14–15 but in areas CA4 and DG at PND20–21 (Zhang et al. 2002; Guilarte and McGlothan 1998; Guilarte et al. 2000). On the other hand, Pb^{2+}-induced decreased expression of NR2A was observed in areas CA1, CA2, CA3, and DG at PND15 and areas CA1, CA3, and DG at PND20. Similarly, NR3A mRNA levels were also significantly decreased in CA1, CA4, and DG subfields at PND15 and CA1 and DG subfields at PND20 in Pb^{2+}-exposed rats (Zhang et al. 2002). These regional and developmental regulations of NMDAR mRNA splicing may lead to abnormality of natural NMDAR stoichiometry, control sensitivities to phosphorylation, and therefore kinetic properties of the NMDA channels and their involvement in LTP and synaptic plasticity (Zhang et al. 2002). Thus, this disruption of the ontogenetically defined pattern of NMDAR subunit expression and NMDAR-mediated calcium signaling in glutamatergic synapses appears to be the mechanism of Pb^{2+}-induced neurotoxicity and deficits in learning and memory. These changes are associated with deficits in long-term potentiation in the hippocampus and impairment of spatial learning (Nihei and Guilarte 2001; Toscano and Guilarte 2005; Zhang et al. 2002; Gilbert et al. 1996; Neal and Guilarte 2010).

10 Lead and NMDA Signaling

It has been reported that synaptic NMDARs mediate cyclic AMP response element binding protein (CREB) and extracellular signal-regulated kinases (ERK) activation, synaptic plasticity, and survival pathways. On the other hand, extra-synaptic receptors are associated with a CREB-shutoff pathway, ERK inactivation, and induction of cellular death pathways (Hardingham et al. 2002; Vanhoutte and Bading 2003; Ivanov et al. 2006). Changes in NMDAR subunit composition can result in altered NMDAR-dependent signaling. Many signaling pathways are dependent on NMDAR subunit composition and/or localization. Pb^{2+}-induced alterations in NMDAR subunit composition could interfere with the downstream signaling pathways including MAPK signaling (Cordova et al. 2004), calcium/calmodulin kinase II (CaMKII) activity (Toscano et al. 2005), protein kinase C (PKC) activity (Bressler et al. 1999), and CREB phosphorylation status and binding affinity (Toscano et al. 2002; 2003). CREB is a transcription factor for many NMDAR activity-dependent immediate early genes (IEGs), which play an essential role in memory consolidation (Athos et al. 2002; Bourtchuladze et al. 1994). Thus, CREB plays an important role in signal propagation from synapses to the nucleus by linking NMDAR activation and calcium-dependent signaling to the

expression of genes necessary for synaptic plasticity (Bredt et al. 1992). Altered IEG expression in animals exposed to Pb^{2+} has been observed (Kim et al. 2002), indicating that altered CREB activity due to Pb^{2+}-mediated disruption of NMDAR signaling may result in impaired learning and memory processes.

CREB phosphorylation is an important element of LTP. Induction of LTP (and learning) increases CREB phosphorylation (Mizuno et al. 2002; Schultz et al. 1999; Viola et al. 2000), whereas pharmacological inhibition of NMDAR-mediated signaling decreases CREB phosphorylation (Athos et al. 2002). CREB phosphorylation at serine-133 facilitates the recruitment of CREB-binding proteins and the assembly of transcriptionally active complex at the start site of CRE containing genes (Chirivia et al. 1993). Pb^{2+} exposure decreases CREB phosphorylation without affecting the total CREB levels and alters the ability of CREB family proteins to bind with CRE (Toscano et al. 2003), suggesting that the mechanisms that regulate the phosphorylation of CREB at serine-133 and the binding ability of CREB family proteins may be altered by Pb^{2+} exposure. This may affect the transcription of genes associated with learning, memory, and synaptic plasticity.

11 Lead and BDNF Signaling

Another potential signaling pathway that may be involved in Pb^{2+}-induced neurotoxicity is impaired NMDAR-dependent retrograde signaling of neurotrophic factors, particularly of brain-derived neurotrophic factor (BDNF). Pb^{2+}-exposed hippocampal neurons exhibit reduced proBDNF expression and BDNF release. Furthermore, complete recovery of Pb^{2+}-induced changes in presynaptic protein levels and vesicular neurotransmitter release has been reported in hippocampal neurons incubated with exogenous BDNF (Neal et al. 2010). Retrograde BDNF signal from the postsynaptic side has been implicated in axon morphology, synaptic connectivity, and synaptic ultrastructure (Neal and Guilarte 2010; Neal et al. 2010). NMDAR activation results in the generation and release of BDNF (Hartmann et al. 2001; Jiang et al. 2005; Walz et al. 2006), which may be essential to the generation or unmasking of presynaptic neurotransmitter release sites (Walz et al. 2006). BDNF signaling is known to modulate the expression of several pre- and postsynaptic proteins (Pozzo-Miller et al. 1999; Tartaglia et al. 2001). Of particular interest is the enhancement of NR2A expression but not the expression of NR2B in hippocampal slices exposed to exogenous BDNF (Small et al. 1998; Caldeira et al. 2007). These results are further supported by the reduced expression of NR2A but not the NR2B subunit and reduced vesicular release in BDNF knockout mice (Margottil and Domenici 2003). These observations suggest that NR2A-NMDARs may be preferentially linked to BDNF signaling. The altered expression of NR2A subunit by both Pb^{2+} exposure and impaired BDNF signaling strongly supports the hypothesis that the disruption of NMDAR activity-dependent BDNF signaling in involved in Pb^{2+}-induced toxicity in synapses (Neal et al. 2010).

12 Lead and Other Brain Cells

Most of the work on Pb^{2+}-induced neurotoxicity has focused on neuronal cells. Other support cells, which are severalfold greater in number than neurons and regulate/modulate the function of neurons, may also be affected by Pb^{2+} and thus may be involved in Pb-induced neurotoxicity. Of the support cells, astroglia are capable of uptaking 14-fold more Pb^{2+} than neurons. The uptake of Pb^{2+} by astroglia is suggested to be through voltage-dependent Ca^{2+} channels (Simons and Pocock 1987). This Ca^{2+}-dependent uptake of Pb^{2+} by astroglia may be induced by their interaction with neurons (Tiffany-Castiglioni 1993; Lindhal et al. 1999) and may involve glutamate-dependent increase in intracellular calcium (Cornell-Bell et al. 1990). The accumulation and storage of Pb^{2+} in astroglia may be a mechanism of protection for neurons which are more sensitive than astroglia to the toxic effects of Pb^{2+} (Holtzman et al. 1987; Tiffany-Castiglioni et al. 1986; Tiffany-Castiglioni 1993). On the other hand, such storage of Pb^{2+} in astroglia may provide a reservoir for its continuous release and thereby may contribute to the toxicity of adjacent neurons or astroglia themselves (Struzynska 2009).

13 Lead and Metabotropic Glutamate Receptors

Of the metabotropic glutamate receptor, mGluR5 has also been linked to Pb^{2+}-induced deficits in learning and memory. The impact of developmental lead exposure on hippocampal mGluR5 expression and its potential role in Pb^{2+}-induced neurotoxicity has been investigated both in vitro and in vivo (Xu et al. 2009a, b). Decreased expression of mGluR5 mRNA and protein by Pb^{2+} dose dependently suggests that mGluR5 might be involved in Pb^{2+}-induced neurotoxicity. Impairment of mGluR5-dependent long-term depression (LTD) (Huang and Hsu 2006) and decreasing NMDAR-dependent or protein synthesis-dependent long-term potentiation have been hypothesized as potential mechanisms of Pb^{2+}-induced neurotoxicity (Toscano and Guilarte 2005; Topolnik et al. 2006; Manahan-Vaughan et al. 2003; Manahan-Vaughan and Braunewell 2005; Naie and Manahan-Vaughan 2004; Neyman and Manahan-Vaughan 2008).

14 Protein Phosphatases and Lead-Induced NMDAR-Dependent Neurotoxicity

The major Ser/Thr protein phosphatases (PPs) in mammalian brain are PP1, PP2A, and PP2B (Liu et al. 2005). Overactivation/overexpression of these Ser/Thr PPs has been implicated in impairment of learning and memory. PPs are strong molecular constraints on learning and memory (Lee and Silva 2009). PPs are also involved in cognitive decline in aging (Mansuy and Shenolikar 2006; Knobloch et al. 2007) and in favoring forgetting (Genoux et al. 2002). Overactivation of PP1 is associated

with learning and memory impairment (Genoux et al. 2002, 2011; Koshibu et al. 2009, 2011; Graff et al. 2010; Haege et al. 2010). PP2A and PP2B also adversely affect learning and memory (Bennett et al. 2003; Havekes et al. 2006; Yamashita et al. 2006; Mauna et al. 2010; Oberbeck et al. 2010). Overactivation of PP2A induces LTD in vivo (Thiels et al. 1998), and inhibition of PP2A abrogates LTD induction (Mauna et al. 2010).

Whether the overactivation/overexpression of these PPs is involved in Pb^{2+}-induced impairment of learning and memory has largely remained uninvestigated until now. This issue was recently addressed by investigating the effect of Pb^{2+}-exposure on PPs both in cultured human neurons and in rats exposed to Pb^{2+} during early development. In the Pb^{2+}-exposed cultured human fetal neurons, a significant increase in total PPs and PP2A activities was observed. Pb^{2+} exposure significantly increased the expression of PP1 and PP2B but significantly decreased the expression of PP2A and PP5 in cultured human fetal neurons (Rahman et al. 2010). In rats exposed to Pb^{2+} during early development, learning, short-term memory (STM), and long-term memory (LTM) were significantly impaired at PND 21, and this impairment of learning and LTM was associated with decreased synaptogenesis and increased expression and activity of PP1 and PP2A in the hippocampus. On the other hand, at PND 30, learning and short-term memory (STM), but not the LTM, was impaired by Pb^{2+}. At this developmental stage, expression and activity of hippocampal PP1 was increased but that of PP2A was decreased. These results suggest that increased PP1 activity in hippocampus is involved in the impairment of learning and LTM, whereas, increased PP2A activity is involved in the impairment of STM (Rahman et al. 2012).

LTM involves protein synthesis, growth, and formation of new synapses (Martin et al. 1997; Ma et al. 1999; Toni et al. 1999; Bozdagi et al. 2000; De Roo et al. 2008). By contrast, STM involves covalent modification of proteins in the presynaptic or postsynaptic structures (Kandel 2001; Malinow and Malenka 2002). The major covalent modification underlying these long-lasting changes in synaptic communication is protein phosphorylation, which is regulated by a balance between protein kinases (PKs) and PPs. Increased activity of PKs results in increased protein phosphorylation which has been implicated in LTP. On the other hand, increased activity of PPs results in decreased protein phosphorylation, which has been implicated in LTD (Roberson et al. 1996; Winder and Sweatt 2001; Blitzer et al. 2005). Reversible protein phosphorylation regulates presynaptic and postsynaptic events in excitatory and inhibitory neurons. The major substrates for PPs at these synaptic sites include ligand-gated ion channels and G-protein-coupled receptors, whose functional properties, trafficking, and synaptic organization are controlled by reversible phosphorylation (Swope et al. 1999).

Several mechanisms have been proposed to explain the role of PPs in learning and memory. These include dephosphorylation and the subsequent deacetylation of histone proteins and chromatin remodelling and altered expression of CREB and NF-κB (Kandel 2001; Fischer et al. 2007; Miller et al. 2008; Koshibu et al. 2009, 2011; Oberbeck et al. 2010). Both PP1 and PP2A dephosphorylate CREB

(Mauna et al. 2010) and thereby reduce CREB-mediated gene expression (Wadzinski et al. 1993; Alberts et al. 1994; Genoux et al. 2002; Oberbeck et al. 2010). Other mechanisms include dephosphorylation by PP1 and PP2A of NMDA receptor and MAPK (Chan and Sucher 2001; Oberbeck et al. 2010), calcium-/calmodulin-dependent protein kinase IV (CaMKIV) (Westphal et al. 1998; Anderson et al. 2004), ERK (Davis et al. 2000; Norman et al. 2000; Silverstein et al. 2002; Ho et al. 2007), and the AMPA receptor (Thiels et al. 2002).

Of particular significance in the Pb^{2+}-induced excitotoxicity is the dephosphorylation and subsequent inhibition of the NMDARs-associated signaling pathways. Protein phosphorylation has been established as an important mechanism for the regulation of NMDAR function. LTP is accompanied by increased glutamate receptor phosphorylation through various protein kinases and a concomitant decrease in protein phosphatase activity (Bliss and Collingridge 1993; Mulkey et al. 1993; Soderling and Derkach 2000). In contrast, LTD has been shown to be dependent on glutamate receptor dephosphorylation mediated by an increase in the activity of protein phosphatases, possibly PP1 and PP2A (Lee et al. 1998, 2000; Thiels et al. 1998). Thus, coordination of kinase and phosphatase activities is crucial for the modulation of synaptic plasticity. Both kinases (PKA) and phosphatases (PP1 and PP2A) are located in physical proximity to NMDAR (Chan and Sucher 2001). Synaptic NMDA receptors are phosphorylated or dephosphorylated depending on synaptic stimulation. Phosphorylated NMDA receptors have enhanced channel openings and a consequent increase in Ca^{2+} influx, and this influx is necessary for inducing long-term neuronal changes (Levitan 1999; Prybylowski and Wenthold 2004; Raymond et al. 1994; Roche et al. 1994). Following excitatory neurotransmission and Ca^{2+} influx, NMDA receptors are phosphorylated by PKA then rapidly dephosphorylated by PP1, PP2A, or PP2B. This reversible phosphorylation controls synaptic strength, memory formation, and storage by the induction of LTP or LTD (Blitzer et al. 2005). Several PKs, including CaMKII, PKC, PKA, and ERKs, have been implicated in LTP induction and maintenance, whereas PPs are implicated in the induction of LTD. Previous studies have shown that LTD is dependent on the change of the phosphorylation state of glutamate receptors in general (Lee et al. 1998, 2000) and the activity of PPs, particularly PP1 and PP2A (Mulkey et al. 1993, 1994; Thiels et al. 1998). Stimulation of NMDARs activates PP1, PP2A, and PP2B whose substrate specificity is primarily determined through interaction with regulatory and targeting proteins (Mansuy and Shenolikar 2006).

NMDAR activity is attenuated by PP1, PP2A (Wang et al. 1994), and PP2B (Mulkey et al. 1994; Wang and Kelly 1997). PP1 diminishes NMDA-receptor-mediated synaptic currents in an activity-dependent manner in the hippocampus (Wang et al. 1994; Westphal et al. 1999). PP1 and PP2A appear to decrease the single channel open time in cultured rat hippocampal neurons and thus reduce NMDAR activity (Wang et al. 1994). PP1 has been reported to have more involvement than PP2A in dephosphorylating the NMDAR.

Both positive and negative effects of PP2B on glutamate receptor functions have been reported. PP2B reduces NMDA-receptor-mediated currents by

dephosphorylating the NR2A subunit of the NMDAR and reduction of the open time of individual channels (Lieberman and Mody 1994) and desensitization of the receptor (Krupp et al. 2002; Tong et al. 1995). On the other hand, PP2B has been reported to potentiate NMDAR-induced metabotropic mGluR5 receptor activity by dephosphorylating PKC-dependent sites on the mGluR5 C-terminus and attenuating mGluR5 desensitization (Alagarsamy et al. 2005). PP2B-mediated dephosphorylation also contributes to the developmental switch of NMDAR subunits and thus modulates NMDA receptor subunit composition. In the developing cerebellum, it counteracts the TrkB- and ERK1/2-dependent upregulation of the NR2C subunit, which then exchanges with NR2B to promote synaptic transmission from mature mossy fibers onto granule cells (Suzuki et al. 2005).

The role of PP2A in NMDAR regulation and functioning has been well-studied and best understood. There are intriguing functional similarities between PP2A and NMDAR. Both contribute to the regulation of neural functions such as synaptic transmission and plasticity. Immunoprecipitation studies indicated that the carboxyl domain of NR3A subunit of NMDAR forms a stable complex with the catalytic subunit of PP2A in the rat brain in vivo (Chan and Sucher 2001). Further studies demonstrated that NR3A constitutively associates with the PP2A holoenzyme but not the core enzyme in rat brain synaptic plasma membranes. A sequence of six amino acids between leucine 958 and histidine 974 of the NR3A is critical for binding to PP2A, as deletion or mutation of this sequence disrupted the NR3A-PP2A interaction (Ma and Sucher 2004). Association of PP2A with NMDARs leads to an increase in the activity of PP2A and the dephosphorylation of Ser 897 of the NMDAR subunit NR1. Stimulation of NMDARs leads to the dissociation of PP2A from the complex and the reduction of PP2A activity (Chan and Sucher 2001).

The NR1 subunit is phosphorylated by both PKA and PKC (Tingley et al. 1997). PKA is associated with NMDAR through the scaffold protein yotiao (Westphal et al. 1999), and this NMDAR-associated PKA has been shown to phosphorylate the Ser 897 of NR1 (Chan and Sucher 2001). PP2A associated with NR3A dephosphorylate this serine residue of NR1. PP1 has also been shown to be associated with NMDAR through the same scaffold protein. These PP2A- (and possibly, PP1-) induced changes in phosphorylation of NR1 decrease NMDA currents through the attenuated opening of the NMDAR-gated channels (Wang et al. 1994). PP2A may also indirectly modulate the phosphorylation of NR1 subunits by modulating the function of PKC. It has been reported that PP2A dephosphorylates and inactivates PKC in vitro, and inhibition of PP2A by okadaic acid prevents dephosphorylation of PKC and enhances the function of PKC (Hansra et al. 1996; Millward et al. 1999; Ricciarelli and Azzi 1998).

In addition to NR1, PP2A is also known to dephosphorylate NR2B subunit. It is known that CaMKII can bind to the C-terminal region of the NR2B subunit of NMDAR and phosphorylate it at serine-1303 (Mayadevi et al. 2002). PP2A may regulate the phosphorylation of this site of NR2B subunits by modulating the function of CaMKII. CaMKII autophosphorylated at Thr286 is active, whereas the activity is decreased by dephosphorylation of this site (Colbran 2004; Otmakhov et al. 2004). CaMKII autophosphorylated at Thr286 was first reported to be exclusively dephosphorylated by PP1 in isolated synaptosomes (Shields et al. 1985).

From the literature reviewed above, it is evident that our knowledge about the mechanism of Pb^{2+}-induced neurotoxicity is patchy, and there is a need to put these isolated pieces of information together to understand how the pathway(s) involved in learning and memory are affected by Pb^{2+}. Only when we understand the problem, we will be able to devise an effective intervention.

References

Alagarsamy, S., Saugstad, J., Warren, L., Mansuy, I. M., Gereau, R. W., 4th, & Conn, P. J. (2005). NMDA-induced potentiation of mGluR5 is mediated by activation of protein phosphatase 2B/calcineurin. *Neuropharmacology, 49*(Suppl. 1), 135–145.

Alberts, A.S., Montminy, M., Shenolikar, S., & Feramisco, J.R. (1994). Expression of a peptide inhibitor of protein phosphatase 1 increases phosphorylation and activity of CREB in NIH 3T3 fibroblasts. *Mol Cell Biol 14*(7), 4398–4407.

Altmann, L., Sveinsson, K., & Wiegand, H. (1991). Long-term potentiation in rat hippocampal slices is impaired following acute lead perfusion. *Neuroscience Letters, 128*, 109–112.

Altmann, L., Weinsberg, F., Sveinsson, K., Lilienthal, H., Wiegand, H., & Winneke, G. (1993). Impairment of long-term potentiation and learning following chronic lead exposure. *Toxicology Letters, 66*, 105–112.

Altmann, L., Gutowski, M., & Wiegand, H. (1994). Effects of maternal lead exposure on functional plasticity in the visual cortex and hippocampus of immature rats. *Brain Research. Developmental Brain Research, 81*(1), 50–56.

Anderson, K. A., Noeldner, P. K., Reece, K., Wadzinski, B. E., & Means, A. R. (2004). Regulation and function of the calcium/calmodulin-dependent protein kinase IV/protein serine/threonine phosphatase 2A signaling complex. *The Journal of Biological Chemistry, 279*(30), 31708–31716.

Antonio, M. T., & Lert, M. L. (2000). Study of the neurochemical alterations produced in discrete brain areas by perinatal low-level lead exposure. *Life Sciences, 67*(6), 635–642.

Atchison, W. D. (2003). Effects of toxic environmental contaminants on voltage-gated calcium channel function: From past to present. *Journal of Bioenergetics and Biomembranes, 35*, 507–532.

Atchison, W. D., & Narahashi, T. (1984). Mechanism of action of lead on neuromuscular junction. *Neurotoxicology, 5*, 267–282.

Athos, J., Impey, S., Pineda, V. V., Chen, X., & Storm, D. R. (2002). Hippocampal CRE-mediated gene expression is required for contextual memory formation. *Nature Neuroscience, 5*, 1119–1120.

Audesirk, G. (1993). Electrophysiology of lead intoxication: Effects on voltage-sensitive ion channels. *Neurotoxicology, 14*, 137–147.

Bellinger, D. (1995). Lead and neuropsychological function in children: Progress and problems in establishing brain– behavior relationships. *Advanced Child Neuropsychology, 3*, 12–45.

Bellingr, D. C., & Bellinger, A. M. (2006). Childhood lead poisoning: The torturous path from science to policy. *The Journal of Clinical Investigation, 116*(4), 853–857.

Bennett, P. C., Moutsoulas, P., Lawen, A., Perini, E., & Ng, K. T. (2003). Novel effects on memory observed following unilateral intracranial administration of okadaic acid, cyclosporine A, FK506 and [MeVal4]CyA. *Brain Research, 988*(1–2), 56–68.

Bielarczyk, H., Tian, X., & Suszkiw, J. B. (1996). Cholinergic denervation-like changes in rat hippocampus following developmental lead exposure. *Brain Research, 708*, 108–115.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long term potentiation in the hippocampus. *Nature, 361*, 31–39.

Blitzer, R. D., Iyengar, R., & Landau, E. M. (2005). Postsynaptic signaling networks: Cellular cogwheels underlying long-term plasticity. *Biological Psychiatry, 57*(2), 113–119.

Borja-Aburto, V. H., Hertz-Picciotto, I., Lopez, M. R., Farias, P., Rios, C., & Blanco, J. (1999). Blood lead levels measured prospectively and risk of spontaneous abortion. *American Journal of Epidemiology, 150*, 590–597.

Bourtchuladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schultz, G., & Silva, A. J. (1994). Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell, 79*, 59–68.

Bouton, C. M. L. S., Frelin, L. P., Forde, C. E., Godwin, H. A., & Pevsner, J. (2001). Synaptotagmin i is a molecular target for lead. *Journal of Neurochemistry, 76*, 1724–1735.

Bozdagi, O., Shan, W., Tanaka, H., Benson, D. L., & Huntley, G. W. (2000). Increasing numbers of synaptic puncta during late-phase LTP: N-cadherin is synthesized, recruited to synaptic sites, and required for potentiation. *Neuron, 28*, 245–259.

Braga, M. F. M., Pereira, E. F. R., & Albuquerque, E. X. (1999a). Nanomolar concentrations of lead inhibit glutamatergic and GABAergic transmission in hippocampal neurons. *Brain Research, 826*, 22–34.

Braga, M. F. M., Pereira, E. F. R., Marchioro, M., & Albuquerque, E. X. (1999b). Lead increases tetrodotoxin-insensitive spontaneous release of glutamate and GABA from hippocampal neurons. *Brain Research, 826*, 10–21.

Braga, M. F., Pereira, E. F., Mike, A., & Albuquerque, E. X. (2004). Pb^{2+} via protein kinase C inhibits nicotinic cholinergic modulation of synaptic transmission in the hippocampus. *The Journal of Pharmacology and Experimental Therapeutics, 311*(2), 700–710.

Bredt, D. S., Ferris, C. D., & Snyder, S. H. (1992). Nitric oxide synthase regulatory sites. Phosphorylation by cyclic AMP-dependent protein kinase, protein kinase C, and calcium/calmodulin protein kinase; identification of flavin and calmodulin binding sites. *The Journal of Biological Chemistry, 267*, 10976–10981.

Bressler, J., Kim, K. A., Chakraborti, T., & Goldstein, G. (1999). Molecular mechanisms of lead neurotoxicity. *Neurochemical Research, 24*, 595–600.

Busselberg, D., Evans, M. L., Haas, H. L., & Carpenter, D. O. (1993). Blockade of mammalian and invertebrate calcium channels by lead. *Neurotoxicology, 14*, 249–258.

Caldeira, M. V., Melo, C. V., Pereira, D. B., Carvalho, R. F., Carvalho, A. L., & Duarte, C. B. (2007). BDNF regulates the expression and traffic of NMDA receptors in cultured hippocampal neurons. *Molecular and Cellular Neurosciences, 35*, 208–219.

Canfield, R. L., Henderson, C. R., Jr., Cory-Slechta, D. A., Cox, C., Jusko, T. A., & Lanphear, B. P. (2003). Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. *The New England Journal of Medicine, 348*, 1517–1526.

Chan, S. F., & Sucher, N. J. (2001). An NMDA receptor signaling complex with protein phosphatase 2A. *The Journal of Neuroscience, 21*(20), 7985–7992.

Chirivia, J., Kwok, R., Lamb, N., Hagiwara, M., Montminy, M., & Goodman, R. (1993). Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature, 365*, 855–859.

Chisolm, J. J., Jr. (2001). Evolution of the management and prevention of childhood lead poisoning: Dependence of advances in public health on technological advances in the determination of lead and related biochemical indicators of its toxicity. *Environmental Research, 86*, 111–121.

Clarkson, T. W. (1987). Metal toxicity in the central nervous system. *Environmental Health Perspectives, 75*, 59–64.

Colbran, R. J. (2004). Targeting of calcium/calmodulin-dependent protein kinase II. *The Biochemical Journal, 378*, 1–16.

Collingridge, G. L., & Bliss, T. V. P. (1987). NMDA receptors – Their role in long-term potentiation. *Trends in Neuropharmacology, 10*, 288–293.

Collingridge, G. L., & Lester, R. A. (1989). Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacological Reviews, 41*, 143–210.

Cordova, F. M., Rodrigues, L. S., Giocomelli, M. B. O., Oliveira, C. S., Posser, T., Dunkley, P. R., & Leal, R. B. (2004). Lead stimulates ERK1/2 and p38MAPK phosphorylation in the hippocampus of immature rats. *Brain Research, 998*, 65–72.

Cornell-Bell, A. H., Finkbeiner, S. M., Cooper, M. S., & Smith, S. J. (1990). Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science, 247*, 470–473.

Davis, S., Vanhoutte, P., Pages, C., Caboche, J., & Laroche, S. (2000). The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. *The Journal of Neuroscience, 20*(12), 4563–4572.

De Roo, M., Klauser, P., & Muller, D. (2008). LTP promotes a selective long-term stabilization and clustering of dendritic spines. *PLoS Biology, 6*, 1850–1860.

DeMichele, S. J. (1984). Nutrition of lead. *Comparative Biochemistry and Physiology A, 78*(3), 401–408.

Dietrich, K. N., Ware, J. H., Salganik, M., Radcliffe, J., Rogan, W. J., Rhoads, G. G., Fay, M. E., Davoli, C. T., Denckla, M. B., Bornschein, R. L., Schwarz, D., Dockery, D. W., Adubato, S., & Jones, R. L. (2004). Effect of chelation therapy on the neuropsychological and behavioral development of lead-exposed children after school entry. *Pediatrics, 114*, 19–26.

Dorea, J. G. (2004). Mercury and lead during breast-feeding. *The British Journal of Nutrition, 92*(1), 21–40.

Downing, J. E., & Role, L. W. (1987). Activators of protein kinase C enhance acetylcholine receptor desensitization in sympathetic ganglion neurons. *Proceedings of the National Academy of Sciences, 84*, 7739–7743.

Durand, G. M., Gregor, P., Zheng, Z., Bennett, M. V. L., Uhl, G. R., & Zukin, R. S. (1992). Cloning of an apparent splice variant of the rat *N*-methyl-D-aspartate receptor NMDAR1 with altered sensitivity to polyamines and activators of protein kinase C. *Proceedings of the National Academy of Sciences, 89*, 9359–9363.

Ehlers, M. D. (2003). Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nature Neuroscience, 6*, 231–242.

Evans, M. L., Busselberg, D., & Carpenter, D. O. (1991). Pb^{2+} blocks calcium currents of cultured dorsal root ganglion cells. *Neuroscience Letters, 129*, 103–106.

Fenster, C. P., Beckman, M. L., Parker, J. C., Sheffield, E. B., Whitworth, T. L., Quick, M. W., & Lester, R. A. (1999). Regulation of alpha4beta2 nicotinic receptor desensitization by calcium and protein kinase C. *Molecular Pharmacology, 55*, 432–443.

Finkelstein, Y., Markowitz, M. E., & Rosen, J. F. (1998). Low-level lead-induced neurotoxicity in children: An update on central nervous system effects. *Brain Research Reviews, 27*, 168–176.

Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., & Tsai, L. H. (2007). Recovery of learning and memory is associated with chromatin remodelling. *Nature, 447*(7141), 178–182.

Gasparini, F., Lingenhöhl, K., Stoehr, N., Flor, P. J., Heinrich, M., Vranesic, I., Biollaz, M., Allgeier, H., Heckendorn, R., Urwyler, S., Varney, M. A., Johnson, E. C., Hess, S. D., Rao, S. P., Sacaan, A. I., Santori, E. M., Velicelebi, G., & Kuhn, R. (1999). 2-Methyl-6-(phenylethyl)-pyridine (MPEP), a potent, selective and systematically active mGluR5 receptor antagonist. *Neuropharmacology, 38*, 1493–1503.

Gavazzo, P., Gazzoli, A., Mazzolini, M., & Marchetti, C. (2001). Lead inhibition of NMDA channels in native and recombinant receptors. *Neuroreport, 12*(14), 3121–3125.

Gavazzo, P., Zanardi, I., Baranowska-Bosiacka, I., & Marchetti, C. (2008). Molecular determinants of Pb^{2+} interaction with NMDA receptor channels. *Neurochemistry International, 52*, 329–337.

Genoux, D., Haditsch, U., Knobloch, M., Michalon, A., Storm, D., & Mansuy, I. M. (2002). Protein phosphatase 1 is a molecular constraint on learning and memory. *Nature, 418*(6901), 970–975.

Genoux, D., Bezerra, P., & Montgomery, J. M. (2011). Intra-spaced stimulation and protein phosphatase 1 dictate the direction of synaptic plasticity. *The European Journal of Neuroscience, 33*(10), 1761–1770.

Gilbert, M. E., & Lasley, S. M. (2002). Long-term consequences of developmental exposure to lead or polychlorinated biphenyls: Synaptic transmission and plasticity in the rodent CNS. *Environmental Toxicology and Pharmacology, 12*, 105–117.

Gilbert, M. E., & Lasley, S. M. (2007). Developmental lead (Pb) exposure reduces the ability of the NMDA antagonist MK-801 to suppress long-term potentiation (LTP) in the rat dentate gyrus, in vivo. *Neurotoxicology and Teratology, 29*(2007), 385–393.

Gilbert, M. E., & Mack, C. M. (1990). The NMDA antagonist, MK-801, suppresses long-term potentiation, kindling, and kindling-induced potentiation in the perforant path of the unanesthetized rat. *Brain Research, 519*, 89–96.

Gilbert, M., & Mack, C. (1998). Chronic lead exposure accelerates decay of long-term potentiation in rat dentate gyrus in vivo. *Brain Research, 789*, 139–149.

Gilbert, M. E., Mack, C. M., & Lasley, S. M. (1996). Chronic developmental lead exposure increases the threshold for long-term potentiation in rat dentate gyrus in vivo. *Brain Research, 736*, 118–124.

Gilbert, M. E., Mack, C. M., & Lasley, S. M. (1999a). The influence of developmental period of lead exposure on long-term potentiation in the rat dentate gyrus in vivo. *Neurotoxicology, 20*, 57–70.

Gilbert, M. E., Mack, M. E., & Lasley, S. M. (1999b). Developmental lead exposure reduces the magnitude of long-term potentiation: A dose–response analysis. *Neurotoxicology, 20*, 71–82.

Goyer, R. A. (1995). Nutrition and metal toxicity. *The American Journal of Clinical Nutrition, 61* (Suppl), 646S–650S.

Goyer, R. A., & Clarkson, T. W. (2001). Toxic effects of metals. In C. D. Klaassen (Ed.), *Casarett and Doull's toxicology: The basic science of poisons* (6th ed., pp. 811–867). New York: McGraw-Hill.

Gräff, J., Koshibu, K., Jouvenceau, A., Dutar, P., & Mansuy, I. M. (2010). Protein phosphatase 1-dependent transcriptional programs for long-term memory and plasticity. *Learning & Memory, 17*(7), 355–363.

Guilarte, T. R. (1997). Glutamatergic system and developmental lead neurotoxicity. *Neurotoxicology, 18*, 665–672.

Guilarte, T. R., & McGlothan, J. L. (1998). Hippocampal NMDA receptor mRNA undergoes subunit specific changes during developmental lead exposure. *Brain Research, 790*, 98–107.

Guilarte, T. R., & McGlothan, J. L. (2003). Selective decrease in NR1 subunit splice variant mRNA in the hippocampus of Pb^{2+}-exposed rats: Implications for synaptic targeting and cell surface expression of NMDAR complexes. *Brain Research. Molecular Brain Research, 113* (1–2), 37–43.

Guilarte. T.R., Miceli, R.C. & Jett, D.A. (1994). Neurochemical aspects of hippocampal and cortical Pb^{2+} neurotoxicity. *Neurotoxicology. 15*(3), 459–66.

Guilarte, T. R., Miceli, R. C., & Jett, D. A. (1995). Biochemical evidence of an interaction of lead at the zinc allosteric sites of the NMDA receptor complex: Effects of neuronal development. *Neurotoxicology, 16*, 63–71.

Guilarte, T. R., McGlothan, J. L., & Nihei, M. K. (2000). Hippocampal expression of *N*-methyl-D-aspartate receptor (NMDAR1) subunit splice variant mRNA is altered by developmental exposure to Pb^{2+}. *Molecular Brain Research, 76*, 299–305.

Gutowski, M., Altmann, L., Sveinsson, K., & Wiegand, H. (1998). Synaptic plasticity in the CA1 and CA3 hippocampal region of pre- and postnatally lead-exposed rats. *Toxicology Letters, 95*, 195–203.

Haege, S., Galetzka, D., Zechner, U., Haaf, T., Gamerdinger, M., Behl, C., Hiemke, C., & Schmitt, U. (2010). Spatial learning and expression patterns of PP1 mRNA in mouse hippocampus. *Neuropsychobiology, 61*(4), 188–196.

Hansra, G., Bornancin, F., Whelan, R., Hemmings, B. A., & Parker, P. J. (1996). 12-*O*-Tetradecanoylphorbol-13-acetate-induced dephosphorylation of protein kinase Calpha correlates with the presence of a membrane-associated protein phosphatase 2A heterotrimer. *The Journal of Biological Chemistry, 271*, 32785–32788.

Hardingham, G. E., Fukunaga, Y., & Bading, H. (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nature Neuroscience, 5*(5), 405–414.

Hartmann, M., Heumann, R., & Lessmann, V. (2001). Synaptic secretion of BDNF after high-frequency stimulation of glutamatergic synapses. *The EMBO Journal, 20*, 5887–5897.

Hashemzadeh-Gargari, H., & Guilarte, T. R. (1999). Divalent cations modulate *N*-methyl-D-aspartate receptor function at the glycine site. *The Journal of Pharmacology and Experimental Therapeutics, 290*, 1356–1362.

Hassel, B., & Dingledine, R. (2006). Glutamate. In G. J. Siegel, R. W. Albers, S. T. Brady, & D. L. Price (Eds.), *Basic neurochemistry: Molecular, cellular and medical aspects* (7th ed., pp. 267–290). San Diego, CA: Elsevier/Academic.

Havekes, R., Nijholt, I. M., Luiten, P. G., & Van der Zee, E. A. (2006). Differential involvement of hippocampal calcineurin during learning and reversal learning in a Y-maze task. *Learning & Memory, 13*(6), 753–759.

Ho, Y., Logue, E., Callaway, C. W., & DeFranco, D. B. (2007). Different mechanisms account for extracellular-signal regulated kinase activation in distinct brain regions following global ischemia and reperfusion. *Neuroscience, 145*(1), 248–255.

Hoffmann, H., Gremme, T., Hatt, H., & Gottmann, K. (2000). Synaptic activity dependent developmental regulation of NMDA receptor subunit expression in cultured neocortical neurons. *Journal of Neurochemistry, 75*, 1590–1599.

Holtzman, D., Olson, J. E., DeVries, C., & Bensch, K. (1987). Lead toxicity in primary cultured cerebral astrocytes and cerebellar granular neurones. *Toxicology and Applied Pharmacology, 89*, 211–225.

Huang, C. C., & Hsu, K. S. (2006). Sustained activation of metabotropic glutamate receptor 5 and protein tyrosine phosphatases mediate the expression of (*S*)-3,5-dihydroxyphenylglycine-induced long-term depression in the hippocampal CA1 region. *Journal of Neurochemistry, 96*, 179–194.

Ishihara, K., Alkondon, M., Montes, J. G., & Albuquerque, E. X. (1995). Ontogenically related properties of *N*-methyl-D-aspartate receptors in rat hippocampal neurons and the age-specific sensitivity of developing neurons to lead. *The Journal of Pharmacology and Experimental Therapeutics, 279*, 1459–1470.

Ivanov, A., Pellegrino, C., Rama, S., Dumalska, I., Salyha, Y., Ben-Ari, Y., & Medina, I. (2006). Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons. *The Journal of Physiology, 572*(Pt 3), 789–798.

Izquierdo, I. (1993). Long-term potentiation and the mechanism of memory. *Drug Development Research, 30*, 1–17.

Jiang, X., Tian, F., Mearow, K., Okagaki, P., Lipsky, R. H., & Marini, A. M. (2005). The excitoprotective effect of *N*-methyl-D-aspartate receptors is mediated by a brain-derived neurotrophic factor autocrine loop in cultured hippocampal neurons. *Journal of Neurochemistry, 94*, 713–722.

Kandel, E. R. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. *Science, 294*, 1030–1038.

Kawamura, Y., Manita, S., Nakamura, T., Inoue, M., Kudo, Y., & Miyakawa, H. (2004). Glutamate release increases during mossy-CA3 LTP but not during Schaffer-CA1 LTP. *The European Journal of Neuroscience, 19*, 1591–1600.

Kim, K. A., Chakraborti, T., Golstein, G., Johnston, M., & Bressler, J. (2002). Exposure to lead elevates induction of zif268 and ARC mRNA in rats after electroconvulsive shock: The involvement of protein kinase C. *Journal of Neuroscience Research, 69*, 268–277.

Knobloch, M., Farinelli, M., Konietzko, U., Nitsch, R. M., & Mansuy, I. M. (2007). Aβ oligomer-mediated long-term potentiation impairment involves protein phosphatase 1-dependent mechanisms. *The Journal of Neuroscience, 27*, 7648–7653.

Kobayashi, T., & Mori, Y. (1998). Ca^{2+} channel antagonists and neuroprotection from cerebral ischemia. *European Journal of Pharmacology, 363*, 1–15.

Kober, T. E., & Cooper, G. P. (1976). Lead competitively inhibits calcium-dependent synaptic transmission in the bullfrog sympathetic ganglion. *Nature, 262*, 704–705.

Koshibu, K., Gräff, J., Beullens, M., Heitz, F. D., Berchtold, D., Russig, H., Farinelli, M., Bollen, M., & Mansuy, I. M. (2009). Protein phosphatase 1 regulates the histone code for long-term memory. *The Journal of Neuroscience, 29*(41), 13079–13089.

Koshibu, K., Gräff, J., & Mansuy, I. M. (2011). Nuclear protein phosphatase-1: An epigenetic regulator of fear memory and amygdala long-term potentiation. *Neuroscience, 173*, 30–36.

Krupp, J. J., Vissel, B., Thomas, C. G., Heinemann, S. F., & Westbrook, G. L. (2002). Calcineurin acts via the C-terminus of NR2A to modulate desensitization of NMDA receptors. *Neuropharmacology, 42*(5), 593–602.

Kuhlmann, A. C., McGlothan, J. L., & Guilarte, T. R. (1997). Developmental lead exposure causes spatial learning deficits in adult rats. *Neuroscience Letters, 233*, 101–104.

Lanphear, B. P., Dietrich, K., Auinger, P., & Cox, C. (2000). Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. *Public Health Reports, 115*, 521–529.

Lasley, S. M., & Gilbert, M. E. (1996). Presynaptic glutamatergic function in dentate gyrus in vivo is diminished by chronic exposure to inorganic lead. *Brain Research, 736*, 125–134.

Lasley, S. M., & Gilbert, M. E. (1999). Lead inhibits the rat *N*-methyl-D-aspartate receptor channel by binding to a site distinct from the zinc allosteric site. *Toxicology and Applied Pharmacology, 159*(3), 224–233.

Lasley, S. M., & Gilbert, M. E. (2002). Rat hippocampal glutamate and GABA release exhibit biphasic effects as a function of chronic lead exposure level. *Toxicological Sciences, 66*(1), 139–147.

Lasley, S. M., Green, M. C., & Gilbert, M. E. (2001). Rat hippocampal NMDA receptor binding as a function of chronic lead exposure level. *Neurotoxicology and Teratology, 23*, 185–189.

Lau, W. K., Yeung, C. W., Lui, P. W., Cheung, L. H., Poon, N. T., & Yung, K. K. (2002). Different trends in modulation of NMDAR1 and NMDAR2B gene expression in cultured cortical and hippocampal neurons after lead exposure. *Brain Research, 932*(1–2), 10–24.

Laurie, D. J., & Seeburg, P. H. (1994). Regional and developmental heterogeneity in splicing of the rat brain NMDAR1 mRNA. *The Journal of Neuroscience, 14*, 3180–3194.

Lee, Y. S., & Silva, A. J. (2009). The molecular and cellular biology of enhanced cognition. *Nature Reviews. Neuroscience, 10*, 126–140.

Lee, H. K., Kameyama, K., Huganir, R. L., & Bear, M. F. (1998). NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. *Neuron, 21*, 1067–1078.

Lee, H. K., Barbarosie, M., Kameyama, K., Bear, M. F., & Huganir, R. L. (2000). Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature, 405*, 955–959.

Levitan, I. B. (1999). Modulation of ion channels by protein phosphorylation. How the brain works. *Advances in Second Messenger and Phosphoprotein Research, 33*, 3–22.

Lieberman, D. N., & Mody, I. (1994). Regulation of NMDA channel function by endogenous Ca^{2+}-dependent phosphatase. *Nature, 369*, 235–239.

Lindhal, L. S., Bird, L., Legare, M. E., Mikeska, G., Bratton, G. R., & Tiffany-Castiglioni, E. (1999). differential ability of astroglia and neuronal cells to accumulate lead: Dependence on cell type and on degree of differentiation. *Toxicological Sciences, 50*, 236–243.

Lindlbauer, R., Mohrmann, R., Hatt, H., & Gottmann, K. (1998). Regulation of kinetic and pharmacological properties of synaptic NMDA receptors depends on presynaptic exocytosis in rat hippocampal neurones. *The Journal of Physiology, 508*(Pt. 2), 495–502.

Liu, L., Wong, T. P., Pozza, M. F., Lingenhoehl, K., Wang, Y., Sheng, M., Auberson, Y. P., & Wang, Y. T. (2004). Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science, 304*(5673), 1021–1024.

Liu, F., Grundke-Iqbal, I., Iqbal, K., & Gong, C. X. (2005). Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *The European Journal of Neuroscience, 22*(8), 1942–1950.

Loikkanen, J., Naarala, J., Vahakangas, K. H., & Savolainen, K. H. (2003). Glutamate increases toxicity of inorganic lead in GT1-7 neurons: Partial protection induced by flunarizine. *Archives of Toxicology, 77*, 663–671.

Ma, O. K., & Sucher, N. J. (2004). Molecular interaction of NMDA receptor subunit NR3A with protein phosphatase 2A. *Neuroreport, 15*(9), 1447–1450.

Ma, L., Zablow, L., Kandel, E. R., & Siegelbaum, S. A. (1999). Cyclic AMP induces functional presynaptic boutons in hippocampal CA3-CA1 neuronal cultures. *Nature Neuroscience, 2*, 24–30.

Madison, D. V., Malenka, R. C., & Nicoll, R. A. (1991). Mechanisms underlying long-term potentiation of synaptic transmission. *Annual Review of Neuroscience, 14*, 379–397.

Malenka, R. C., & Nicoll, R. A. (1993). NMDA-receptor-dependent synaptic plasticity: Multiple forms and mechanisms. *Trends in Neurosciences, 16*, 521–527.

Malenka, R. C., & Nicoll, R. A. (1999). Long-term potentiation – A decade of progress? *Science, 285*, 1870–1874.

Malinow, R., & Malenka, R. C. (2002). AMPA receptor trafficking and synaptic plasticity. *Annual Review of Neuroscience, 25*, 103–126.

Manahan-Vaughan, D., & Braunewell, K. H. (2005). The metabotropic glutamate receptor, mGluR5, is a key determinant of good and bad spatial learning performance and hippocampal synaptic plasticity. *Cerebral Cortex, 15*, 1703–1713.

Manahan-Vaughan, D., Ngomba, R. T., Storto, M., Kulla, A., Catania, M. V., Chiechio, S., Rampello, L., Passarelli, F., Capece, A., Reymann, K. G., & Nicoletti, F. (2003). An increased expression of the mGlu5 receptor protein following LTP induction at the perforant path-dentate gyrus synapse in freely moving rats. *Neuropharmacology, 44*, 17–25.

Mansuy, I. M., & Shenolikar, S. (2006). Protein serine/threonine phosphatases in neuronal plasticity and disorders of learning and memory. *Trends in Neurosciences, 29*(12), 679–686.

Marchetti, C. (2003). Molecular targets of lead in brain neurotoxicity. *Neurotoxicity Research, 5*(3), 221–236.

Margottil, E., & Domenici, L. (2003). NR2A but not NR2B *n*-methyl-D-aspartate receptor subunit is altered in the visual cortex of BDNF-knock-out mice. *Cellular and Molecular Neurobiology, 23*, 165–174.

Martin, K. C., Casadio, A., Zhu, H., Yaping, E., Rose, J. C., Chen, M., Bailey, C. H., & Kandel, E. R. (1997). Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: A function for local protein synthesis in memory storage. *Cell, 91*, 927–938.

Massicotte, G., & Baudry, M. (1991). Triggers and substrates of hippocampal synaptic plasticity. *Neuroscience and Biobehavioral Reviews, 15*, 415–423.

Mauna, J. C., Miyamae, T., Pulli, B., & Thiels, E. (2010). Protein phosphatases 1 and 2A are both required for long-term depression and associated dephosphorylation of cAMP response element binding protein in hippocampal area CA1 in vivo. *Hippocampus, 21*(10), 1093–1104.

Mayadevi, M., Praseeda, M., Kumar, K. S., & Omkumar, R. V. (2002). Sequence determinants on the NR2A and NR2B subunits of NMDA receptor responsible for specificity of phosphorylation by CaMKII. *Biochimica et Biophysica Acta, 1598*, 40–45.

McNamara, R. K., & Skelton, R. W. (1993). The neuropharmacological and neurochemical basis of place learning in the Morris water maze. *Brain Research. Brain Research Reviews, 18*, 33–49.

McNaughton, B. L. (1993). The mechanism of expression of long-term enhancement of hippocampal synapses: Current issues and theoretical implications. *Annual Review of Physiology, 55*, 375–396.

Mike, A., Pereira, E. F., & Albuquerque, E. X. (2000). Ca(2+)-sensitive inhibition by Pb(2+) of alpha7-containing nicotinic acetylcholine receptors in hippocampal neurons. *Brain Research, 873*(1), 112–123.

Miller, C. A., Campbell, S. L., & Sweatt, J. D. (2008). DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. *Neurobiology of Learning and Memory, 89*(4), 599–603.

Millward, T. A., Zolnierowicz, S., & Hemmings, B. A. (1999). Regulation of protein kinase cascades by protein phosphatase 2A. *Trends in Biochemical Sciences, 24*, 186–191.

Minnema, D. J., Michaelson, I. A., & Cooper, G. P. (1988). Calcium efflux and neurotransmitter release from rat hippocampal synaptosomes exposed to lead. *Toxicology and Applied Pharmacology, 92*, 351–357.

Mizuno, M., Yamada, K., Maekawa, N., Saito, K., Seishima, M., & Nabeshima, T. (2002). CREB phosphorylation as a molecular marker of memory processing in the hippocampus for spatial learning. *Behavioural Brain Research, 133*, 135–141.

Monaghan, D. T., Holets, V. R., Toy, D. W., & Cotman, C. W. (1983). Anatomical distributions of four pharmacologically distinct ^{3}H-l-glutamate binding sites. *Nature, 306*, 176–179.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., & Seeburg, P. H. (1992). Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science, 256*, 1217–1221.

Monyer, H., Burnashev, N., Laurie, D. J., Sakmann, B., & Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron, 12*, 529–540.

Moody, W. J. (1998). Control of spontaneous activity during development. *Journal of Neurobiology, 37*, 97–109.

Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N., & Nakanishi, S. (1991). Molecular cloning and characterization of the rat NMDA receptor. *Nature, 354*, 31–37.

Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297*, 681–683.

Morris, R. G., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. *Nature, 319*, 774–776.

Moss, S. J., McDonald, B. J., Rudhard, Y., & Schoepfer, R. (1996). Phosphorylation of the predicted major intracellular domains of the rat and the chick neuronal nicotinic acetylcholine receptor 7 subunit by cAMP-dependent protein kinase. *Neuropharmacology, 35*, 1023–1028.

Mulkey, R. M., Herron, C. E., & Malenka, R. C. (1993). An essential role for protein phosphatases in hippocampal long-term depression. *Science, 261*, 1051–1055.

Mulkey, R. M., Endo, S., Shenolikar, S., & Malenka, R. C. (1994). Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature, 369*, 486–488.

Naie, K., & Manahan-Vaughan, D. (2004). Regulation by metabotropic glutamate receptor 5 of LTP in the dentate gyrus of freely moving rats: Relevance for learning and memory formation. *Cerebral Cortex, 14*, 189–198.

Neal, A. P., & Guilarte, T. R. (2010). Molecular neurobiology of lead (Pb2+): Effects on synaptic function. *Molecular Neurobiology, 42*(3), 151–160.

Neal, A. P., Stansfield, K. H., Worley, P. F., Thompson, R. E., & Guilarte, T. R. (2010). Lead exposure during synaptogenesis alters vesicular proteins and impairs vesicular release: Potential role of NMDA receptor-dependent BDNF signaling. *Toxicological Sciences, 116*(1), 249–263.

Neal, A. P., Worley, P. F., & Guilarte, T. R. (2011). Lead exposure during synaptogenesis alters NMDA receptor targeting via NMDA receptor inhibition. *Neurotoxicology, 32*, 281–289.

Needleman, H. L., Gunnoe, C., Leviton, A., Reed, R., Peresie, H., Maher, C., & Barrett, P. (1979). Deficits in psychologic and classroom performance of children with elevated dentine lead levels. *The New England Journal of Medicine, 300*, 689–695.

Nevin, R. (2000). How lead exposure relates to temporal changes in IQ, violent crime, and unwed pregnancy. *Environmental Research, 83*, 1–22.

Neyman, S., & Manahan-Vaughan, D. (2008). Metabotropic glutamate receptor 1 (mGluR1) and 5 (mGluR5) regulate late phases of LTP and LTD in the hippocampal CA1 region in vitro. *The European Journal of Neuroscience, 27*, 1345–1352.

Nihei, M. K., & Guilarte, T. R. (1999). NMDAR-2A subunit protein expression is reduced in the hippocampus of rats exposed to Pb^{2+} during development. *Molecular Brain Research, 66*, 42–49.

Nihei, M. K., & Guilarte, T. R. (2001). Molecular changes in glutamatergic synapses induced by Pb^{2+}: Association with deficits of LTP and spatial learning. *Neurotoxicology, 22*, 635–643.

Nihei, M. K., Desmond, N. L., McGlothan, J. L., Kuhlmann, A. C., & Guilarte, R. T. (2000). *N*-methyl-D-aspartate receptor subunit changes are associated with lead-induced deficits of long-term potentiation and spatial learning. *Neuroscience, 99*, 233–242.

Nihei, M. K., McGlothan, J. L., Toscano, C. D., & Guilarte, T. R. (2001). Low level Pb^{2+} exposure affects hippocampal protein kinase C gamma gene and protein expression in rats. *Neuroscience Letters, 298*, 212–216.

Norman, E. D., Thiels, E., Barrionuevo, G., & Klann, E. (2000). Long-term depression in the hippocampus in vivo is associated with protein phosphatase-dependent alterations in extracellular signal-regulated kinase. *Journal of Neurochemistry, 74*(1), 192–198.

Oberbeck, D. L., McCormack, S., & Houpt, T. A. (2010). Intra-amygdalar okadaic acid enhances conditioned taste aversion learning and CREB phosphorylation in rats. *Brain Research, 1348*, 84–94.

Omelchenko, I. A., Nelson, C. S., Marino, J. L., & Allen, C. N. (1996). The sensitivity of *N*-methyl-D-aspartate receptors to lead inhibition is dependent on the receptor subunit composition. *The Journal of Pharmacology and Experimental Therapeutics, 278*(1), 15–20.

Omelchenko, I. A., Nelson, C. S., & Allen, C. N. (1997). Lead inhibition of *N*-methyl-D-aspartate receptors containing NR2A NR2C, and NR2D subunits. *The Journal of Pharmacology and Experimental Therapeutics, 282*, 1458–1464.

Otmakhov, N., Tao-Cheng, J. H., Carpenter, S., Asrican, B., Dosemeci, A., Reese, T. S., & Lisman, J. (2004). Persistent accumulation of calcium/calmodulin-dependent protein kinase II in dendritic spines after induction of NMDA receptor-dependent chemical long-term potentiation. *The Journal of Neuroscience, 24*(42), 9324–9331.

Ozawa, S., Kamiya, H., & Tsuzuki, K. (1998). Glutamate receptors in the mammalian central nervous system. *Progress in Neurobiology, 54*, 581–618.

Paoletti, P., Perin-Dureau, F., Fayyazuddin, A., Le Goff, A., Callebaut, I., & Neyton, J. (2000). Molecular organization of a zinc binding N-terminal modulatory domain in a NMDA receptor subunit. *Neuron, 28*(3), 911–925.

Peng, S., Hajela, R. K., & Atchison, W. D. (2002). Characteristics of block by Pb^{2+} of function of human neuronal L-, N-, and R-type Ca^{2+} channels transiently expressed in human embryonic kidney 293 cells. *Molecular Pharmacology, 62*, 1418–1430.

Perez-Otano, I., & Ehlers, M. (2004). Learning from NMDA receptor trafficking: Clues to the development and maturation of glutamatergic synapses. *Neuro-Signals, 13*, 175–189.

Pozzo-Miller, L. D., Gottschalk, W., Zhang, L., McDermott, K., Du, J., Gopalakrishnan, R., Oho, C., Sheng, Z. H., & Lu, B. (1999). Impairments in high-frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice. *The Journal of Neuroscience, 19*, 4972–4983.

Prybylowski, K., & Wenthold, R. J. (2004). *N*-methyl-D-aspartate receptors: Subunit assembly and trafficking to the synapse. *The Journal of Biological Chemistry, 279*, 9673–9676.

Rachline, J., Perin-Dureau, F., Le Goff, A., Neyton, J., & Paoletti, P. (2005). The micromolar zinc-binding domain on the NMDA receptor subunit NR2B. *The Journal of Neuroscience, 25*(2), 308–317.

Rahman, A., Brew, B. J., & Guillemin, G. J. (2010). Lead dysregulates serine/threonine protein phosphatases in human neurons. *Neurochemical Research, 36*, 195–204.

Rahman, A., Khan, K. M., Al-Khaledi, G., Khan, I., & Al-Shemary, T. (2012). Over activation of hippocampal serine/threonine protein phosphatases PP1 and PP2A is involved in lead-induced deficits in learning and memory in young rats. *Neurotoxicology*, *33*, 370–383 *[Epub ahead of print]:* DOI:10.1016/j.neuro.2012.02.014.

Rajanna, B., Rajanna, S., Hall, E., & Yallapragada, P. R. (1997). In vitro metal inhibition of *N*-methyl-D-aspartate specific glutamate receptor binding in neonatal and adult rat brain. *Drug and Chemical Toxicology, 20*(1–2), 21–29.

Raymond, L. A., Tingley, W. G., Blackstone, C. D., Roche, K. W., & Huganir, R. L. (1994). Glutamate receptor modulation by protein phosphorylation. *The Journal of Physiology, 88*, 181–192.

Reddy, G. R., Devi, B. C., & Chetty, C. S. (2007). Developmental lead neurotoxicity: Alterations in brain cholinergic system. *Neurotoxicology, 28*(2), 402–407.

Ricciarelli, R., & Azzi, A. (1998). Regulation of recombinant PKC alpha activity by protein phosphatase 1 and protein phosphatase 2A. *Archives of Biochemistry and Biophysics, 355*, 197–200.

Roberson, E. D., English, J. D., & Sweatt, J. D. (1996). A biochemist's view of long-term potentiation. *Learning & Memory, 3*(1), 1–24.

Robinson, G. B., & Reed, G. D. (1992). Effect of MK-801 on the induction and subsequent decay of long-term potentiation in the unanesthetized rabbit hippocampal dentate gyrus. *Brain Research, 569*, 78–85.

Roche, K. W., Tingley, W. G., & Huganir, R. L. (1994). Glutamate receptor phosphorylation and synaptic plasticity. *Current Opinion in Neurobiology, 4*, 383–388.

Rogan, W. J., Dietrich, K. N., Ware, J. H., Dockery, D. W., Salganik, M., Radcliffe, J., Jones, R. L., Ragan, N. B., Chisolm, J. J., & Rhoads, G. G. (2001). The effect of chelation therapy with succimer on neuropsychological development in children exposed to lead. *The New England Journal of Medicine, 344*, 1421–1426.

Savolainen, K. M., Loikkanen, J., Eerikainen, S., & Naarala, J. (1998a). Glutamate-stimulated ROS production in neuronal cultures: Interactions with lead and the cholinergic system. *Neurotoxicology, 19*, 669–674.

Savolainen, K. M., Loikkanen, J., Eerikainen, S., & Naarala, J. (1998b). Interactions of excitatory neurotransmitters and xenobiotics in excitotoxicity and oxidative stress: Glutamate and lead. *Toxicology Letters, 102–103*, 363–367.

Scheetz, A. J., & Constantine-Paton, M. (1994). Modulation of NMDA receptor function: Implications for vertebrate neural development. *The FASEB Journal, 8*, 745–752.

Schultz, S., Siemer, H., Krug, M., & Hollt, V. (1999). Direct evidence for biphasic cAMP responsive element-binding protein phosphorylation during long-term potentiation in the rat dentate gyrus in vivo. *The Journal of Neuroscience, 19*, 5683–5692.

Seguela, P., Wadiche, J., Dineley-Miller, K., Dani, J. A., & Patrick, J. W. (1993). Molecular cloning, functional properties and distribution of rat brain alpha 7: A nicotinic cation channel highly permeable to calcium. *The Journal of Neuroscience, 13*, 596–604.

Selevan, S. G., Rice, D. C., Hogan, K. A., Euling, S. Y., Pfahles- Hutchens, A., & Bethel, J. (2003). Blood lead concentration and delayed puberty in girls. *The New England Journal of Medicine, 348*, 1527–1536.

Sharifi, A. M., Baniasadi, S., Jorjani, M., Rahimi, F., & Bakhshayesh, M. (2002). Investigation of acute lead poisoning on apoptosis in rat hippocampus in vivo. *Neuroscience Letters, 329*, 45–48.

Shields, S. M., Ingebritsen, T. S., & Kelly, P. T. (1985). Identification of protein phosphatase 1 in synaptic junctions: Dephosphorylation of endogenous calmodulin-dependent kinase II and synapse-enriched phosphoproteins. *The Journal of Neuroscience, 5*(12), 3414–3422.

Shimizu, E., Tang, Y. P., Rampon, C., & Tsien, J. Z. (2000). NMDA receptor-dependent synaptic reinforcement as a process for memory consolidation. *Science, 290*, 1170–1174.

Silbergeld, E. K. (1992). Mechanisms of lead neurotoxicity, or looking beyond the lamppost. *The FASEB Journal, 6*(13), 3201–3206.

Silverstein, A. M., Barrow, C. A., Davis, A. J., & Mumby, M. C. (2002). Actions of PP2A on the MAP kinase pathway and apoptosis are mediated by distinct regulatory subunits. *Proceedings of the National Academy of Sciences, 99*(7), 4221–4226.

Simons, T. J. B. (1993). Lead-calcium interactions in cellular lead toxicity. *Neurotoxicology, 14*, 77–85.

Simons, T. J. B., & Pocock, G. (1987). Lead enters bovine adrenal medullary cells through calcium channels. *Journal of Neurochemistry, 48*, 383–389.

Small, D. L., Murray, C. L., Mealing, G. A., Poulter, M. O., Buchan, A. M., & Morley, P. (1998). Brain derived neurotrophic factor induction of *N*-methyl-D-aspartate receptor subunit NR2A expression in cultured rat cortical neurons. *Neuroscience Letters, 252*, 211–214.

Soderling, T. R., & Derkach, V. A. (2000). Postsynaptic protein phosphorylation and LTP. *Trends in Neurosciences, 23*, 75–80.

Struzynska, L. (2009). A glutamatergic component of lead toxicity in adult brain: The role of astrocytic glutamate transporters. *Neurochemistry International, 55*, 151–156.

Sui, L., Ge, S. Y., Ruan, D. Y., Chen, J. T., Xu, Y. Z., & Wang, M. (2000). Age-related impairment of long-term depression in area CA1 and dentate gyrus of rat hippocampus following developmental lead exposure in vitro. *Neurotoxicology and Teratology, 22*, 381–387.

Sun, L., Zhao, Z. Y., Hu, J., & Zhou, X. L. (2005). Potential association of lead exposure during early development of mice with alteration of hippocampus nitric oxide levels and learning memory. *Biomedical and Environmental Sciences, 18*, 375–378.

Suszkiw, J. B. (2004). Presynaptic disruption of transmitter release by lead. *Neurotoxicology, 25*, 599–604.

Suzuki, K., Sato, M., Morishima, Y., & Nakanishi, S. (2005). Neuronal depolarization controls brain-derived neurotrophic factor-induced upregulation of NR2C NMDA receptor via calcineurin signaling. *The Journal of Neuroscience, 25*(41), 9535–9543.

Swanson, K. L., Marchioro, M., Ishihara, K., Alkondon, M., Pereira, E. F. R., & Albuquerque, E. X. (1997). Neuronal targets of lead in the hippocampus: Relationship to low-level lead intoxication. *Comprehensive Toxicology, 11*, 470–491.

Swope, S. L., Moss, S. J., Raymond, L. A., & Huganir, R. L. (1999). Regulation of ligand-gated ion channels by protein phosphorylation. *Advances in Second Messenger and Phosphoprotein Research, 33*, 49–78.

Tartaglia, N., Du, J., Tyler, W. J., Neale, E., Pozzo-Miller, L., & Lu, B. (2001). Protein synthesis-dependent and -independent regulation of hippocampal synapses by brain-derived neurotrophic factor. *The Journal of Biological Chemistry, 276*, 37585–37593.

Teyler, T. J., & DiScenna, P. (1987). Long-term potentiation. *Annual Review of Neuroscience, 10*, 131–161.

Thiels, E., Norman, E. D., Barrionuevo, G., & Klann, E. (1998). Transient and persistent increases in protein phosphatase activity during long-term depression in the adult hippocampus in vivo. *Neuroscience, 86*(4), 1023–1029.

Thiels, E., Kanterewicz, B. I., Norman, E. D., Trzaskos, J. M., & Klann, E. (2002). Long-term depression in the adult hippocampus in vivo involves activation of extracellular signal-regulated kinase and phosphorylation of Elk-1. *The Journal of Neuroscience, 22*(6), 2054–2062.

Tiffany-Castiglioni, E. (1993). Cell culture models for lead toxicity in neuronal and glial cells. *Neurotoxicology, 14*, 513–536.

Tiffany-Castiglioni, E., Zmudzki, J., & Bratton, G. R. (1986). Cellular targets of lead toxicity: In vitro models. *Toxicology, 42*, 305–315.

Tingley, W. G., Ehlers, M. D., Kameyama, K., Doherty, C., Ptak, J. B., Riley, C. T., & Huganir, R. L. (1997). Characterization of protein kinase A and protein kinase C phosphorylation of the *N*-methyl-D-aspartate receptor NR1 subunit using phosphorylation site-specific antibodies. *The Journal of Biological Chemistry, 272*, 5157–5166.

Tong, G., Shepherd, D., & Jahr, C. E. (1995). Synaptic desensitization of NMDA receptors by calcineurin. *Science, 267*(5203), 1510–1512.

Toni, N., Buchs, P. A., Nikonenko, I., Bron, C. R., & Muller, D. (1999). LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. *Nature, 402*, 421–425.

Topolnik, L., Azzi, M., Morin, F., Kougioumoutzakis, A., & Lacaille, J. C. (2006). MGluR1/5 subtype-specific calcium signalling and induction of long-term potentiation in rat hippocampal oriens/alveus interneurones. *The Journal of Physiology, 575*, 115–131.

Toscano, C. D., & Guilarte, T. R. (2005). Lead neurotoxicity: From exposure to molecular effects. *Brain Research Reviews, 49*, 529–555.

Toscano, C. D., Hashemzadeh-Gargari, H., McGlothan, J. L., & Guilarte, T. R. (2002). Developmental Pb^{2+} exposure alters NMDAR subtypes and reduces CREB phosphorylation in the rat brain. *Developmental Brain Research, 139*, 217–226.

Toscano, C. D., McGlothan, J. L., & Guilarte, T. R. (2003). Lead exposure alters cyclic-AMP response element binding protein phosphorylation and binding activity in the developing rat brain. *Developmental Brain Research, 145*, 219–228.

Toscano, C. D., O'Callaghan, J. P., & Guilarte, T. R. (2005). Calcium/calmodulin-dependent protein kinase II activity and expression are altered in the hippocampus of Pb^{2+}-exposed rats. *Brain Research, 1044*, 51–58.

Ujihara, H., & Albuquerque, E. X. (1992). Developmental change of the inhibition by lead of NMDA activated currents in cultured hippocampal neurons. *The Journal of Pharmacology and Experimental Therapeutics, 263*, 868–875.

United States Centers for Disease Control and Prevention. Atlanta: CDC; (2002). Managing elevated blood lead levels among young children: Recommendations from the advisory committee on childhood lead poisoning prevention. CDC, Atlanta, GA, United States.

Vanhoutte, P., & Bading, H. (2003). Opposing roles of synaptic and extrasynaptic NMDA receptors in neuronal calcium signalling and BDNF gene regulation. *Current Opinion in Neurobiology, 13*(3), 366–371.

Viola, H., Furman, M., Izquierdo, L. A., Alonso, M., Barros, D. M., de Souza, M. M., Izquierdo, I., & Medina, J. H. (2000). Phosphorylated cAMP response element-binding protein as a molecular marker of memory processing in rat hippocampus: Effect of novelty. *The Journal of Neuroscience, 20*(23), RC112.

Wadzinski, B. E., Wheat, W. H., Jaspers, S., Peruski, L. F., Jr., Lickteig, R. L., Johnson, G. L., & Klemm, D. J. (1993). Nuclear protein phosphatase 2A dephosphorylates protein kinase A-phosphorylated CREB and regulates CREB transcriptional stimulation. *Molecular and Cellular Biology, 13*(5), 2822–2834.

Walz, C., Jungling, K. L., & Gottmann, K. (2006). Presynaptic plasticity in an immature neocortical network requires NMDA receptor activation and BDNF release. *Journal of Neurophysiology, 96*, 3512–3516.

Wang, J. H., & Kelly, P. T. (1997). Postsynaptic calcineurin activity downregulates synaptic transmission by weakening intracellular Ca^{2+} signaling mechanisms in hippocampal CA1 neurons. *The Journal of Neuroscience, 17*, 4600–4611.

Wang, L. Y., Orser, B. A., Brautigan, D. L., & MacDonald, J. F. (1994). Regulation of NMDA receptors in cultured hippocampal neurons by protein phosphatases 1 and 2A. *Nature, 369*, 230–232.

Wang, L., Luo, L., Luo, Y. Y., Gu, Y., & Ruan, D. Y. (2007). Effects of Pb^{2+} on muscarinic modulation of glutamatergic synaptic transmission in rat hippocampal CA1 area. *Neurotoxicology, 28*(3), 499–507.

Waters, K.A. & Machaalani, R. (2004). NMDA receptors in the developing brain and effects of noxious insults. *Neurosignals 13*,162–174.

Westphal, R. S., Anderson, K. A., Means, A. R., & Wadzinski, B. E. (1998). A signaling complex of Ca^{2+}-calmodulin-dependent protein kinase IV and protein phosphatase 2A. *Science, 280*(5367), 1258–1261.

Westphal, R. S., Tavalin, S. J., Lin, J. W., Alto, N. M., Fraser, I. D., Langeberg, L. K., Sheng, M., & Scott, J. D. (1999). Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science, 285*(5424), 93–96.

White, L. D., Cory-Slechta, D. A., Gilbert, M. E., Tiffany-Castiglioni, E., Zawia, N. H., Virgolini, M., Rossi-George, A., Lasley, S. M., Qian, Y. C., & Basha, M. R. (2007). New and evolving concepts in the neurotoxicology of lead. *Toxicology and Applied Pharmacology, 225*(1), 1–27.

Winder, D. G., & Sweatt, J. D. (2001). Roles of serine/threonine phosphatases in hippocampal synaptic plasticity. *Nature Reviews. Neuroscience, 2*(7), 461–474.

Xiao, C., Gu, Y., Zhou, C. Y., Wang, L., Zhang, M. M., & Ruan, D. Y. (2006). Pb^{2+} impairs GABAergic synaptic transmission in rat hippocampal slices: A possible involvement of presynaptic calcium channels. *Brain Research, 1088*, 93–100.

Xu, S., & Rajanna, B. (2006). Glutamic acid reverses Pb^{2+}-induced reductions of NMDA receptor subunits in vitro. *Neurotoxicology, 27*, 169–175.

Xu, S. Z., Bullock, L., Shan, C. J., Cornelius, K., & Rajanna, B. (2005). PKC isoforms were reduced by lead in the developing rat brain. *International Journal of Developmental Neuroscience, 23*(1), 53–64.

Xu, J., Yan, C. H., Yang, B., Xie, H. F., Zou, X. Y., Zhong, L., Gao, Y., Tian, Y., & Shen, X. M. (2009a). The role of metabotropic glutamate receptor 5 in developmental lead neurotoxicity. *Toxicology Letters, 191*(2–3), 223–230.

Xu, J., Zhu, Y., Contractor, A., & Heinemann, S. F. (2009b). mGluR5 has a critical role in inhibitory learning. *The Journal of Neuroscience, 29*, 3676–3684.

Xy, Z., Liu, A. P., Ruan, D. Y., & Liu, J. (2002). Effect of developmental lead exposure on the expression of specific NMDA receptor subunit mRNAs in the hippocampus of neonatal rats by digoxigenin-labeled in situ hybridization histochemistry. *Neurotoxicology and Teratology, 24*, 149–160.

Yamashita, T., Inui, S., Maeda, K., Hua, D. R., Takagi, K., Fukunaga, K., & Sskaguchi, N. (2006). Regulation of CamKII by alpha4/PP2Ac contributes to learning and memory. *Brain Research, 1082*(1), 1–10.

Zalutsky, R. A., & Nicoll, R. A. (1990). Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science, 248*, 1619–1624.

Zhang, W., Shen, H., Blaner, W. S., Zhao, Q., Ren, X., & Graziano, J. H. (1996). Chronic lead exposure alters transthyretin concentration in rat cerebrospinal fluid: The role of the choroid plexus. *Toxicology and Applied Pharmacology, 139*(2), 445–450.

Zhang, X. Y., Liu, A. P., Ruan, D. Y., & Liu, J. (2002). Effect of developmental lead exposure on the expression of specific NMDA receptor subunit mRNAs in the hippocampus of neonatal rats by digoxigenin-labeled in situ hybridization histochemistry. *Neurotoxicology and Teratology, 24*(2), 149–160.

Zukin, R. S., & Bennett, M. V. (1995). Alternatively spliced isoforms of the NMDARI receptor subunit. *Trends in Neurosciences, 18*, 306–313.

MS and Excitotoxicity

Chai K. Lim

Contents

Abstract

In this section, it will be studied how excitotoxicity contributes to neurodegeneration in the context of multiple sclerosis (MS). MS has long been regarded as an autoimmune inflammatory disease targeting oligodendrocytes of the central nervous system (CNS). However, recent advancement in research questioned whether MS is merely an inflammatory disease despite pathological evidences of degeneration are clearly present. More recent findings further challenge the research focus of MS which previously thought oligodendrocytes as the main target, whereas current debate points towards pathology of MS being primarily dysfunctional glial interaction, i.e., astrocytes and microglia. Herein, a brief overview will be provided on how various aspects of excitotoxicity are evident in MS pathology and its possible roles between interactions of cells in the CNS that ultimately lead to neurodegeneration in inflammatory condition of MS.

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1 Introduction

Multiple sclerosis (MS) is a chronic degenerative disease of the central nervous system (CNS) characterized by the presence of multifocal lesions with immune cells infiltration, inflammation, demyelination, and neural damages. Although the cause of MS is unknown, very often, MS begins with an autoimmune response to myelin components and progressively leads to oligodendrocytes and axonal degeneration in the chronic phase. The early stages of MS are often resembled by the cycle of inflammation described as relapsing-remitting (RR) phase where the recovery (remitting) period can generally occur from several months to years. About half of RRMS patients will experience exacerbation of the disease progression, of which the remitting period progressively shortens while the relapsing phase persists, leading to the secondary progressive (SP) stage. It remains unclear when and how this switch occurs, but the current hypothesis is that the early stage of MS is driven by the adaptive immunity through inflammation, whereas in the later stage of MS, the innate immunity mediating through neurodegeneration predominates.

2 MS, an Autoimmune Disease or Neurodegenerative Disease?

MS has long been considered an autoimmune demyelinating disease of the CNS. The concept of immune-mediated response in the pathogenesis of MS is indisputable. Presence of autoreactive antibodies and T-cell-mediated destruction on CNS myelin had been the core of this concept. Studies using immunized animals with myelin-related peptides known as experimental autoimmune encephalomyelitis (EAE) exhibiting similar phenotype to MS further highlight the importance of the immune regulation in MS progression. As a result, large number of transacted axons arises from inflammatory insult, but most of these demyelinated axons initially survive and subsequently the damages are reversed. However, in late stages of MS, i.e., SPMS, the brain undergoes continuous atrophy where new inflammatory demyelinating lesions are rare. Progressive neuronal and axonal loss provided a logical explanation for the transition from RRMS to SPMS and for continuous and irreversible neurological decline in SPMS. However, these observations present a more typical hallmark of neurodegeneration. More importantly, neuronal and axonal degeneration must continue in environments other than the inflammatory demyelinating lesion. Furthermore, the inflammatory microenvironment contains a variety of substances that could injure axons, such as proteolytic enzymes, cytokines, oxidative products, and free radicals produced by activated immune

and glial cells. Hence, mechanisms other than inflammatory demyelination of white matter, therefore, must contribute to axonal and neuronal degeneration. It has been well established that excitotoxicity has played a significant role in neurodegeneration, and the next few sections will look at past and recent evidences of excitotoxicity participating in MS pathogenesis.

3 Clinical Evidences of Glutamate-Induced Excitotoxicity in MS

It was notable that the concentration in one of the main excitatory amino acids, glutamate, was significantly elevated in the CNS of MS implicated from several clinical studies. Stover et al. showed that the glutamate level in the CSF of active relapsing phase of MS patients was significantly higher than those who are in the silent (remitting) phase of MS (Stover et al. 1997; Sarchielli et al. 2003). This increase was also associated with the severity and progression of the disease (Sarchielli et al. 2003; Barkhatova et al. 1998). More importantly, Srinivasan et al. demonstrated that the increase of glutamate levels was present in acute lesions and normal-appearing white matter but not in chronic lesions of MS patients using in vivo magnetic resonance spectroscopy imaging technique (Srinivasan et al. 2005). In consistent with the clinical data, MS animal model, EAE lesions are also primarily localized in rich glutamatergic area such as cerebellum and the subpial region of the spinal cord (Brown et al. 1982; Tran et al. 1997).

4 Focus on Oligodendrocytes

The potential role of glutamate-induced excitotoxicity in MS was observed in experimental data demonstrating the beneficial effect of NMDA and AMPA/kainate antagonist, memantine in rat EAE showing reduction of axonal and oligodendrocyte damage but do not dampen CNS inflammation (Wallstrom et al. 1996). This indicates that the mechanism of axonal damage and reversible neurological deficit is likely to act through NMDA receptor and independent of autoimmune neuroinflammation. In addition, two independent studies using NBQX, a selective inhibitor of AMPA/kainate, led to significant reduction in clinical impairment with increased survival of oligodendrocytes in EAE (Pitt et al. 2000; Smith et al. 2000). These data further indicate that AMPA/kainate receptor-mediated glutamate excitotoxicity is a chief factor in oligodendrocyte death in EAE. Indeed, oligodendrocytes express functional AMPA/kainate-type glutamate receptor (Holtzclaw et al. 1995; Steinhauser and Gallo 1996), and exposure to low concentration of AMPA, kainate, or glutamate results in death of oligodendrocytes which can be reversed by addition of NBQX (McDonald et al. 1998). Furthermore, the degree of vulnerability in oligodendrocytes to glutamate excitotoxicity is similar to cortical neuron cultures (McDonald et al. 1998). In addition, oligodendrocytes, like astrocytes, also express functional glutamate transporters for glutamate uptake

(Domercq et al. 1999) and glutamine synthetase (D'Amelio et al. 1990; Cammer 1990). This further subjects oligodendrocytes to glutamate excitotoxicity should their ability to regulate glutamate be compromised (Domercq et al. 2005).

It was found that GluR1, a calcium-permeable AMPA subunit, was elevated on oligodendrocytes in the borders and within active MS plaques in comparison with white matter reflected by positive GluR1-immuneopositive oligodendrocytes (Newcombe et al. 2008). This upregulation of GluR1 on oligodendrocytes around and within plaques may render these cells vulnerable to glutamate excitotoxic damage and death mediated by intracellular calcium ions. One possible mechanism for the increase in GluR1 may be due to presence of growth factor that is facilitated by recruitment of progenitor neural cells at the lesion sites. Growth factors can induce robust and selective enhancement of GluR1 expression in functional channel of oligodendrocytes (Chew et al. 1997). Hence, growth factor-induced upregulation of GluR1 may increase cell vulnerability to glutamate and indirectly limit remyelination at lesion sites.

5 Focus on Microglia and Astrocytes

During inflammation, glutamate is produced and released into the extracellular space by activated leukocytes and microglia in the CNS (Piani et al. 1991; Werner et al. 2001). Astrocytes, on the other hand, are the main glial cells capable of catabolizing glutamate within the CNS (Schousboe et al. 1993) and are thus strategically situated in the vicinity of neurons and oligodendrocytes to prevent damage caused by variation in glutamate extracellular level. Astrocytes express two critical glutamate transporters, GLAST and GLT-1, to maintain glutamate homeostasis in the CNS (Rothstein et al. 1996). In the course of EAE, although high astrocytic reaction is observed reflected by increased GFAP (marker for astrocytes), there is a decrease in expression of astrocytic glutamate catabolic enzymes, glutamine synthetase, and glutamate dehydrogenase, suggesting reduction in the capacity of astrocytes to break down glutamate (Hardin-Pouzet et al. 1997). Further, Ohgoh et al. also found significant downregulation of expression in the protein level of the glial glutamate transporter GLAST (EAAT1) and GLT-1 (EAAT2) in EAE, possibly contributing to increase extracellular glutamate (Ohgoh et al. 2002).

It is inevitable not to mention inflammatory cytokines in regard to microglia as these two entities are closely linked. Inflammatory cytokines can also fuel excitotoxicity in MS. Two proinflammatory cytokines of MS, interleukin (IL)-1β and tumor necrosis factor (TNF)-α, dose-dependently inhibited glutamate uptake in astrocytes, whereas treatment with interferon (IFN)-γ alone promotes glutamate uptake. Hu et al. showed that this paradigm can be antagonized by nitric oxide synthase (NOS) blocker, N^G-monomethyl-L-arginine, suggesting a mechanistic involvement of NO in IL-1β-mediated inhibition of glutamate uptake (Hu et al. 2000). In addition, anti-inflammatory cytokine IFN-β, a common treatment therapy

used in RRMS, was also found to mimic NOS inhibitor by limiting NO production while promoting glutamate uptake in astrocytes (Hu et al. 2000). This phenomenon may partly explain the beneficial effects of the current IFN-β therapy in MS. TNF-α had also shown to disrupt the glutamate homeostasis by promoting glutamate exocytosis in CXCR4-activated astrocytes (Bezzi et al. 2001). Bezzi et al. also showed that glutamate release is further enhanced by presence of activated microglia (Bezzi et al. 2001). TNF-α was also found to inhibit the uptake of glutamate in a dose-dependent fashion by human primary astrocytes; however, the exact mechanism remains to be resolved (Fine et al. 1996). Hence, it is likely that astrocytes-microglia interaction during MS inflammation, under the influences of proinflammatory cytokines, forms an amplification cascade leading to abnormal glutamate homeostasis. Indeed, Takahashi et al. showed that IL-1β and TNF-α are not detrimental in pure oligodendrocyte culture; however, in co-culture with astrocytes and microglia, they evoke apoptosis in oligodendrocyte mediating through glutamate excitotoxicity (Takahashi et al. 2003). Collectively, all the above evidences suggest that loss of ability to modify the local glutamate levels between glial-glial interactions is likely to contribute to neurodegeneration in MS.

6 Quinolinic Acid, Another Potential Endogenous Culprit in Excitotoxicity of MS

One of the NMDA receptor agonists that is worth mentioning and progressively received significant attention in the field of excitotoxicity is quinolinic acid (QUIN). This glutamatergic agonist acts preferentially on distinct population of NMDA receptors containing the NR2A and NR2B subunits. QUIN has had described to be the most potent agonist for NMDA receptors among the others such as NMDA, ibotenate, and glutamate (Monaghan and Beaton 1991). QUIN is one of the downstream metabolites of the kynurenine pathway (KP) which is a major catabolic route of the tryptophan metabolism. It is one of the several metabolites in the KP that are both neuroactive and neurotoxic. Under physiological condition, QUIN is present at nanomolar concentrations in human and rodent brain (Chen and Guillemin 2009). However, during inflammatory state, activation of the KP under the influence of proinflammatory cytokines in the presence of macrophages and microglia can lead to production of up to submicromolar concentration of QUIN (Guillemin et al. 2003, 2005). Hence, its endogenous level in the CNS may be of concern for excitotoxicity given its potency over any other agonists. Indeed, Schwarcz et al. showed that nanomolar concentrations of QUIN injected into rat striatum and hippocampus region of the brain resulted in axon-sparing lesions (Schwarcz et al. 1983). Several in vitro studies also demonstrated that neuronal cells are vulnerable to QUIN excitotoxicity similar to glutamate at pathophysiological concentration including neurons (Kerr et al. 1998), astrocytes (Ting et al. 2009), and oligodendrocytes (Cammer 2001). QUIN can further enhance glutamate excitotoxicity by increasing the release of glutamate by neurons,

decrease uptake by astrocytes (Tavares et al. 2002), and inhibit astroglial glutamine synthetase (Ting et al. 2009) consequently disrupting glutamate-glutamine cycle and ultimately leading to excitotoxicity. Detrimental effects of QUIN can be reversed either through increased endogenous of kynurenic acid (KYNA) (Miranda et al. 1997; Harris et al. 1998), an antagonist of the NMDA receptor, or using potent selective NMDA blocker, MK801 and memantine (Braidy et al. 2009). KYNA is also a KP metabolite and its endogenous level in the mammalian system plays a pivotal role in regulating QUIN excitotoxicity. KYNA was found to be present at subnormal concentration in several neurodegenerative diseases (Chen and Guillemin 2009) including MS (Rejdak et al. 2002). It was suggested that it requires twice as much KYNA to antagonist the same concentration of QUIN (Lekieffre et al. 1990). This further suggests that QUIN is a potent NMDA agonist mediating excitotoxicity at pathophysiological conditions such as inflammation. In the context of MS, QUIN had been implicated to be involved in the pathogenesis of MS associated with neurodegeneration (reviewed (Lim et al. 2010)). Flanagan et al. was the first to demonstrate that QUIN is elevated in EAE and is selective to CNS (Flanagan et al. 1995). They further showed that the time course of QUIN production was similar to the neurological signs of the disorder apart from the initial increased in QUIN before onset of any prominent abnormal behavioral sign of EAE. This suggests that QUIN may be involved in the pathogenesis of EAE. Another study reporting similar results further identified the source of QUIN to be coming from infiltrating macrophages resulting in an accumulation of QUIN at a level considered to be toxic to both neurons and glial cells (Chiarugi et al. 2001). In addition, treatment of EAE mice with a KP modulator (Ro61-8048) that selectively inhibits kynurenine monooxygenase (enzyme that breaks down upstream KP metabolite) results in decreased downstream production of QUIN, while upstream metabolite, KYNA, was significantly increased (Chiarugi et al. 2001). Interestingly, this paradigm was associated with reverse paralysis of the EAE which further suggests a detrimental role of QUIN in progression of EAE. These data implied that altered QUIN homeostasis is likely to contribute to MS pathogenesis. However, the level of QUIN in MS patients remains to be elucidated. Clinical data of QUIN involvement in MS may have pharmacological implication for late stages of MS (i.e., PMS) associated with neurodegeneration since there is currently no effective treatment.

7 Conclusion

This chapter presented various evidences on the roles of excitotoxicity in a complex multifactorial disease, MS. We have highlighted the various components involved in glutamate excitotoxicity and their interactions in cells and animal model of MS. We also looked at how excitotoxicity may interact with some of the immune components of MS. It is indisputable that all these evidences point towards the pivotal roles of excitotoxicity participating in MS pathogenesis. However, the presence of demyelination, the hallmark of MS, whether it is a cause or effect due to autoimmune response or neurodegeneration remains to be elucidated.

References

Barkhatova, V. P., Zavalishin, I. A., Askarova, L., Shavratskii, V., & Demina, E. G. (1998). Changes in neurotransmitters in multiple sclerosis. *Neuroscience and Behavioral Physiology, 28*, 341–344.

Bezzi, P., et al. (2001). CXCR4-activated astrocyte glutamate release via TNFalpha: Amplification by microglia triggers neurotoxicity. *Nature Neuroscience, 4*, 702–710.

Braidy, N., Grant, R., Adams, S., Brew, B. J., & Guillemin, G. J. (2009). Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons. *Neurotoxicity Research, 16*, 77–86.

Brown, A., McFarlin, D. E., & Raine, C. S. (1982). Chronologic neuropathology of relapsing experimental allergic encephalomyelitis in the mouse. *Laboratory Investigation, 46*, 171–185.

Cammer, W. (1990). Glutamine synthetase in the central nervous system is not confined to astrocytes. *Journal of Neuroimmunology, 26*, 173–178.

Cammer, W. (2001). Oligodendrocyte killing by quinolinic acid in vitro. *Brain Research, 896*, 157–160.

Chen, Y., & Guillemin, G. J. (2009). Kynurenine pathway metabolites in humans: Disease and healthy states. *International Journal of Tryptophan Research, 2*, 1–19.

Chew, L. J., et al. (1997). Growth factor-induced transcription of GluR1 increases functional AMPA receptor density in glial progenitor cells. *Journal of Neuroscience, 17*, 227–240.

Chiarugi, A., Cozzi, A., Ballerini, C., Massacesi, L., & Moroni, F. (2001). Kynurenine 3-monooxygenase activity and neurotoxic kynurenine metabolites increase in the spinal cord of rats with experimental allergic encephalomyelitis. *Neuroscience, 102*, 687–695.

D'Amelio, F., Eng, L. F., & Gibbs, M. A. (1990). Glutamine synthetase immunoreactivity is present in oligodendroglia of various regions of the central nervous system. *Glia, 3*, 335–341.

Domercq, M., Sanchez-Gomez, M. V., Areso, P., & Matute, C. (1999). Expression of glutamate transporters in rat optic nerve oligodendrocytes. *European Journal of Neuroscience, 11*, 2226–2236.

Domercq, M., Etxebarria, E., Perez-Samartin, A., & Matute, C. (2005). Excitotoxic oligodendrocyte death and axonal damage induced by glutamate transporter inhibition. *Glia, 52*, 36–46.

Fine, S. M., et al. (1996). Tumor necrosis factor alpha inhibits glutamate uptake by primary human astrocytes. Implications for pathogenesis of HIV-1 dementia. *Journal of Biological Chemistry, 271*, 15303–15306.

Flanagan, E. M., Erickson, J. B., Viveros, O. H., Chang, S. Y., & Reinhard, J. F., Jr. (1995). Neurotoxin quinolinic acid is selectively elevated in spinal cords of rats with experimental allergic encephalomyelitis. *Journal of Neurochemistry, 64*, 1192–1196.

Guillemin, G. J., Smith, D. G., Smythe, G. A., Armati, P. J., & Brew, B. J. (2003). Expression of the kynurenine pathway enzymes in human microglia and macrophages. *Advances in Experimental Medicine and Biology, 527*, 105–112.

Guillemin, G. J., Smythe, G., Takikawa, O., & Brew, B. J. (2005). Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. *Glia, 49*, 15–23.

Hardin-Pouzet, H., et al. (1997). Glutamate metabolism is down-regulated in astrocytes during experimental allergic encephalomyelitis. *Glia, 20*, 79–85.

Harris, C. A., et al. (1998). Modulation of striatal quinolinate neurotoxicity by elevation of endogenous brain kynurenic acid. *British Journal of Pharmacology, 124*, 391–399.

Holtzclaw, L. A., Gallo, V., & Russell, J. T. (1995). AMPA receptors shape Ca^{2+} responses in cortical oligodendrocyte progenitors and CG-4 cells. *Journal of Neuroscience Research, 42*, 124–130.

Hu, S., Sheng, W. S., Ehrlich, L. C., Peterson, P. K., & Chao, C. C. (2000). Cytokine effects on glutamate uptake by human astrocytes. *Neuroimmunomodulation, 7*, 153–159.

Kerr, S. J., Armati, P. J., Guillemin, G. J., & Brew, B. J. (1998). Chronic exposure of human neurons to quinolinic acid results in neuronal changes consistent with AIDS dementia complex. *AIDS, 12*, 355–363.

Lekieffre, D., Plotkine, M., Allix, M., & Boulu, R. G. (1990). Kynurenic acid antagonizes hippocampal quinolinic acid neurotoxicity: Behavioral and histological evaluation. *Neuroscience Letters, 120*, 31–33.

Lim, C. K., Bruce, B. J., & Guillemin, G. J. (2010). Understanding the roles of the kynurenine pathway in multiple sclerosis progression. *International Journal of Tryptophan Research, 3*, 157–167.

McDonald, J. W., Althomsons, S. P., Hyrc, K. L., Choi, D. W., & Goldberg, M. P. (1998). Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. *Nature Medicine, 4*, 291–297.

Miranda, A. F., Boegman, R. J., Beninger, R. J., & Jhamandas, K. (1997). Protection against quinolinic acid-mediated excitotoxicity in nigrostriatal dopaminergic neurons by endogenous kynurenic acid. *Neuroscience, 78*, 967–975.

Monaghan, D. T., & Beaton, J. A. (1991). Quinolinate differentiates between forebrain and cerebellar NMDA receptors. *European Journal of Pharmacology, 194*, 123–125.

Newcombe, J., et al. (2008). Glutamate receptor expression in multiple sclerosis lesions. *Brain Pathology, 18*, 52–61.

Ohgoh, M., et al. (2002). Altered expression of glutamate transporters in experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology, 125*, 170–178.

Piani, D., Frei, K., Do, K. Q., Cuenod, M., & Fontana, A. (1991). Murine brain macrophages induced NMDA receptor mediated neurotoxicity in vitro by secreting glutamate. *Neuroscience Letters, 133*, 159–162.

Pitt, D., Werner, P., & Raine, C. S. (2000). Glutamate excitotoxicity in a model of multiple sclerosis. *Nature Medicine, 6*, 67–70.

Rejdak, K., et al. (2002). Decreased level of kynurenic acid in cerebrospinal fluid of relapsing-onset multiple sclerosis patients. *Neuroscience Letters, 331*, 63–65.

Rothstein, J. D., et al. (1996). Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron, 16*, 675–686.

Sarchielli, P., Greco, L., Floridi, A., & Gallai, V. (2003). Excitatory amino acids and multiple sclerosis: Evidence from cerebrospinal fluid. *Archives of Neurology, 60*, 1082–1088.

Schousboe, A., Westergaard, N., & Hertz, L. (1993). Neuronal-astrocytic interactions in glutamate metabolism. *Biochemical Society Transactions, 21*, 49–53.

Schwarcz, R., Whetsell, W. O., Jr., & Mangano, R. M. (1983). Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. *Science, 219*, 316–318.

Smith, T., Groom, A., Zhu, B., & Turski, L. (2000). Autoimmune encephalomyelitis ameliorated by AMPA antagonists. *Nature Medicine, 6*, 62–66.

Srinivasan, R., Sailasuta, N., Hurd, R., Nelson, S., & Pelletier, D. (2005). Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T. *Brain, 128*, 1016–1025.

Steinhauser, C., & Gallo, V. (1996). News on glutamate receptors in glial cells. *Trends in Neurosciences, 19*, 339–345.

Stover, J. F., et al. (1997). Neurotransmitters in cerebrospinal fluid reflect pathological activity. *European Journal of Clinical Investigation, 27*, 1038–1043.

Takahashi, J. L., Giuliani, F., Power, C., Imai, Y., & Yong, V. W. (2003). Interleukin-1beta promotes oligodendrocyte death through glutamate excitotoxicity. *Annals of Neurology, 53*, 588–595.

Tavares, R. G., et al. (2002). Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochemistry International, 40*, 621–627.

Ting, K. K., Brew, B. J., & Guillemin, G. J. (2009). Effect of quinolinic acid on human astrocytes morphology and functions: Implications in Alzheimer's disease. *Journal of Neuroinflammation, 6*, 36.

Tran, E. H., Hardin-Pouzet, H., Verge, G., & Owens, T. (1997). Astrocytes and microglia express inducible nitric oxide synthase in mice with experimental allergic encephalomyelitis. *Journal of Neuroimmunology, 74*, 121–129.

Wallstrom, E., et al. (1996). Memantine abrogates neurological deficits, but not CNS inflammation, in Lewis rat experimental autoimmune encephalomyelitis. *Journal of the Neurological Sciences, 137*, 89–96.

Werner, P., Pitt, D., & Raine, C. S. (2001). Multiple sclerosis: Altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. *Annals of Neurology, 50*, 169–180.

Neuroprotectants Targeting NMDA Receptor Signaling

Sandra M. Vetiska and Michael Tymianski

Contents

Abstract

N-Methyl-D-aspartate (NMDA) receptors are key mediators of fast excitatory synaptic transmission within the mammalian central nervous system (CNS) and play vital roles in learning, memory, and synaptic development. Overactivation of NMDA receptors (NMDARs) at glutamatergic synapses often results in excitotoxicity, which can cause neuronal injury and death, and is associated with many neurological disorders such as ischemic stroke, epilepsy, neuropathic pain, traumatic brain and spinal injuries; ocular disorders such as glaucoma; as well as neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson's, Huntington's, and Alzheimer's

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diseases. Numerous NMDAR antagonists have been developed that have been effective in reducing cell damage and death in both in vitro and in vivo experimental models of neurological disease, such as ischemia and traumatic brain injury. Unfortunately, clinical use of these NMDAR antagonists has been limited by intolerable side effects, likely due to the necessity of NMDARs in normal brain function. Intracellular signaling pathways that couple to NMDAR activation can promote either cell survival or cell death, depending on NMDAR subunit composition and/or subcellular localization. Ideal therapeutic strategies targeting NMDAR-mediated neurotoxicity should selectively block pro-death signaling, while sparing pro-survival signals. Neuroprotectants targeting downstream effectors of NMDAR-mediated cell death, rather than NMDARs directly, would maintain normal NMDAR function and minimize adverse effects commonly associated with traditional NMDAR antagonists in the treatment of neurological disorders.

1 Introduction

In the mammalian central nervous system (CNS), the principal excitatory neurotransmitter is glutamate. This neurotransmitter acts on two classes of receptors: ionotropic glutamate receptors that form a central ion channel pore and mediate fast excitatory synaptic transmission and metabotropic glutamate receptors, which are G protein-coupled receptors that play neuromodulatory roles within the CNS (Traynelis et al. 2010). Three types of ionotropic glutamate receptors have been identified and classified based on electrophysiological and pharmacological properties: *N*-Methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainate, each being named for their preferred pharmacologic agonist (Nakanishi 1992; Hollmann and Heinemann 1994). Activation of ionotropic glutamate receptors leads to the opening of their associated ion channel, which, depending on receptor type, is permeable to sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) ions. While AMPA and kainate receptors have modest Ca^{2+} permeability, NMDA receptors (NMDARs) are highly Ca^{2+}-permeable (MacDermott et al. 1986; McBain and Mayer 1994; Dingledine et al. 1999), and it is this Ca^{2+} influx that is thought to play a major role in both the physiological as well as pathophysiological functions of the NMDAR (Choi 1988a, b; Tymianski et al. 1993; Ghosh and Greenberg 1995).

The term "excitotoxicity," first coined by Olney (1969), refers to the overactivation of glutamate receptors due to excessive synaptic release of glutamate. Initial studies on glutamate toxicity were done by Lucas and Newhouse (1957), who showed that systemic injections of L-glutamate into mice destroy the inner neural layers of the retina. These observations of neuronal death following excessive glutamate administration were later extended to the brain (Olney 1969; Olney and de Gubareff 1978) and spinal cord (Regan and Choi 1991). Excitotoxicity is now considered an important mechanism involved in many acute CNS insults, such as ischemia and traumatic brain injury (TBI) as

well as in chronic neurodegenerative diseases, such as Parkinson's (PD), Huntington's (HD), and Alzheimer's (AD) diseases (Lipton and Rosenberg 1994; Arundine and Tymianski 2004; Lau and Tymianski 2010).

Glutamate-mediated neurotoxicity arises from excessive Ca^{2+} influx through NMDARs (Berdichevsky et al. 1983; Choi 1987, 1988a, b; Sattler and Tymianski 2000). Ca^{2+} overload can trigger many detrimental cellular processes, including activation of calcium-dependent proteases, mitochondrial dysfunction, and death signals (Pivovarova and Andrews 2010; Szydlowska and Tymianski 2010). Additionally, excessive cellular Ca^{2+} can induce the production of harmful reactive oxygen species. For example, nitric oxide (NO), synthesized by the Ca^{2+}-dependent enzyme neuronal nitric oxide synthase (nNOS), can react with the superoxide anion (O_2^-) to form the highly reactive species peroxynitrite, which can cause damage to proteins, lipids, and DNA (Radi et al. 1991a, b; Aarts et al. 2003b). NMDARs are key mediators of excitotoxicity in the CNS and have thus been the targets of many neuroprotective strategies.

2 NMDA Receptors and Their Antagonists

Structurally, NMDARs exist as heterotetramers, typically consisting of two obligatory NR1 subunits and two regulatory NR2 subunits and less commonly including NR3 subunits (Das et al. 1998; Chatterton et al. 2002; Paoletti and Neyton 2007; Cavara and Hollmann 2008; Pachernegg et al. 2012). Four subtypes of NR2 (NR2A-D) can assemble with the NR1 subunit, most commonly NR2A or NR2B in the forebrain. These receptors are typically diheteromeric in nature; that is, they are comprised of either NR1/NR2A or NR1/NR2B subunits. Some NMDARs can also incorporate two different NR2 subunits (Chazot et al. 1994; Chazot and Stephenson 1997); however, few studies have addressed the functional implications of these receptors.

The type of NR2 subunit determines the biophysical and pharmacological properties of NMDARs; furthermore, these subunits govern distinct protein-protein interactions and downstream signaling pathways, by way of the structural diversity of their carboxyl (C)-terminus (Hardingham and Bading 2003; Cull-Candy and Leszkiewicz 2004; Prybylowski and Wenthold 2004; Kohr 2006; Ryan et al. 2008). Alternatively spliced isoforms also exist for some subunits, such as NR1, creating additional functional diversity (Sugihara et al. 1992; Zukin and Bennett 1995). At resting membrane potential, the channel pore of the NMDAR is blocked by magnesium (Mg^{2+}) which prevents ion flow through the channel. Upon membrane depolarization, Mg^{2+} is expelled, allowing for the passage of ions through the channel. Thus, NMDAR activation requires not only the presynaptic release of glutamate but also the depolarization of the postsynaptic membrane. NMDARs also require simultaneous binding of glutamate and glycine (or D-serine) for activation (Johnson and Ascher 1987; Kleckner and Dingledine 1988; Lerma et al. 1990). The glutamate-binding site is located on the NR2 subunits, while the NR1 subunits provide the glycine-binding site (Furukawa and Gouaux 2003; Furukawa et al. 2005).

Evidence from preclinical research has demonstrated a critical role for NMDAR-mediated neurotoxicity in cellular and animal models of neurological disorders, such as ischemic stroke and TBI (Choi 1988a, b; Tymianski et al. 1993; Arundine et al. 2003). NMDA receptor antagonists targeting the glutamate-binding site, the glycine-binding site, or the channel pore, as well as NR2B subunit-selective antagonists, all showed neuroprotection in preclinical studies of excitotoxicity-related CNS diseases (Danysz and Parsons 2002; Wang and Shuaib 2005). Unfortunately, all clinical trials for neuroprotection in acute disorders including stroke and TBI using NMDAR antagonists, such as Selfotel, Aptiganel, and Gavestinel, have failed. This was largely due either to adverse effects or to limited clinical efficacy (Wood and Hawkinson 1997; Morris et al. 1999; Gladstone et al. 2002; Ikonomidou and Turski 2002; Hoyte et al. 2004; Muir 2006; Kalia et al. 2008; Lau and Tymianski 2010). In addition to undesirable side effects, a short therapeutic window may be a limiting factor with the use of NMDAR antagonists as neuroprotectants in stroke and TBI (Ikonomidou and Turski 2002; Roesler et al. 2003).

In the case of more chronic disorders, an anti-excitotoxic approach using NMDAR antagonists may have a role. For example, memantine is the only NMDAR antagonist currently approved for the treatment of moderate to severe Alzheimer's disease (AD) in most of Europe, the USA, and Canada, either as a monotherapy or in combination with cholinesterase inhibitors (Thomas and Grossberg 2009). Memantine is a moderate affinity, uncompetitive NMDAR antagonist with strong voltage-dependency and fast kinetics, which allow rapid binding to and quick dissociation from NMDARs. These properties allow memantine to be well tolerated and have few side effects, unlike other NMDAR channel blockers (Danysz and Parsons 2012); however, there is no clear benefit to date for the use of memantine in milder stages of AD and only a small beneficial effect on cognition in vascular dementia (Parsons et al. 1999; McShane et al. 2006; Rammes et al. 2008; Herrmann et al. 2011). Memantine has been studied in several other neurological diseases, both at the preclinical and clinical levels (Stieg et al. 1999; Rao et al. 2001; Volbracht et al. 2006; Rojas et al. 2008; Thomas and Grossberg 2009; Milnerwood et al. 2010), and may have clinical benefit in some disorders, including PD (Aarsland et al. 2009), HD (Beister et al. 2004; Ondo et al. 2007) and HIV-associated dementia (Schifitto et al. 2007; Zhao et al. 2010). Further work is required to establish the full potential of memantine in these diseases.

3 Dual Roles of NMDARs in Health and Disease: Implications for Neuroprotectants

3.1 Importance of NMDAR Subcellular Localization

The critical roles that NMDARs play in normal brain function may help explain why clinical treatment of stroke and brain trauma with NMDAR antagonists has failed. An ideal therapeutic approach to antagonize NMDAR overactivity in

neurological disorders would include blocking downstream excitotoxic signaling without directly blocking NMDARs or inhibiting normal excitatory neurotransmission. Recently, dual roles for the NMDAR in both neuronal death and survival have been increasingly appreciated. These depend, at least in part, on the subcellular NMDAR pool that is activated (Hardingham and Bading 2010). At the neuronal plasma membrane, NMDARs are localized to both the synapse and to extrasynaptic regions, and these two receptor populations may have different roles in physiological and pathophysiological processes. Although NMDARs at both locations can mediate excitotoxicity (Sattler et al. 2000; Liu et al. 2007), studies in cultured cortical and hippocampal neurons showed that stimulation of synaptic NMDARs promotes cell survival pathways, whereas extrasynaptic NMDAR activation is more strongly associated with neuronal death (Vanhoutte and Bading 2003; Soriano and Hardingham 2007; Leveille et al. 2008; Hardingham and Bading 2010). This dual role of NMDARs in cell survival and death stems from distinct intracellular signaling pathways that are coupled to synaptic and extrasynaptic NMDARs. Synaptically localized NMDARs promote cell survival by suppressing apoptotic gene expression (Leveille et al. 2010) and activating pro-survival factors such as cyclic AMP response element-binding protein (CREB), a transcription factor that induces expression of genes such as brain-derived neurotrophic factor (BDNF) that is important for survival (Hardingham et al. 2002). In contrast, extrasynaptic NMDARs can mediate cell death by attenuating the CREB pathway, blocking BDNF expression, and promoting mitochondrial dysfunction and cell death (Hardingham et al. 2002; Gouix et al. 2009). Recent work has demonstrated that, similar to cortical neurons, striatal medium-sized spiny neurons also show increased and decreased CREB signaling after stimulation of synaptic and extrasynaptic NMDARs, respectively (Kaufman et al. 2012).

Alterations in synaptic and extrasynaptic NMDAR localization, leading to an imbalance between pro-survival and proapoptotic signaling, have been associated with several neurological disorders (Gladding and Raymond 2011; Sanz-Clemente et al. 2012). For example, enhanced activity of extrasynaptically localized NMDARs was observed in cerebral ischemia (Tu et al. 2010) and Huntington's disease (HD; Okamoto et al. 2009; Milnerwood et al. 2010). In the striatum of transgenic HD mice, increased NR2B-containing extrasynaptic NMDAR expression, increased current, and associated reductions in nuclear CREB activation were found. Furthermore, this reduction in CREB activity, along with associated motor learning deficits, was reversed by treatment of HD mice with memantine, which was shown to preferentially block extrasynaptic NMDARs (Leveille et al. 2008; Okamoto et al. 2009; Xia et al. 2010). The selective block of extrasynaptic NMDARs by memantine may contribute to its clinical tolerability; that is, memantine may spare physiological synaptic transmission needed for normal brain function but selectively antagonize extrasynaptic NMDARs that are excessively activated under pathological conditions.

3.2 Importance of NMDAR NR2 Subunit Subtype

Seemingly contrary to the idea that neuronal survival and death pathways are mediated by synaptic and extrasynaptic NMDARs, respectively, the well-characterized synapse-specific protein PSD95 (postsynaptic density-95) is required for NMDAR-mediated excitotoxic neuronal death (Sattler et al. 1999; Aarts et al. 2002; Zhou et al. 2010). This apparent paradox may be explained by the temporally and spatially regulated expression of different NMDAR NR2 subunits (Traynelis et al. 2010). In adult forebrain neurons, NR2A-containing NMDARs are preferentially localized to synapses, while NR2B-containing NMDARs are primarily expressed at extrasynaptic sites (Tovar and Westbrook 1999; Liu et al. 2007; Groc et al. 2009). In addition, NR2A-containing NMDARs are thought to mediate cell survival signals, whereas NR2B-containing NMDARs are associated with cell death pathways, in both in vitro and in vivo models of stroke and TBI (DeRidder et al. 2006; Zhou and Baudry 2006; Liu et al. 2007; Chen et al. 2008; Terasaki et al. 2010). However, this distribution profile of NMDAR NR2 subunits is not absolute. NR2A-containing receptors can be found at extrasynaptic sites; likewise, NR2B-containing NMDARs are expressed in the postsynaptic membrane, where they associate with other postsynaptic density proteins, such as PSD95 (Groc et al. 2006; Thomas et al. 2006; Harris and Pettit 2007). Therefore, neuronal death pathways may be activated by either synaptic or extrasynaptic NR2B-containing NMDARs, while activation of either synaptic or extrasynaptic NR2A-containing NMDARs promotes neuronal survival (Liu et al. 2007).

NMDARs are localized in the cell membrane, including that of synapses, by an array of scaffolding proteins. Among these is the abundant PSD95 protein, discussed in greater detail in sections below. In synapses, PSD95 binds the C-termini of NMDAR NR2 subunits and links NMDARs to other proteins found in the multiprotein complex (MPC) with which NMDARs associate. In doing so, PSD95 links NMDARs to signaling proteins within the MPC that mediate excitotoxic neuronal death (Sattler et al. 1999; Aarts et al. 2002; Lai and Wang 2010; Zhou et al. 2010). The linkage between NMDARs and downstream toxic signaling pathways may be strongest with NR2B-containing NMDARs. Lending support to this idea is a recent study (Martel et al. 2012) that used chimeric constructs of NMDAR NR2A and NR2B subunits with reciprocal exchanges of their C-terminal domains to demonstrate that the NR2B C-terminal domain is important for NMDAR-mediated toxicity, regardless of receptor location. As discussed above in the case of PSD95, the precise mechanisms by which NMDAR subunit composition determines functional outcome of the receptor may lie in the specific intracellular signaling proteins that couple to NMDARs, most often via their C-terminal tails. Ideally, a therapeutic strategy for excitotoxicity-related neurological disorders would selectively target only those NMDARs or their downstream signaling components associated with neuronal death, while sparing the cell survival-promoting NMDAR population.

4 NMDAR Signaling at the Postsynaptic Density

At the postsynaptic membrane of central excitatory synapses, ionotropic glutamate receptors are localized into specialized MPCs in an electron-dense region termed the postsynaptic density (PSD) (Kennedy 1997; Sheng 2001). NMDARs are abundantly expressed in association with the PSD (Moon et al. 1994), which is composed of both membranous and cytoplasmic proteins (Ziff 1997; Okabe 2007). Scaffolding proteins, which are major components of the MPC, associate with each other and other MPC proteins via highly specific and often unique protein-protein interactions. Such interactions govern cell-to-cell adhesion, regulation of receptor clustering, and modulation of receptor function (Feng and Zhang 2009; Verpelli et al. 2012). PSD95 (also known as SAP90), the first PSD scaffolding protein to be identified (Cho et al. 1992), is a member of a larger group of scaffolding proteins known as membrane-associated guanylate kinase (MAGUK) proteins (Gardoni 2008; Zheng et al. 2011). MAGUKs contain modular protein interaction domains, such as SH3 (Src homology 3), GK (guanylate kinase), and PDZ (Postsynaptic density-95/Discs large homolog/Zona occludens-1) domains (Sheng and Sala 2001; Gardoni 2008; Zheng et al. 2011).

Many surface receptors, such as the NMDAR, bind to MAGUK PDZ domains via their C-terminal PDZ-binding domains. For example, through its first two PDZ domains, PSD95 can bind NMDAR NR2 subunits at their C-terminus PDZ-binding motif (ESDV) (Kornau et al. 1995; Niethammer et al. 1996). PSD95 connects NMDARs to intracellular signaling proteins, such as neuronal nitric oxide synthase (nNOS). PSD95 also binds directly to nNOS via a PDZ-PDZ interaction that involves the second PDZ domain of PSD95 (Brenman et al. 1996; Craven and Bredt 1998; Christopherson et al. 1999; Tezuka et al. 1999). By physically bringing together the NMDAR and nNOS, PSD95 allows Ca^{2+} that permeates through NMDARs to preferentially induce the activation of nNOS and thus couples NMDAR activity to the production of nitric oxide (NO), a signaling molecule that mediates NMDAR-dependent excitotoxicity (Dawson et al. 1991, 1993). Disrupting the NMDAR-PSD95-nNOS complex in cultured cortical neurons using antisense oligonucleotides against PSD95 selectively attenuated NMDAR-mediated excitotoxicity, without affecting NMDAR expression or function (Sattler et al. 1999).

5 NMDAR NR2B Signaling: Downstream Effectors of Excitotoxicity as Therapeutic Targets in Neurological Diseases

5.1 NR2B-PSD95-nNOS Signaling Complex

Because suppression of PSD95 using antisense oligonucleotides is impractical in the treatment of excitotoxicity-related disorders in humans, an alternative strategy

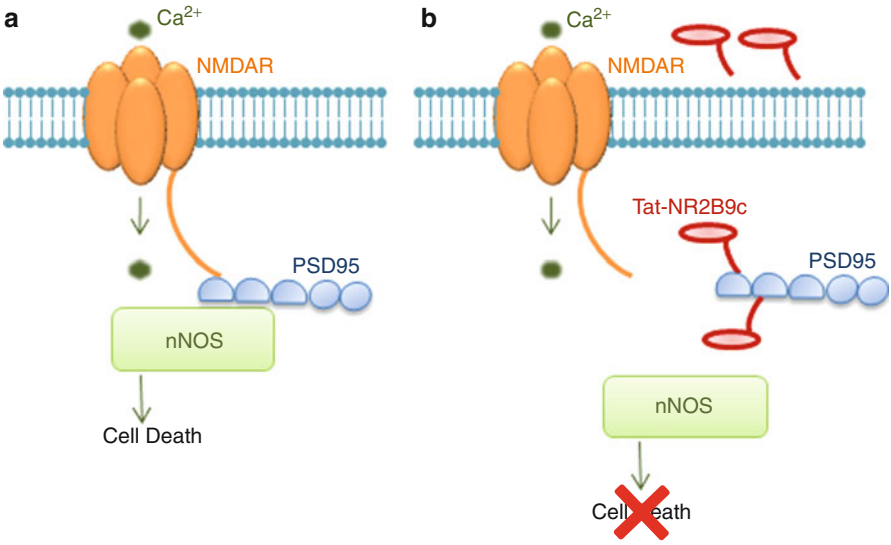

Fig. 1 The NMDAR-PSD95-nNOS signaling complex. (**a**) By binding to both the NMDAR and nNOS, PSD95 brings nNOS in close proximity to the NMDAR and allows Ca^{2+} entering the neuron through the NMDAR to preferentially induce the activation of nNOS, leading to cell death. (**b**) The Tat-NR2B9c peptide disrupts the NMDAR-PSD95-nNOS complex, dissociating NMDARs from downstream neurotoxic signaling, without blocking synaptic activity or calcium influx

for disrupting the NR2B-PSD95-nNOS signaling complex is to use drug compounds that block protein interactions within this complex. One such drug compound is NR2B9c (Aarts et al. 2002). The NR2B9c peptide, comprising the nine C-terminal residues of NR2B (KLSSIESDV), can be rendered cell permeant by fusion to the 11 residue protein transduction domain of the human immunodeficiency virus type 1 (HIV-1) Tat protein (Schwarze et al. 1999). This produces a 20-amino acid peptide termed Tat-NR2B9c. This peptide permeates through cell membranes and disrupts the intracellular interaction of NMDARs with PSD95 (See Fig. 1). This effectively dissociates NMDARs from downstream neurotoxic signaling. However, unlike NMDAR antagonists, this occurs without blocking synaptic activity or calcium influx. This approach is feasible in vivo: when applied either before, 1 or 3 h after an insult, Tat-NR2B9c protected cultured neurons from excitotoxicity and reduced transient middle cerebral artery occlusion (MCAO)-mediated ischemic brain damage in rats (Aarts et al. 2002; Sun et al. 2008).

A proteomic and biochemical analysis of the interactions of Tat-NR2B9c with most or all PDZs in the human genome revealed that the disruption of the NMDAR-PSD95-nNOS complex by Tat-NR2B9c was highly specific. Because the peptide targets the first and second PDZ domains of PSD95, where both the NMDAR and nNOS bind, Tat-NR2B9c inhibited the interaction between both PSD95 and NR2 subunits as well as between PSD95 and nNOS (Cui et al. 2007).

Importantly, Tat-NR2B9c administered 3 h after both permanent and transient focal ischemia reduced infarct volumes in rats and improved long-term neurobehavior, including sensorimotor function, emotionality, and cognition (Sun et al. 2008), raising the possibility for clinical usefulness in a wide therapeutic window. Further downstream, the consequences of administering Tat-NR2B9c are to ameliorate excitotoxic neuronal loss in vitro and ischemic cortical damage in vivo by impairing pro-death p38 signaling, without affecting NMDAR-mediated pro-survival pathways involving CREB or Akt (Soriano et al. 2008; Martel et al. 2009). Other studies have proposed that compounds other than Tat-NR2B9c that target the NMDAR-PSD95 interaction may also have neuroprotective effects. Disruption of the PSD95-nNOS interaction using a small molecule inhibitor (ZL006) is suggested to be neuroprotective in ischemia (Zhou et al. 2010). In addition, the use of a dimeric inhibitor, Tat-*N*PEG4(IETDV)$_2$ (Tat-*N*-dimer), which binds the tandem PDZ1-2 domain of PSD95, reduced infarct volume in mice subjected to cerebral ischemia (Bach et al. 2012).

Recently, Tat-NR2B9c was used for the treatment of stroke in cynomolgus macaques, higher-order gyrencephalic nonhuman primates, which bear anatomical and behavioral similarities to humans (Cook et al. 2012). Animals treated with Tat-NR2B9c up to 3 h after MCAO showed significant reduction in infarct volume, as measured by magnetic resonance imaging and histology. In addition, Tat-NR2B9c-treated macaques showed improved neurobehavioral outcomes assessed using the nonhuman primate stroke scale (NHPSS). This neuroprotection preserved cellular functionality, as gauged by the capacity for gene transcription in ischemic brain tissue. The 3-h time frame for neuroprotection with Tat-NR2B9c is significant as it suggests a broader clinical applicability. Tat-NR2B9c has already entered clinical trials; the ENACT (Evaluating Neuroprotection in Aneurysm Coiling Therapy) Phase 2 clinical trial (ClinicalTrials.gov number, NCT00728182) to determine the safety and efficacy of Tat-NR2B9c in reducing embolic strokes in patients undergoing endovascular repair of brain aneurysms was recently successfully completed (Hill et al. 2012). The encouraging results from this trial warrant further investigation of Tat-NR2B9c in the clinical treatment of stroke.

Since the first study (Aarts et al. 2002) utilizing blocking peptides against PSD95 in stroke, subsequent work using similar strategies has been carried out to investigate other excitotoxicity-mediated neurological diseases, such as epilepsy, AD, and neuropathic pain. Administration of Tat-NR2B9c in rats 3 h after the termination of status epilepticus reduced cell loss in regions of the hippocampus (Dykstra et al. 2009). Perturbing the NR2B-PSD95 association with Tat-NR2B9c ameliorated Aβ-mediated toxicity in vitro and was sufficient to prevent lethality and memory deficits in an AD mouse model (Ittner et al. 2010). The importance of the NR2B9c-PSD95-nNOS signaling complex in neuropathic pain has been demonstrated using a variety of genetic, biochemical, and proteomic techniques. These include the use of antisense oligonucleotides against PSD95 (Tao et al. 2001, 2003), small molecule inhibitors that block the interaction between PSD95 and nNOS (Florio et al. 2009), or Tat-fusion peptides comprising regions of PSD95 (Tao et al. 2008), nNOS (Florio et al. 2009), or the NMDAR NR2B subunit (D'Mello et al. 2011).

5.2 NR2B-ND2-Src Signaling Complex

NMDAR activity is governed in part by the balance between phosphorylation and dephosphorylation (Salter and Kalia 2004). Tyrosine phosphorylation upregulates the activity of NMDARs (Wang and Salter 1994; Zheng et al. 1998), and the nonreceptor tyrosine kinase Src (Ohnishi et al. 2011) can mediate this upregulation. Within the NMDAR complex, Src, along with the phosphotyrosine phosphatase STEP (striatal enriched tyrosine phosphatase), act in opposition to phosphorylate or dephosphorylate the NMDAR, respectively. $STEP_{61}$, the membrane-associated STEP isoform (Sharma et al. 1995; Bult et al. 1996), suppresses NMDAR activity by opposing the actions of Src (Pelkey et al. 2002). Src-mediated enhanced phosphorylation of NMDARs is associated with synaptic plasticity (Rostas et al. 1996; Salter 1998) as well as pathophysiological conditions such as ischemia (Cheung et al. 2003), HD (Song et al. 2003), epilepsy (Sanna et al. 2000; Moussa et al. 2001), and neuropathic pain (Salter and Pitcher 2012). Inhibition of Src activity may attenuate brain injury in ischemia (Jiang et al. 2008; Liang et al. 2009), supporting a role for Src in excitotoxic processes.

Src is anchored to the NMDAR via the adaptor protein NADH dehydrogenase 2 (ND2) (Gingrich et al. 2004). ND2 brings Src in close proximity to the NMDAR and allows Src to upregulate NMDAR function through phosphorylation of NR2 subunits. Disruption of the NMDAR-ND2-Src signaling complex has been shown to suppress pain behaviors. A 10-amino acid peptide, Src40-49, comprising the region of Src needed to bind ND2, was able to inhibit the binding of Src to ND2, thus releasing Src from the NMDAR complex and inhibiting Src-mediated enhancement of NMDAR activity (Liu et al. 2008). Importantly, uncoupling of Src from the NMDAR complex with the membrane-permeable Src40-49Tat fusion peptide suppressed pain hypersensitivity induced by inflammation and peripheral nerve injury (see Fig. 2). Thus, by regulating the function of NMDARs through phosphorylation and dephosphorylation, Src and STEP play vital roles in both physiological as well as pathophysiological glutamatergic neurotransmission. Furthermore, both Src and STEP themselves are subject to regulation. For example, differential regulation of $STEP_{61}$ may play an important role in mediating the dual roles of the NMDAR in cell survival and death (Xu et al. 2009). Synaptic NMDAR activation promotes the ubiquitination and degradation of $STEP_{61}$, concomitant with ERK1/2 activation and cell survival. In contrast, extrasynaptic NMDAR stimulation leads to calpain-mediated proteolysis of $STEP_{61}$, activation of p38, and cell death. Aberrant regulation of both Src and STEP may therefore be involved in NMDAR-mediated pathophysiological conditions (Hossain et al. 2012; Salter and Pitcher 2012). Although targeting STEP or the Src:ND2 interaction has not been tested in ischemia, it is another example of a protein-protein interaction that may be targeted therapeutically in disorders that depend on NMDAR overactivity.

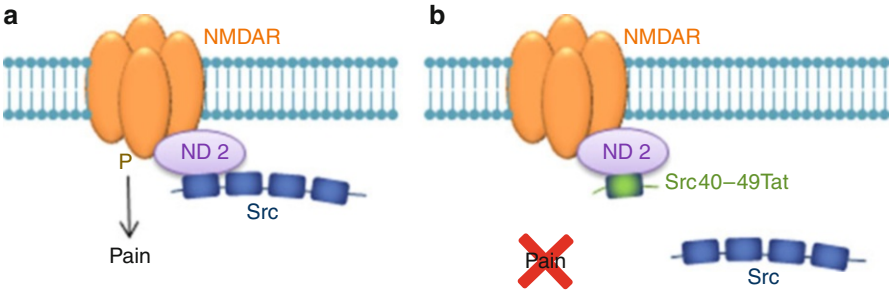

Fig. 2 The NMDAR-ND2-Src signaling complex. (**a**) Src is anchored within the NMDAR complex via the adaptor protein NADH dehydrogenase 2 (ND2). By bringing Src in close proximity to the NMDAR, ND2 allows Src to phosphorylate the receptor and enhance NMDAR activity, resulting in hypersensitivity to pain. (**b**) Using a Src40–49Tat peptide to block the interaction between Src and ND2 dissociates Src from ND2, thereby inhibiting Src-mediated upregulation of NMDAR activity and suppressing pain behaviors (Figure adapted from Liu et al. 2008)

5.3 Other Signaling Pathways Implicated in NMDAR-Mediated Neurotoxicity

Other signaling pathways downstream of NMDARs have been identified to play roles in excitotoxic signaling. For example, during cerebral ischemia, death-associated protein kinase 1 (DAPK1) is recruited to extrasynaptic NMDAR NR2B subunits in the cortex of mice (Tu et al. 2010). DAPK1 directly binds to NR2B, phosphorylates it, and enhances NMDAR channel conductance, leading to increased excitotoxic signaling. Peptides that inhibit the interaction between DAPK1 and the NMDAR NR2B subunit reduced brain infarction and improved neurological function in mice. DAPK1 itself is subject to regulation by other signaling molecules, such as ERK1/2, which may affect NMDAR activity (Liu et al. 2012). Another signaling protein involved in cell death downstream from the NMDAR is PTEN (phosphatase and tensin homolog deleted on chromosome 10). PTEN is recruited selectively to NMDAR NR2B subunits (Ning et al. 2004), potentiates neurotoxic NMDAR activity, and inhibits the PI3K (phosphatidylinositol 3-kinase) survival pathway, thus contributing to excitotoxic neuronal death in stroke. PTEN-induced kinase 1 (PINK1) in a ubiquitous kinase thought to function in cell survival-promoting pathways (Wilhelmus et al. 2012). Overactivation of NR2B-containing NMDARs may contribute to ischemic cell death by suppressing PINK1-dependent survival signaling (Shan et al. 2009). Thus, drug compounds that are able to increase PINK1 levels and activity may prove to be neuroprotective in NMDAR-mediated neurotoxicity.

Activation of NMDARs has been linked to the modulation of several transcription factors that can mediate either pro-survival or pro-death pathways (Hardingham et al. 2002; Zou and Crews 2006; Lai et al. 2011), and these may be useful targets for therapeutic intervention. One example is the transcription factor

SREBP-1 (sterol regulatory element-binding protein-1) (Taghibiglou et al. 2009), which typically controls lipid biosynthesis genes but whose activation is essential in NMDAR-mediated excitotoxic neuronal death in both in vitro and in vivo models of stroke. Therapies aimed at pro-death transcription factors downstream of NMDAR-mediated excitotoxic pathways may offer some novel therapeutic targets in the treatment of neurological diseases. Since activation of these transcription factors is often delayed, relative to NMDAR activation, neuroprotective strategies targeting these proteins may offer a wider therapeutic window if such pathways participate in the cell injury process.

Cell death pathways also exist that may occur in parallel with and converge upon NMDAR-mediated excitotoxic signaling mechanisms (Tymianski 2011). TRPM7 and TRPM2 are members of the transient receptor potential (TRP) channel superfamily (Nilius and Owsianik 2011) that are widely expressed and function in diverse cellular processes, including death, survival, and proliferation. Both TRPM2 and TRPM7 are nonselective cation channels that are activated by oxidative stress and are thought to play significant roles in ischemic cell death (Aarts et al. 2003a; MacDonald et al. 2006; Sun et al. 2009; Xie et al. 2010). TRPM2 and TRPM7 may represent additional Ca^{2+} influx pathways, other than NMDARs, in mediating neurotoxicity in ischemia. Neurotoxic signals such as reactive oxygen species downstream of NMDARs may in turn activate TRPM2 and TRPM7 channels to form a positive feedback loop that perpetuates ischemic cell death.

6 Conclusion

NMDARs have important physiological roles mediating synaptic plasticity and neurodevelopment. However, overactivation of NMDARs leads to excitotoxicity, common to many neurological diseases such as ischemic stroke, epilepsy, neuropathic pain, traumatic brain and spinal injuries; ocular disorders such as glaucoma; as well as neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson's, Huntington's, and Alzheimer's diseases. Antagonists that directly inhibit NMDARs have limited use clinically, possibly due to intolerable side effects at anti-excitotoxic doses. Intracellular signaling pathways that couple to NMDAR activation can promote either cell survival or cell death, depending on NMDAR subunit composition and/or subcellular localization. Extrasynaptic NMDARs, and especially those receptors containing NR2B subunits, engage in cell death pathways upon excessive glutamate stimulation. Conversely, activation of synaptically localized NMDARs, and especially those receptors containing NR2A subunits, may result in neuroprotection. Therapeutic strategies targeting NMDAR-mediated neurotoxicity should preferentially block pro-death signaling while sparing pro-survival signals. Since neuroprotectants that selectively target the molecular components involved in pro-death signaling downstream of the NMDAR do not directly inhibit NMDARs themselves, they may be better tolerated than traditional NMDAR antagonists.

The last decade of scientific research has shed light on the molecular mechanisms involved in NMDAR-mediated excitotoxicity and has revealed additional targets for neuroprotective strategies in the treatment of neurological disorders; however, many questions remain unanswered. First, in addition to NR1/NR2A and NR1/NR2B diheteromeric NMDARs, triheteromeric receptors comprising NR1/NR2A/N2B are also believed to exist. The roles of these triheteromeric receptors, in both health and disease, have yet to be elucidated. Second, several signaling molecules downstream of NMDARs, such as nNOS, DAPK1, and PTEN, play roles in excitotoxic signaling to promote cell damage and death. Whether and how these signaling molecules cross talk with one another is not known. Third, in conditions such as ischemia, excessive Ca^{2+} influx through NMDARs leads to the formation of reactive oxygen species, which can activate other plasma membrane channels, such as TRPM2 and TRPM7 channels; these channels thus represent additional therapeutic targets. Lastly, a major part of our understanding about NMDAR-mediated cell death pathways comes from models of cerebral ischemia. Future work will need to determine whether these pathways also play a role in other neurological diseases that involve excitotoxic mechanisms and whether preclinical neuroprotective therapies can be translated to clinical use.

References

Aarsland, D., Ballard, C., Walker, Z., Bostrom, F., Alves, G., Kossakowski, K., Leroi, I., Pozo-Rodriguez, F., Minthon, L., & Londos, E. (2009). Memantine in patients with Parkinson's disease dementia or dementia with Lewy bodies: A double-blind, placebo-controlled, multicentre trial. *Lancet Neurology, 8*, 613–618.

Aarts, M., Liu, Y., Liu, L., Besshoh, S., Arundine, M., Gurd, J. W., Wang, Y. T., Salter, M. W., & Tymianski, M. (2002). Treatment of ischemic brain damage by perturbing NMDA receptor-PSD-95 protein interactions. *Science, 298*, 846–850.

Aarts, M., Iihara, K., Wei, W. L., Xiong, Z. G., Arundine, M., Cerwinski, W., MacDonald, J. F., & Tymianski, M. (2003a). A key role for TRPM7 channels in anoxic neuronal death. *Cell, 115*, 863–877.

Aarts, M. M., Arundine, M., & Tymianski, M. (2003b). Novel concepts in excitotoxic neurodegeneration after stroke. *Expert Reviews in Molecular Medicine, 5*, 1–22.

Arundine, M., & Tymianski, M. (2004). Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. *Cellular and Molecular Life Sciences, 61*, 657–668.

Arundine, M., Chopra, G. K., Wrong, A., Lei, S., Aarts, M. M., MacDonald, J. F., & Tymianski, M. (2003). Enhanced vulnerability to NMDA toxicity in sublethal traumatic neuronal injury in vitro. *Journal of Neurotrauma, 20*, 1377–1395.

Bach, A., Clausen, B. H., Moller, M., Vestergaard, B., Chi, C. N., Round, A., Sorensen, P. L., Nissen, K. B., Kastrup, J. S., Gajhede, M., Jemth, P., Kristensen, A. S., Lundstrom, P., Lambertsen, K. L., & Stromgaard, K. (2012). A high-affinity, dimeric inhibitor of PSD-95 bivalently interacts with PDZ1-2 and protects against ischemic brain damage. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 3317–3322.

Beister, A., Kraus, P., Kuhn, W., Dose, M., Weindl, A., & Gerlach, M. (2004). The *N*-methyl-D-aspartate antagonist memantine retards progression of Huntington's disease. *Journal of Neural Transmission. Supplementum, 68*, 117–122.

Berdichevsky, E., Riveros, N., Sanchez-Armass, S., & Orrego, F. (1983). Kainate, *N*-methylaspartate and other excitatory amino acids increase calcium influx into rat brain cortex cells in vitro. *Neuroscience Letters, 36*, 75–80.

Brenman, J. E., Chao, D. S., Gee, S. H., McGee, A. W., Craven, S. E., Santillano, D. R., Wu, Z., Huang, F., Xia, H., Peters, M. F., Froehner, S. C., & Bredt, D. S. (1996). Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains. *Cell, 84*, 757–767.

Bult, A., Zhao, F., Dirkx, R., Jr., Sharma, E., Lukacsi, E., Solimena, M., Naegele, J. R., & Lombroso, P. J. (1996). STEP61: A member of the family of brain-enriched PTPs is localized to the endoplasmic reticulum. *The Journal of Neuroscience, 16*, 7821–7831.

Cavara, N. A., & Hollmann, M. (2008). Shuffling the deck anew: How NR3 tweaks NMDA receptor function. *Molecular Neurobiology, 38*, 16–26.

Chatterton, J. E., Awobuluyi, M., Premkumar, L. S., Takahashi, H., Talantova, M., Shin, Y., Cui, J., Tu, S., Sevarino, K. A., Nakanishi, N., Tong, G., Lipton, S. A., & Zhang, D. (2002). Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature, 415*, 793–798.

Chazot, P. L., & Stephenson, F. A. (1997). Molecular dissection of native mammalian forebrain NMDA receptors containing the NR1 C2 exon: Direct demonstration of NMDA receptors comprising NR1, NR2A, and NR2B subunits within the same complex. *Journal of Neurochemistry, 69*, 2138–2144.

Chazot, P. L., Coleman, S. K., Cik, M., & Stephenson, F. A. (1994). Molecular characterization of *N*-methyl-D-aspartate receptors expressed in mammalian cells yields evidence for the coexistence of three subunit types within a discrete receptor molecule. *The Journal of Biological Chemistry, 269*, 24403–24409.

Chen, M., Lu, T. J., Chen, X. J., Zhou, Y., Chen, Q., Feng, X. Y., Xu, L., Duan, W. H., & Xiong, Z. Q. (2008). Differential roles of NMDA receptor subtypes in ischemic neuronal cell death and ischemic tolerance. *Stroke, 39*, 3042–3048.

Cheung, H. H., Teves, L., Wallace, M. C., & Gurd, J. W. (2003). Inhibition of protein kinase C reduces ischemia-induced tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor. *Journal of Neurochemistry, 86*, 1441–1449.

Cho, K. O., Hunt, C. A., & Kennedy, M. B. (1992). The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. *Neuron, 9*, 929–942.

Choi, D. W. (1987). Ionic dependence of glutamate neurotoxicity. *The Journal of Neuroscience, 7*, 369–379.

Choi, D. W. (1988a). Glutamate neurotoxicity and diseases of the nervous system. *Neuron, 1*, 623–634.

Choi, D. W. (1988b). Calcium-mediated neurotoxicity: Relationship to specific channel types and role in ischemic damage. *Trends in Neurosciences, 11*, 465–469.

Christopherson, K. S., Hillier, B. J., Lim, W. A., & Bredt, D. S. (1999). PSD-95 assembles a ternary complex with the *N*-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. *The Journal of Biological Chemistry, 274*, 27467–27473.

Cook, D. J., Teves, L., & Tymianski, M. (2012). Treatment of stroke with a PSD-95 inhibitor in the gyrencephalic primate brain. *Nature, 483*, 213–217.

Craven, S. E., & Bredt, D. S. (1998). PDZ proteins organize synaptic signaling pathways. *Cell, 93*, 495–498.

Cui, H., Hayashi, A., Sun, H. S., Belmares, M. P., Cobey, C., Phan, T., Schweizer, J., Salter, M. W., Wang, Y. T., Tasker, R. A., Garman, D., Rabinowitz, J., Lu, P. S., & Tymianski, M. (2007). PDZ protein interactions underlying NMDA receptor-mediated excitotoxicity and neuroprotection by PSD-95 inhibitors. *The Journal of Neuroscience, 27*, 9901–9915.

Cull-Candy, S. G. & Leszkiewicz, D. N. (2004). Role of distinct NMDA receptor subtypes at central synapses. *Science STKE, 2004*, re16.

Danysz, W., & Parsons, C. G. (2002). Neuroprotective potential of ionotropic glutamate receptor antagonists. *Neurotoxicity Research, 4*, 119–126.

Danysz, W., & Parsons, C. G. (2012). Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine – Searching for the connections. *British Journal of Pharmacology, 167*, 324–352.

Das, S., Sasaki, Y. F., Rothe, T., Premkumar, L. S., Takasu, M., Crandall, J. E., Dikkes, P., Conner, D. A., Rayudu, P. V., Cheung, W., Chen, H. S. V., Lipton, S. A., & Nakanishi, N. (1998). Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature, 393*, 377–381.

Dawson, V. L., Dawson, T. M., London, E. D., Bredt, D. S., & Snyder, S. H. (1991). Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proceedings of the National Academy of Sciences of the United States of America, 88*, 6368–6371.

Dawson, V. L., Dawson, T. M., Bartley, D. A., Uhl, G. R., & Snyder, S. H. (1993). Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. *The Journal of Neuroscience, 13*, 2651–2661.

DeRidder, M. N., Simon, M. J., Siman, R., Auberson, Y. P., Raghupathi, R., & Meaney, D. F. (2006). Traumatic mechanical injury to the hippocampus in vitro causes regional caspase-3 and calpain activation that is influenced by NMDA receptor subunit composition. *Neurobiology of Disease, 22*, 165–176.

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacological Reviews, 51*, 7–61.

D'Mello, R., Marchand, F., Pezet, S., McMahon, S. B., & Dickenson, A. H. (2011). Perturbing PSD-95 interactions with NR2B-subtype receptors attenuates spinal nociceptive plasticity and neuropathic pain. *Molecular Therapy, 19*, 1780–1792.

Dykstra, C. M., Ratnam, M., & Gurd, J. W. (2009). Neuroprotection after status epilepticus by targeting protein interactions with postsynaptic density protein 95. *Journal of Neuropathology and Experimental Neurology, 68*, 823–831.

Feng, W., & Zhang, M. (2009). Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density. *Nature Reviews Neuroscience, 10*, 87–99.

Florio, S. K., Loh, C., Huang, S. M., Iwamaye, A. E., Kitto, K. F., Fowler, K. W., Treiberg, J. A., Hayflick, J. S., Walker, J. M., Fairbanks, C. A., & Lai, Y. (2009). Disruption of nNOS-PSD95 protein-protein interaction inhibits acute thermal hyperalgesia and chronic mechanical allodynia in rodents. *British Journal of Pharmacology, 158*, 494–506.

Furukawa, H., & Gouaux, E. (2003). Mechanisms of activation, inhibition and specificity: Crystal structures of the NMDA receptor NR1 ligand-binding core. *EMBO Journal, 22*, 2873–2885.

Furukawa, H., Singh, S. K., Mancusso, R., & Gouaux, E. (2005). Subunit arrangement and function in NMDA receptors. *Nature, 438*, 185–192.

Gardoni, F. (2008). MAGUK proteins: New targets for pharmacological intervention in the glutamatergic synapse. *European Journal of Pharmacology, 585*, 147–152.

Ghosh, A., & Greenberg, M. E. (1995). Calcium signaling in neurons: Molecular mechanisms and cellular consequences. *Science, 268*, 239–247.

Gingrich, J. R., Pelkey, K. A., Fam, S. R., Huang, Y., Petralia, R. S., Wenthold, R. J., & Salter, M. W. (2004). Unique domain anchoring of Src to synaptic NMDA receptors via the mitochondrial protein NADH dehydrogenase subunit 2. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 6237–6242.

Gladding, C. M., & Raymond, L. A. (2011). Mechanisms underlying NMDA receptor synaptic/extrasynaptic distribution and function. *Molecular and Cellular Neurosciences, 48*, 308–320.

Gladstone, D. J., Black, S. E., & Hakim, A. M. (2002). Toward wisdom from failure: Lessons from neuroprotective stroke trials and new therapeutic directions. *Stroke, 33*, 2123–2136.

Gouix, E., Leveille, F., Nicole, O., Melon, C., Had-Aissouni, L., & Buisson, A. (2009). Reverse glial glutamate uptake triggers neuronal cell death through extrasynaptic NMDA receptor activation. *Molecular and Cellular Neurosciences, 40*, 463–473.

Groc, L., Bard, L., & Choquet, D. (2009). Surface trafficking of *N*-methyl-D-aspartate receptors: Physiological and pathological perspectives. *Neuroscience, 158*, 4–18.

Groc, L., Heine, M., Cousins, S. L., Stephenson, F. A., Lounis, B., Cognet, L., & Choquet, D. (2006). NMDA receptor surface mobility depends on NR2A-2B subunits. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 18769–18774.

Hardingham, G. E., & Bading, H. (2003). The Yin and Yang of NMDA receptor signaling. *Trends in Neurosciences, 26*, 81–89.

Hardingham, G. E., & Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signaling: Implications for neurodegenerative disorders. *Nature Reviews Neuroscience, 11*, 682–696.

Hardingham, G. E., Fukunaga, Y., & Bading, H. (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nature Neuroscience, 5*, 405–414.

Harris, A. Z., & Pettit, D. L. (2007). Extrasynaptic and synaptic NMDA receptors form stable and uniform pools in rat hippocampal slices. *The Journal of Physiology, 584*, 509–519.

Herrmann, N., Li, A., & Lanctot, K. (2011). Memantine in dementia: A review of the current evidence. *Expert Opinion on Pharmacotherapy, 12*, 787–800.

Hill, M. D. M., Martin, R. H., Mikulis, D., Wong, J. H., Silver, F. L., terBrugge, K. G., Milot, G., Clarke, W. M., MacDonald, R. L., Kelly, M. E., Boulton, M., Fleetwood, I., MacDougall, C., Gunnarsson, T., Chow, M., Lum, C., Dodd, R., Poublanc, J., Krings, T., Demchuk, A. M., Goyal, M., Anderson, R., Bishop, J., Garman, D., & Tymianski, M. (2012). Safety and efficacy of NA-1 for neuroprotection in iatrogenic stroke after endovascular aneurysm repair: A randomized controlled trial. *Lancet Neurology, 12*, 70225–70229.

Hollmann, M., & Heinemann, S. (1994). Cloned glutamate receptors. *Annual Review of Neuroscience, 17*, 31–108.

Hossain, M. I., Kamaruddin, M. A., & Cheng, H. C. (2012). Aberrant regulation and function of Src family tyrosine kinases: Their potential contributions to glutamate-induced neurotoxicity. *Clinical and Experimental Pharmacology and Physiology, 39*, 684–691.

Hoyte, L., Barber, P. A., Buchan, A. M., & Hill, M. D. (2004). The rise and fall of NMDA antagonists for ischemic stroke. *Current Molecular Medicine, 4*, 131–136.

Ikonomidou, C., & Turski, L. (2002). Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? *Lancet Neurology, 1*, 383–386.

Ittner, L. M., Ke, Y. D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., Wolfing, H., Chieng, B. C., Christie, M. J., Napier, I. A., Eckert, A., Staufenbiel, M., Hardeman, E., & Gotz, J. (2010). Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell, 142*, 387–397.

Jiang, X., Mu, D., Biran, V., Faustino, J., Chang, S., Rincon, C. M., Sheldon, A., & Ferriero, D. M. (2008). Activated Src kinases interact with the *N*-methyl-D-aspartate receptor after neonatal brain ischemia. *Annals of Neurology, 63*, 632–641.

Johnson, J. W., & Ascher, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature, 325*, 529–531.

Kalia, L. V., Kalia, S. K., & Salter, M. W. (2008). NMDA receptors in clinical neurology: Excitatory times ahead. *Lancet Neurology, 7*, 742–755.

Kaufman, A. M., Milnerwood, A. J., Sepers, M. D., Coquinco, A., She, K., Wang, L., Lee, H., Craig, A. M., Cynader, M., & Raymond, L. A. (2012). Opposing roles of synaptic and extrasynaptic NMDA receptor signaling in cocultured striatal and cortical neurons. *The Journal of Neuroscience, 32*, 3992–4003.

Kennedy, M. B. (1997). The postsynaptic density at glutamatergic synapses. *Trends in Neurosciences, 20*, 264–268.

Kleckner, N. W., & Dingledine, R. (1988). Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. *Science, 241*, 835–837.

Kohr, G. (2006). NMDA receptor function: Subunit composition versus spatial distribution. *Cell and Tissue Research, 326*, 439–446.

Kornau, H. C., Schenker, L. T., Kennedy, M. B., & Seeburg, P. H. (1995). Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science, 269*, 1737–1740.

Lai, T. W., & Wang, Y. T. (2010). Fashioning drugs for stroke. *Nature Medicine, 16*, 1376–1378.

Lai, T. W., Shyu, W. C., & Wang, Y. T. (2011). Stroke intervention pathways: NMDA receptors and beyond. *Trends in Molecular Medicine, 17*, 266–275.

Lau, A., & Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflügers Archiv, 460*, 525–542.

Lerma, J., Zukin, R. S., & Bennett, M. V. (1990). Glycine decreases desensitization of *N*-methyl-D-aspartate (NMDA) receptors expressed in Xenopus oocytes and is required for NMDA responses. *Proceedings of the National Academy of Sciences of the United States of America, 87*, 2354–2358.

Leveille, F., El Gaamouch, F., Gouix, E., Lecocq, M., Lobner, D., Nicole, O., & Buisson, A. (2008). Neuronal viability is controlled by a functional relation between synaptic and extrasynaptic NMDA receptors. *The FASEB Journal, 22*, 4258–4271.

Leveille, F., Papadia, S., Fricker, M., Bell, K. F., Soriano, F. X., Martel, M. A., Puddifoot, C., Habel, M., Wyllie, D. J., Ikonomidou, C., Tolkovsky, A. M., & Hardingham, G. E. (2010). Suppression of the intrinsic apoptosis pathway by synaptic activity. *The Journal of Neuroscience, 30*, 2623–2635.

Liang, S., Pong, D., Gonzales, C., Chen, Y., Ling, H. P., Mark, R. J., Boschelli, F., Boschelli, D. H., Ye, F., Barrios Sosa, A. C., Mansour, T. S., Frost, P., Wood, A., Pangalos, M. N., & Zaleska, M. M. (2009). Neuroprotective profile of novel Src kinase inhibitors in rodent models of cerebral ischemia. *The Journal of Pharmacology and Experimental Therapeutics, 331*, 827–835.

Lipton, S. A., & Rosenberg, P. A. (1994). Excitatory amino acids as a final common pathway for neurologic disorders. *The New England Journal of Medicine, 330*, 613–622.

Liu, Y., Wong, T. P., Aarts, M., Rooyakkers, A., Liu, L., Lai, T. W., Wu, D. C., Lu, J., Tymianski, M., Craig, A. M., & Wang, Y. T. (2007). NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. *The Journal of Neuroscience, 27*, 2846–2857.

Liu, X. J., Gingrich, J. R., Vargas-Caballero, M., Dong, Y. N., Sengar, A., Beggs, S., Wang, S. H., Ding, H. K., Frankland, P. W., & Salter, M. W. (2008). Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex. *Nature Medicine, 14*, 1325–1332.

Liu, S. B., Zhang, N., Guo, Y. Y., Zhao, R., Shi, T. Y., Feng, S. F., Wang, S. Q., Yang, Q., Li, X. Q., Wu, Y. M., Ma, L., Hou, Y., Xiong, L. Z., Zhang, W., & Zhao, M. G. (2012). G-protein-coupled receptor 30 mediates rapid neuroprotective effects of estrogen via depression of NR2B-containing NMDA receptors. *The Journal of Neuroscience, 32*, 4887–4900.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Archives of Ophthalmology, 58*, 193–201.

MacDermott, A. B., Mayer, M. L., Westbrook, G. L., Smith, S. J., & Barker, J. L. (1986). NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature, 321*, 519–522.

MacDonald, J. F., Xiong, Z. G., & Jackson, M. F. (2006). Paradox of Ca^{2+} signaling, cell death and stroke. *Trends in Neurosciences, 29*, 75–81.

Martel, M. A., Soriano, F. X., Baxter, P., Rickman, C., Duncan, R., Wyllie, D. J., & Hardingham, G. E. (2009). Inhibiting pro-death NMDA receptor signaling dependent on the NR2 PDZ ligand may not affect synaptic function or synaptic NMDA receptor signaling to gene expression. *Channels (Austin, Tex.), 3*, 12–15.

Martel, M. A., Ryan, T. J., Bell, K. F. S., Fowler, J. H., McMahon, A., Al-Mubarak, B., Komiyama, N. H., Horsburgh, K., Kind, P. C., Grant, S. G. N., & Wyllie, D. J. A. (2012). The subtype of GluN2 C-terminal domain determines the response to excitotoxic insults. *Neuron, 74*, 543–556.

McBain, C. J., & Mayer, M. L. (1994). *N*-methyl-D-aspartic acid receptor structure and function. *Physiological Reviews, 74*, 723–760.

McShane, R., Areosa, S. A., & Minakaran, N. (2006). Memantine for dementia. *Cochrane Database of Systematic Reviews*, *2*, 1–42.

Milnerwood, A. J., Gladding, C. M., Pouladi, M. A., Kaufman, A. M., Hines, R. M., Boyd, J. D., Ko, R. W. Y., Vasuta, O. C., Graham, R. K., Hayden, M. R., Murphy, T. H., & Raymond, L. A. (2010). Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron, 65*, 178–190.

Moon, I. S., Apperson, M. L., & Kennedy, M. B. (1994). The major tyrosine-phosphorylated protein in the postsynaptic density fraction is *N*-methyl-D-aspartate receptor subunit 2B. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 3954–3958.

Morris, G. F., Bullock, R., Marshall, S. B., Marmarou, A., Maas, A., & Marshall, L. F. (1999). Failure of the competitive *N*-Methyl-D-aspartate antagonist Selfotel (CGS 19755) in the treatment of severe head injury: results of two Phase III clinical trials. The Selfotel Investigators. *Journal of Neurosurgery, 91*, 737–743.

Moussa, R. C., Ikeda-Douglas, C. J., Thakur, V., Milgram, N. W., & Gurd, J. W. (2001). Seizure activity results in increased tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor in the hippocampus. *Brain Research. Molecular Brain Research, 95*, 36–47.

Muir, K. W. (2006). Glutamate-based therapeutic approaches: clinical trials with NMDA antagonists. *Current Opinion in Pharmacology, 6*, 53–60.

Nakanishi, S. (1992). Molecular diversity of glutamate receptors and implications for brain function. *Science, 258*, 597–603.

Niethammer, M., Kim, E., & Sheng, M. (1996). Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. *The Journal of Neuroscience, 16*, 2157–2163.

Nilius, B., & Owsianik, G. (2011). The transient receptor potential family of ion channels. *Genome Biology, 12*, 218–228.

Ning, K., Pei, L., Liao, M., Liu, B., Zhang, Y., Jiang, W., Mielke, J. G., Li, L., Chen, Y., El-Hayek, Y. H., Fehlings, M. G., Zhang, X., Liu, F., Eubanks, J., & Qan, Q. (2004). Dual neuroprotective signaling mediated by downregulating two distinct phosphatase activities of PTEN. *The Journal of Neuroscience, 24*, 4052–4060.

Ohnishi, H., Murata, Y., Okazawa, H., & Matozaki, T. (2011). Src family kinases: modulators of neurotransmitter receptor function and behavior. *Trends in Neurosciences, 34*, 629–637.

Okabe, S. (2007). Molecular anatomy of the postsynaptic density. *Molecular and Cellular Neurosciences, 34*, 503–518.

Okamoto, S., Pouladi, M. A., Talantova, M., Yao, D., Xia, P., Ehrnhoefer, D. E., Zaidi, R., Clemente, A., Kaul, M., Graham, R. K., Zhang, D., Chen, H. S. V., Tong, G., Hayden, M. R., & Lipton, S. A. (2009). Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. *Nature Medicine, 15*, 1407–1413.

Olney, J. W. (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science, 164*, 719–721.

Olney, J. W., & de Gubareff, T. (1978). The fate of synaptic receptors in the kainate-lesioned striatum. *Brain Research, 140*, 340–343.

Ondo, W. G., Mejia, N. I., & Hunter, C. B. (2007). A pilot study of the clinical efficacy and safety of memantine for Huntington's disease. *Parkinsonism & Related Disorders, 13*, 453–454.

Pachernegg, S., Strutz-Seebohm, N., & Hollmann, M. (2012). GluN3 subunit-containing NMDA receptors: Not just one-trick ponies. *Trends in Neurosciences, 35*, 240–249.

Paoletti, P., & Neyton, J. (2007). NMDA receptor subunits: function and pharmacology. *Current Opinion in Pharmacology, 7*, 39–47.

Parsons, C. G., Danysz, W., & Quack, G. (1999). Memantine is a clinically well tolerated *N*-methyl-D-aspartate (NMDA) receptor antagonist–a review of preclinical data. *Neuropharmacology, 38*, 735–767.

Pelkey, K. A., Askalan, R., Paul, S., Kalia, L. V., Nguyen, T. H., Pitcher, G. M., Salter, M. W., & Lombroso, P. J. (2002). Tyrosine phosphatase STEP is a tonic brake on induction of long-term potentiation. *Neuron, 34*, 127–138.

Pivovarova, N. B., & Andrews, S. B. (2010). Calcium-dependent mitochondrial function and dysfunction in neurons. *The FEBS Journal, 277*, 3622–3636.

Prybylowski, K., & Wenthold, R. J. (2004). *N*-Methyl-D-aspartate receptors: subunit assembly and trafficking to the synapse. *The Journal of Biological Chemistry, 279*, 9673–9676.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991a). Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *The Journal of Biological Chemistry, 266*, 4244–4250.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991b). Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics, 288*, 481–487.

Rammes, G., Danysz, W., & Parsons, C. G. (2008). Pharmacodynamics of memantine: An update. *Current Neuropharmacology, 6*, 55–78.

Rao, V. L., Dogan, A., Todd, K. G., Bowen, K. K., & Dempsey, R. J. (2001). Neuroprotection by memantine, a non-competitive NMDA receptor antagonist after traumatic brain injury in rats. *Brain Research, 911*, 96–100.

Regan, R. F., & Choi, D. W. (1991). Glutamate neurotoxicity in spinal cord cell culture. *Neuroscience, 43*, 585–591.

Roesler, R., Quevedo, J., & Schroder, N. (2003). Is it time to conclude that NMDA antagonists have failed? *Lancet Neurology, 2*, 13.

Rojas, J. C., Saavedra, J. A., & Gonzalez-Lima, F. (2008). Neuroprotective effects of memantine in a mouse model of retinal degeneration induced by rotenone. *Brain Research, 1215*, 208–217.

Rostas, J. A., Brent, V. A., Voss, K., Errington, M. L., Bliss, T. V., & Gurd, J. W. (1996). Enhanced tyrosine phosphorylation of the 2B subunit of the *N*-methyl-D-aspartate receptor in long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 10452–10456.

Ryan, T. J., Emes, R. D., Grant, S. G., & Komiyama, N. H. (2008). Evolution of NMDA receptor cytoplasmic interaction domains: Implications for organisation of synaptic signaling complexes. *BMC Neuroscience, 9*, 6.

Salter, M. W. (1998). Src, *N*-methyl-D-aspartate (NMDA) receptors, and synaptic plasticity. *Biochemical Pharmacology, 56*, 789–798.

Salter, M. W., & Kalia, L. V. (2004). Src kinases: A hub for NMDA receptor regulation. *Nature Reviews Neuroscience, 5*, 317–328.

Salter, M. W., & Pitcher, G. M. (2012). Dysregulated Src upregulation of NMDA receptor activity: A common link in chronic pain and schizophrenia. *The FEBS Journal, 279*, 2–11.

Sanna, P. P., Berton, F., Cammalleri, M., Tallent, M. K., Siggins, G. R., Bloom, F. E., & Francesconi, W. (2000). A role for Src kinase in spontaneous epileptiform activity in the CA3 region of the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 8653–8657.

Sanz-Clemente, A., Nicoll, R. A., & Roche, K. W. (2012). Diversity in NMDA receptor composition: Many regulators, many consequences. *Neuroscientist, 19*, 62–75.

Sattler, R., & Tymianski, M. (2000). Molecular mechanisms of calcium-dependent excitotoxicity. *Journal of Molecular Medicine (Berlin), 78*, 3–13.

Sattler, R., Xiong, Z., Lu, W. Y., Hafner, M., MacDonald, J. F., & Tymianski, M. (1999). Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science, 284*, 1845–1848.

Sattler, R., Xiong, Z., Lu, W. Y., MacDonald, J. F., & Tymianski, M. (2000). Distinct roles of synaptic and extrasynaptic NMDA receptors in excitotoxicity. *The Journal of Neuroscience, 20*, 22–33.

Schifitto, G., Navia, B. A., Yiannoutsos, C. T., Marra, C. M., Chang, L., Ernst, T., Jarvik, J. G., Miller, E. N., Ginger, E. J., Ellis, R. J., Kolson, D. L., Simpson, D., Nath, A., Berger, J.,

Shriver, S. L., Millar, L. L., Colquhoun, D., Lenkinski, R., Gonzalez, R. G., Lipton, S. A., & Adult AIDS Clinical Trial Group (ACTG) 301, 700 Teams, HIV MRS Consortium. (2007). Memantine and HIV-associated cognitive impairment: A neuropsychological and proton magnetic resonance spectroscopy study. *AIDS, 21*, 1877–1886.

Schwarze, S. R., Ho, A., Vocero-Akbani, A., & Dowdy, S. F. (1999). In vivo protein transduction: Delivery of a biologically active protein into the mouse. *Science, 285*, 1569–1572.

Shan, Y., Liu, B., Li, L., Chang, N., Li, L., Wang, H., Wang, D., Feng, H., Cheung, C., Liao, M., Cui, T., Sugita, S., & Wan, Q. (2009). Regulation of PINK1 by NR2B-containing NMDA receptors in ischemic neuronal injury. *Journal of Neurochemistry, 111*, 1149–1160.

Sharma, E., Zhao, F., Bult, A., & Lombroso, P. J. (1995). Identification of two alternatively spliced transcripts of STEP: A subfamily of brain-enriched protein tyrosine phosphatases. *Brain Research. Molecular Brain Research, 32*, 87–93.

Sheng, M. (2001). Molecular organization of the postsynaptic specialization. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 7058–7061.

Sheng, M., & Sala, C. (2001). PDZ domains and the organization of supramolecular complexes. *Annual Review of Neuroscience, 24*, 1–29.

Song, C., Zhang, Y., Parsons, C. G., & Liu, Y. F. (2003). Expression of polyglutamine-expanded huntingtin induces tyrosine phosphorylation of *N*-methyl-D-aspartate receptors. *The Journal of Biological Chemistry, 278*, 33364–33369.

Soriano, F. X., & Hardingham, G. E. (2007). Compartmentalized NMDA receptor signaling to survival and death. *The Journal of Physiology, 584*, 381–387.

Soriano, F. X., Martel, M. A., Papadia, S., Vaslin, A., Baxter, P., Rickman, C., Forder, J., Tymianski, M., Duncan, R., Aarts, M., Clarke, P. G. H., Wyllie, D. J. A., & Hardingham, G. E. (2008). Specific targeting of pro-death NMDA receptor signals with differing reliance on the NR2B PDZ ligand. *The Journal of Neuroscience, 28*, 10696–10710.

Stieg, P. E., Sathi, S., Warach, S., Le, D. A., & Lipton, S. A. (1999). Neuroprotection by the NMDA receptor-associated open-channel blocker memantine in a photothrombotic model of cerebral focal ischemia in neonatal rat. *European Journal of Pharmacology, 375*, 115–120.

Sugihara, H., Moriyoshi, K., Ishii, T., Masu, M., & Nakanishi, S. (1992). Structures and properties of seven isoforms of the NMDA receptor generated by alternative splicing. *Biochemical and Biophysical Research Communications, 185*, 826–832.

Sun, H. S., Doucette, T. A., Liu, Y., Fang, Y., Teves, L., Aarts, M., Ryan, C. L., Bernard, P. B., Lau, A., Forder, J. P., Salter, M. W., Wang, Y. T., Tasker, A., & Tymianski, M. (2008). Effectiveness of PSD95 inhibitors in permanent and transient focal ischemia in the rat. *Stroke, 39*, 2544–2553.

Sun, H. S., Jackson, M. F., Martin, L. J., Jansen, K., Teves, L., Cui, H., Kiyonaka, S., Mori, Y., Jones, M., Forder, J. P., Golde, T. E., Orser, B. A., MacDonald, J. F., & Tymianski, M. (2009). Suppression of hippocampal TRPM7 protein prevents delayed neuronal death in brain ischemia. *Nature Neuroscience, 12*, 1300–1307.

Szydlowska, K., & Tymianski, M. (2010). Calcium, ischemia and excitotoxicity. *Cell Calcium, 47*, 122–129.

Taghibiglou, C., Martin, H. G., Lai, T. W., Cho, T., Prasad, S., Kojic, L., Lu, J., Liu, Y., Lo, E., Zhang, S., Wu, J. Z. Z., Li, Y. P., Wen, Y. H., Imm, J. H., Cynader, M. S., & Wang, Y. T. (2009). Role of NMDA receptor-dependent activation of SREBP1 in excitotoxic and ischemic neuronal injuries. *Nature Medicine, 15*, 1399–1406.

Tao, F., Tao, Y. X., Gonzalez, J. A., Fang, M., Mao, P., & Johns, R. A. (2001). Knockdown of PSD-95/SAP90 delays the development of neuropathic pain in rats. *Neuroreport, 12*, 3251–3255.

Tao, F., Tao, Y. X., Mao, P., & Johns, R. A. (2003). Role of postsynaptic density protein-95 in the maintenance of peripheral nerve injury-induced neuropathic pain in rats. *Neuroscience, 117*, 731–739.

Tao, F., Su, Q., & Johns, R. A. (2008). Cell-permeable peptide Tat-PSD-95 PDZ2 inhibits chronic inflammatory pain behaviors in mice. *Molecular Therapy, 16*, 1776–1782.

Terasaki, Y., Sasaki, T., Yagita, Y., Okazaki, S., Sugiyama, Y., Oyama, N., Omura-Matsuoka, E., Sakoda, S., & Kitagawa, K. (2010). Activation of NR2A receptors induces ischemic tolerance through CREB signaling. *Journal of Cerebral Blood Flow and Metabolism, 30*, 1441–1449.

Tezuka, T., Umemori, H., Akiyama, T., Nakanishi, S., & Yamamoto, T. (1999). PSD-95 promotes Fyn-mediated tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor subunit NR2A. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 435–440.

Thomas, S. J., & Grossberg, G. T. (2009). Memantine: A review of studies into its safety and efficacy in treating Alzheimer's disease and other dementias. *Clinical Interventions in Aging, 4*, 367–377.

Thomas, C. G., Miller, A. J., & Westbrook, G. L. (2006). Synaptic and extrasynaptic NMDA receptor NR2 subunits in cultured hippocampal neurons. *Journal of Neurophysiology, 95*, 1727–1734.

Tovar, K. R., & Westbrook, G. L. (1999). The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *The Journal of Neuroscience, 19*, 4180–4188.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., & Dingledine, R. (2010). Glutamate receptor ion channels: Structure, regulation, and function. *Pharmacological Reviews, 62*, 405–496.

Tu, W., Xu, X., Peng, L., Zhong, X., Zhang, W., Soundarapandian, M. M., Balel, C., Wang, M., Jia, N., Zhang, W., Lew, F., Chan, S. L., Chen, Y., & Lu, Y. (2010). DAPK1 interaction with NMDA receptor NR2B subunits mediates brain damage in stroke. *Cell, 140*, 222–234.

Tymianski, M. (2011). Emerging mechanisms of disrupted cellular signaling in brain ischemia. *Nature Neuroscience, 14*, 1369–1373.

Tymianski, M., Charlton, M. P., Carlen, P. L., & Tator, C. H. (1993). Source specificity of early calcium neurotoxicity in cultured embryonic spinal neurons. *The Journal of Neuroscience, 13*, 2085–2104.

Vanhoutte, P., & Bading, H. (2003). Opposing roles of synaptic and extrasynaptic NMDA receptors in neuronal calcium signaling and BDNF gene regulation. *Current Opinion in Neurobiology, 13*, 366–371.

Verpelli, C., Schmeisser, M. J., Sala, C., & Boeckers, T. M. (2012). Scaffold proteins at the postsynaptic density. *Advances in Experimental Medicine and Biology, 970*, 29–61.

Volbracht, C., van Beek, J., Zhu, C., Blomgren, K., & Leist, M. (2006). Neuroprotective properties of memantine in different in vitro and in vivo models of excitotoxicity. *The European Journal of Neuroscience, 23*, 2611–2622.

Wang, Y. T., & Salter, M. W. (1994). Regulation of NMDA receptors by tyrosine kinases and phosphatases. *Nature, 369*, 233–235.

Wang, C. X., & Shuaib, A. (2005). NMDA/NR2B selective antagonists in the treatment of ischemic brain injury. *Current Drug Targets. CNS and Neurological Disorders, 4*, 143–151.

Wilhelmus, M. M., Nijland, P. G., Drukarch, B., de Vries, H. E., & van Horssen, J. (2012). Involvement and interplay of Parkin, PINK1, and DJ1 in neurodegeneration and neuroinflammatory disorders. *Free Radical Biology & Medicine, 53*, 983–992.

Wood, P. L., & Hawkinson, J. E. (1997). *N*-methyl-D-aspartate antagonists for stroke and head trauma. *Expert Opinion on Investigational Drugs, 6*, 389–397.

Xia, P., Chen, H. S., Zhang, D., & Lipton, S. A. (2010). Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. *The Journal of Neuroscience, 30*, 11246–11250.

Xie, Y. F., MacDonald, J. F., & Jackson, M. F. (2010). TRPM2, calcium and neurodegenerative diseases. *International Journal of Physiology, Pathophysiology and Pharmacology, 2*, 95–103.

Xu, J., Kurup, P., Zhang, Y., Goebel-Goody, S. M., Wu, P. H., Hawasli, A. H., Baum, M. L., Bibb, J. A., & Lombroso, P. J. (2009). Extrasynaptic NMDA receptors couple preferentially

to excitotoxicity via calpain-mediated cleavage of STEP. *The Journal of Neuroscience, 29*, 9330–9343.

Zhao, Y., Navia, B. A., Marra, C. M., Singer, E. J., Chang, L., Berger, J., Ellis, R. J., Kolson, D. L., Simpson, D., Miller, E. N., Lipton, S. A., Evans, S. R., Schifitto, G., & The Adult AIDS Clinical Trial Group (ACTG) 301 Team. (2010). Memantine for AIDS dementia complex: Open-label report of ACTG 301. *HIV Clinical Trials, 11*, 59–67.

Zheng, F., Gingrich, M. B., Treynelis, S. F., & Conn, P. J. (1998). Tyrosine kinase potentiates NMDA receptor currents by reducing tonic zinc inhibition. *Nature Neuroscience, 1*, 185–191.

Zheng, C. Y., Seabold, G. K., Horak, M., & Petralia, R. S. (2011). MAGUKs, synaptic development, and synaptic plasticity. *The Neuroscientist, 17*, 493–512.

Zhou, M., & Baudry, M. (2006). Developmental changes in NMDA neurotoxicity reflect developmental changes in subunit composition of NMDA receptors. *The Journal of Neuroscience, 26*, 2956–2963.

Zhou, L., Li, F., Xu, H. B., Luo, C. X., Wu, H. Y., Zhu, M. M., Lu, W., Ji, X., Zhou, Q. G., & Zhu, D. Y. (2010). Treatment of cerebral ischemia by disrupting ischemia-induced interaction of nNOS with PSD-95. *Nature Medicine, 16*, 1439–1443.

Ziff, E. B. (1997). Enlightening the postsynaptic density. *Neuron, 19*, 1163–1174.

Zou, J., & Crews, F. (2006). CREB and NF-kappaB transcription factors regulate sensitivity to excitotoxic and oxidative stress induced neuronal cell death. *Cellular and Molecular Neurobiology, 26*, 385–405.

Zukin, R. S., & Bennett, M. V. (1995). Alternatively spliced isoforms of the NMDARI receptor subunit. *Trends in Neurosciences, 18*, 306–313.

Neuroprotection by Kynurenine Metabolites

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Contents

Abstract

The overexcitation of excitatory amino acid receptors generates excitotoxicity, which plays an important role in the pathophysiology of various neurodegenerative disorders such as Parkinson's and Alzheimer's diseases, stroke, and epilepsy. The prevention of excitotoxic neuronal damage is therefore a major

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objective in neuroprotective strategies. The kynurenine pathway, the main route of the tryptophan metabolism, produces both neuroprotective and neurotoxic metabolites. Kynurenic acid is a broad-spectrum endogenous antagonist of ionotropic excitatory amino acid receptors, and hence it can prevent excitotoxic neuronal damage. The kynurenine metabolites are additionally involved in the regulation of glutamatergic and cholinergic neurotransmission. The pathogeneses of several neurodegenerative disorders have been shown to involve multiple imbalances in the kynurenine metabolism. Kynurenine pathway metabolites may provide important targets for future neuroprotective therapeutic strategies.

Keywords

Glutamate excitotoxicity • Kynurenine pathway • Neurodegeneration • Neuroprotection

List of Abbreviations

3-HANA	3-hydroxyanthranilic acid
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANA	anthranilic acid
3-HK	3-hydroxykynurenine
EAA	excitatory amino acid
IDO	indoleamine 2,3-dioxygenase
KAT	kynurenine aminotransferase
KMO	kynurenine 3-monooxygenase
KYNA	kynurenic acid
L-KYN	L-kynurenine
NAD	nicotinamide adenine dinucleotide
NMDA	*N*-methyl-D-aspartate
QUIN	quinolinic acid
TDO	tryptophan 2,3-dioxygenase

1 Introduction

The term excitotoxicity refers to pathological processes in which excitatory amino acid (EAA) receptors are overactivated, thereby initiating further pathological processes and ending with the death of neurones. This mechanism is involved in the pathophysiology of diverse neurological diseases such as stroke or epilepsy, traumatic brain injury, and various neurodegenerative disorders including Parkinson's disease and Huntington's disease. One of the most important substances participating in these processes is glutamate, which is also the most important excitatory neurotransmitter in the mammalian brain. The results of one of the first studies implicating the toxic effect of glutamate were published in 1957, when Lucas and Newhouse observed that the subcutaneous injection of monosodium glutamate destroyed the neurones in the inner layers of the retina in newborn mice (Lucas and Newhouse 1957). It was later

established that this phenomenon occurs not only in the retina but in several brain regions too: it is restricted to postsynaptic neurones, and glutamate agonists may induce the same effect (Olney 1969). These findings led to emergence of the term excitotoxicity. Glutamate acts on different types of ionotropic and G protein-coupled (metabotropic) receptors. These receptors play essential roles in the normal functioning of the brain, but their excessive stimulation may trigger an extensive influx of calcium (Ca^{2+}) to the neurones, setting into motion various pathological processes, which lead to damage to the cell structures and terminate in cell death (Arundine and Tymianski 2003; Krieglstein 1997). Excitotoxicity is known to be involved in the pathomechanism of a number of neurological diseases, and the search for neuroprotective agents which may prevent neuronal damage has long been an important aim in neuroscience. The kynurenine pathway, the main route in the tryptophan catabolism, involves both an EAA receptor antagonist and an agonist (Beadle et al. 1947), and kynurenines are involved in various physiological and pathophysiological processes, therefore standing at the focus of research interest (Schwarcz 2004).

2 Glutamatergic Excitotoxicity

Ionotropic receptors activated by glutamate can be divided into three major types identified by their preferred agonists: *N*-methyl-D-aspartate (NMDA), kainate, and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), each of them with several subtypes. All three families of ionotropic glutamate receptors allow the permeation of sodium (Na^+) and potassium (K^+), whereas the NMDA-type and some AMPA-type channels also display Ca^{2+} permeability. Metabotropic receptors activated by glutamate exert their effects through the activation of second messenger systems also leading to a cytosolic Ca^{2+} overload. The different receptor families contribute to the excitotoxic processes to different extents, and there is evidence that the NMDA receptors play a pivotal role. AMPA receptors have a heterotetrameric structure built up from four homologous subunits (GluR1-4) (Hollmann and Heinemann 1994). All of the subunits consist of a large extracellular N-terminal domain followed by four transmembrane domains ending in an intracellular C-terminal segment. The second transmembrane domain is responsible for the formation of the cation-permeable channel (Ozawa et al. 1998; Madden 2002). The glutamate-binding domain is formed by a part of the N-terminal domain together with the third membrane domain (Hollmann et al. 1994). The C-terminal domain is responsible for the interaction with the intracellular proteins and contains phosphorylation sites too (Carvalho et al. 2000). The subunits may exist in two distinct forms, flip and flop, which are formed through alternative splicing (Sommer et al. 1990). The activation of AMPA and kainate receptors allows the entry of Na^+ to the neurones, triggering rapid excitatory neurotransmission in the brain. Additionally in the hippocampus, cortex, and retina, various neurones express AMPA receptors which are also permeable to Ca^{2+}. Under physiological conditions, the number of Ca^{2+}-permeable AMPA receptors present in the hippocampal pyramidal neurones (HPNs) is relatively low, but in consequence of ischaemia, this number undergoes a significant increase (Pellegrini-Giampietro et al. 1997).

NMDA receptors are heteromeric complexes consisting of subunits of three major types: the NR1, NR2, and NR3 families. In mammalian tissues, functional NMDA receptors are expressed only when they contain at least one NR1 and one NR2 subunit (Moriyoshi et al. 1991; Ciabarra et al. 1995). NMDA receptors are slow-gating ion channels that are highly permeable to Ca^{2+}. The C-terminal domains of NMDA receptor subunits have an important role in NMDA receptor interactions with intracellular synaptic and cytoskeletal proteins, and these protein–protein interactions initiate various downstream signalling pathways (reviewed by Arundine and Tymianski 2004). It has been demonstrated experimentally that glutamate excitotoxicity has two components: immediate cell damage which is Na^+- and Cl^--dependent and a delayed neuronal degeneration process which can be related to the presence of Ca^{2+}. Na^+ influx causes neuronal damage even in the absence of Ca^{2+}, through the generation of toxic swelling in hippocampal and retinal cells (Rothman 1985; Olney et al. 1986). The neuronal swelling can be avoided by the removal of extracellular Na^+, but neurones exposed to glutamate will still develop neurodegenerative processes unless the extracellular Ca^{2+} is also removed (Berdichevsky et al. 1983; Choi et al. 1987). Other possible ways for Ca^{2+} to enter the cells are voltage-dependent Ca^{2+} channels and Na^+/Ca^{2+} exchangers. Release from internal pools may contribute to toxic Ca^{2+} concentrations. This can occur through mitochondrial damage or dysfunction. An increased intracellular Ca^{2+} level initiates pathological processes such as proteolysis, protein phosphorylation, lipolysis, the activation of nitric oxide synthases, and the generation of free radicals which may destroy neurones (Szydlowska and Tymianski 2010). The activation of proteases, lipases, and endonucleases results in damage to cell structures, degradation of the cytoskeleton and DNA and a mitochondrial dysfunction, and finally leads to cell death.

If neurones suffer an energy impairment, a partial membrane depolarization occurs, which leads to the removal of Mg^{2+} blocking the NMDA receptors, allowing glutamate to exert its downstream effects under physiological concentrations, contributing to neurodegenerative processes (Novelli et al. 1988).

As excitotoxic mechanisms are involved in pathological neurone loss, EAA receptor antagonism has long been at the focus of interest for neuroscientists searching for neuroprotective agents. The early experiments with NMDA antagonists revealed that they are able to prevent the neurotoxic effects of NMDA (Olney et al. 1979) and ibotenate (Schwarcz et al. 1982). In animal models, NMDA antagonism proved to have anticonvulsive properties (Croucher et al. 1982) and to be able to prevent neuronal damage in cerebral ischaemia (Simon et al. 1984). Subsequent to these convincing results, numerous experiments have demonstrated the neuroprotective efficacy of NMDA antagonism.

3 The Kynurenine Pathway and Its Metabolites

3.1 The Kynurenine Pathway

L-Tryptophan, one of the ten essential amino acids, serves as a precursor of several biologically active molecules. Besides its involvement in protein synthesis, it can be

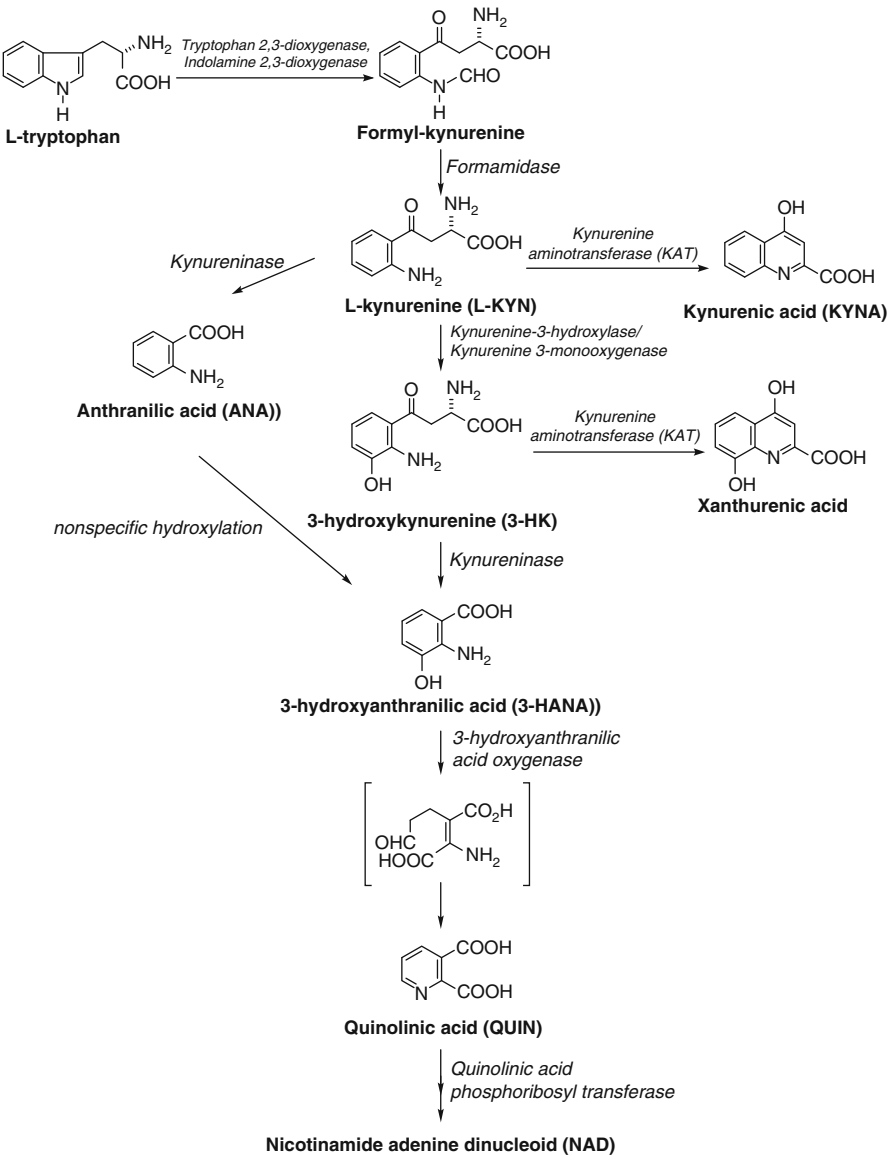

Fig. 1 The kynurenine pathway

metabolized in different pathways. The serotonin pathway is responsible for the synthesis of the important neurotransmitter serotonin, which can be converted to melatonin. In the human brain, as in most mammalian tissues, 95 % of tryptophan is catabolized through the kynurenine pathway (reviewed by Vamos et al. 2009) (Fig. 1). This metabolic cascade is responsible for the synthesis of nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP), coenzymes which are essential for many

biological processes, such as the control of the energy metabolism of cells, DNA repair, and transcription. The other metabolites of this pathway, collectively termed "kynurenines," have been shown to play important roles in the nervous and immune systems and to be involved in the pathophysiology of a growing number of neurological diseases (Ogawa et al. 1992; Stoy et al. 2005; Hartai et al. 2005; Robotka et al. 2008b; Guidetti et al. 2006).

The first step in this cascade is the enzymatic degradation of tryptophan by indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) to formylkynurenine, which is converted by arylformamidase to L-kynurenine (L-KYN). The pathway then divides into three main branches. The first branch ends with the synthesis of kynurenic acid (KYNA) through the transamination of L-KYN, a metabolic step catalyzed by the kynurenine aminotransferases (KATs) (Rossi et al. 2008). It was first believed that there are two main KAT isoforms in the brain (Okuno et al. 1991). The KATs are mainly localized in the astrocytes (Du et al. 1992), but they are also present in neurones in the hippocampus, in the striatum (Knyihar-Csillik et al. 1999), and in most of the neurones in the medulla and spinal cord (Kapoor et al. 1997). Early studies indicated that under physiological pH conditions, KAT-II has higher substrate specificity for KYN than KAT-I and that in most brain regions, KYNA can be attributed primarily to KAT-II activity (Okuno et al. 1991; Guidetti et al. 1997; Kiss et al. 2003). The results of recent experiments relating to the pH profile of human KAT-I indicated that under physiological pH conditions, it is able to catalyze L-KYN to KYNA (Han et al. 2004); hence, it may also play an important role in kynurenine metabolism. Recently, another member of the KAT family was discovered, the KAT-III isoform, which exhibits a number of similarities to KAT-I but appears to have no activity in the human brain (Yu et al. 2006). Whereas the main enzyme of KYNA synthesis in the human and the rat brain is KAT-II, recent studies revealed that the most important enzyme in mice is KAT-IV, which has proved to be identical to mitochondrial aspartate aminotransferase (mtAAT) (Guidetti et al. 2007; Han et al. 2010). The four KAT isoforms have different pH optima and temperature preferences (Han et al. 2010).

L-KYN can also serve as a substrate for kynureninase, giving rise to anthranilic acid (ANA). The third main branch of the kynurenine pathway comprises a sequence of enzymatic steps which ends in the synthesis of NAD. This cascade is responsible for the formation of free radical generators 3-hydroxykynurenine (3-HK) and 3-hydroxyanthranilic acid (3-HANA) and the neurotoxic compound quinolinic acid (QUIN). The first of these steps is to yield 3-HK that involves the action of kynurenine 3-monooxygenase (KMO; kynurenine-3-hydroxylase), which is then degraded by kynureninase into 3-HANA. This metabolite, which may also be generated from ANA, is then converted by 3-HANA-oxygenase to 2-amino-3-carboxymuconate-semialdehyde, an unstable intermediate which is transformed nonenzymatically into QUIN. Finally, QUIN is transformed in consecutive steps involving the enzymatic action of phosphoribosyltransferase to furnish nicotinamide and NAD.

The enzymatic machinery for this pathway is present in both the brain and the periphery, but the metabolic activity differs under physiological and

pathophysiological conditions. Under physiological conditions, this pathway has a much higher capacity at the periphery, while during inflammatory processes localized to the central nervous system (CNS), more than 98 % of the L-KYN and QUIN result from local synthesis in the brain (Kita et al. 2002). KYNA and QUIN are present in nanomolar concentrations in the human cerebrospinal fluid (CSF) (Moroni 1999; Schwarcz and Pellicciari 2002; Erhardt et al. 2004), while the concentration of KYNA in the human brain tissue is assumed to be 0.2–1.5 μM (Moroni et al. 1988; Turski et al. 1988). The neuroactive metabolites KYNA and QUIN cross the blood–brain barrier to only a limited extent, whereas their precursor L-KYN is able to cross it well (Fukui et al. 1991).

Within the CNS, the enzymes are differently distributed between the different subtypes of glial cells. Many studies have indicated that the astrocytes harbor hardly any KMO and therefore contribute more significantly to the synthesis of KYNA. In contrast, the microglial cells contain little KAT and hence are primarily responsible for the formation of QUIN (Guillemin et al. 2001; Lehrmann et al. 2001; Kiss et al. 2003).

3.2 Quinolinic Acid and Other Kynurenines with Neurotoxic Properties

QUIN is a weak competitive agonist of the NMDA receptors containing NR2A and NR2B subunits (Stone and Perkins 1981; de Carvalho et al. 1996). QUIN has been reported to be involved in the pathogenesis of several neurodegenerative disorders (Schwarcz et al. 1984; Klivenyi et al. 2004). Early studies yielded evidence that the administration of QUIN leads to a neurotoxic effect and may result in neuronal death in cortical cell cultures (Kim and Choi 1987). In vivo it has been observed that QUIN produces axon-sparing lesions in the rat brain (Schwarcz et al. 1983), which are similar to those seen in Huntington's disease (Beal et al. 1986). Intrastriatal QUIN injection is therefore a widely used experimental animal model for the study of Huntington's disease. It has proved to be neurotoxic in a number of studies conducted in recent years, but its neurotoxic effect involves several processes besides NMDA receptor agonism. It has become clear from the results that QUIN causes endogenous antioxidant depletion, contributes to the generation of free radicals (Behan et al. 1999; Rodriguez-Martinez et al. 2000), and also induces lipid peroxidation (Rios and Santamaria 1991). Moreover, QUIN has been confirmed to influence the glutamate metabolism, as it can enhance presynaptic glutamate release and inhibit glutamate uptake by astrocytes (Connick and Stone 1988; Tavares et al. 2002). Plasma levels of QUIN are elevated in Parkinson's disease patients (Ravikumar et al. 2000). Human studies revealed that QUIN levels are also increased in early stages of Huntington's disease (Guidetti et al. 2004).

3-HK and 3-HANA are potent neurotoxic agents experimentally demonstrated to be able to induce apoptosis in cultured cells (Morita et al. 1999). 3-HK and 3-HANA also contribute to the production of free radicals and elevation of the oxidative stress level. Under physiological conditions, the concentration of 3-HK is

within the nanomolar range, but there is evidence that under certain pathological conditions, it can reach micromolar levels (Okuda et al. 1998; Pearson and Reynolds 1991). The level of 3-HANA, a metabolite produced from 3-HK or ANA, is elevated in Huntington's disease and Parkinson's disease (Ogawa et al. 1992; Pearson and Reynolds 1992).

3.3 The Endogenous Neuroprotectant Kynurenic Acid

KYNA, one of the neuroactive metabolites of the kynurenine pathway, displays broad-spectrum antagonistic properties of ionotropic EAA receptors (Stone and Connick 1985). KYNA is an endogenous glutamate receptor antagonist of AMPA, NMDA, and kainate types, with the highest affinity for NMDA receptors (Perkins and Stone 1985; Swartz et al. 1990). At low micromolar concentrations (EC50 = 7.9–15 μM), it has a high affinity for the strychnine-insensitive glycine-binding site of the NMDA receptors and blocks it in a competitive way, while at 10–20 times higher concentrations, it exhibits an antagonistic effect of the glutamate-binding site too (EC50 = 200–500 μM) (Birch et al. 1988; Kessler et al. 1989). High concentrations of KYNA exert an anticonvulsant effect and protect the neurones from excitotoxic injury.

In vitro studies have revealed that KYNA may be capable of the direct activation of the G protein-coupled receptor GPR35 (Wang et al. 2006; Ohshiro et al. 2008). KYNA was earlier recognized to display antagonistic properties at the AMPA receptors, and it has only recently become clear that, depending on its concentration, it may also have another mechanism of action. In micromolar concentrations, KYNA inhibits AMPA receptors, whereas in nanomolar concentrations, it acts as a facilitator, binding to a positive modulatory-binding site at the AMPA receptor (Prescott et al. 2006; Rozsa et al. 2008). This dose-dependent dual effect indicates that KYNA may play an important role in the regulation of neurotransmission. KYNA has proved to exert an antagonistic effect on the presynaptic α7-nicotinic acetylcholine (α7-nACh) receptor too, inhibiting its activity in a noncompetitive manner, while increasing the expression of non-α7-nACh receptors (Hilmas et al. 2001). This effect may contribute to the generation of the sensory deficit caused by KYNA (Shepard et al. 2003; Chess and Bucci 2006), and the evidence suggests that it is mediated by its binding to sites located on the N-terminal domain of the α7-nACh receptor subunit (Pereira et al. 2002). Recent studies have revealed that KYNA in low concentrations (30–100 nM) inhibits glutamate release at the presynaptic nACh receptors, thereby contributing to a neuroprotective effect (Carpenedo et al. 2001; reviewed by Zadori et al. 2011). These results support the assumption that the kynurenine pathway is involved in the regulation of glutamatergic and cholinergic neurotransmission in the brain.

An increased level of KYNA was earlier proved to have sedative and anticonvulsive effects (Carpenedo et al. 1994). It was later demonstrated that it is able to reduce neuronal damage in brain ischaemia (Cozzi et al. 1999). There is a growing body of evidence that alterations in the kynurenine metabolism play important roles in the pathomechanisms of neurodegenerative disorders. Human postmortem studies have revealed elevated 3-HK levels in the substantia nigra and the putamen of

Parkinson's disease patients, while the ratio L-KYN/3-HK is decreased (Ogawa et al. 1992). In Huntington's disease patients, the KYNA levels are significantly decreased in the striatum, cortex, and CSF (Beal et al. 1990, 1992). KYNA levels are elevated in Alzheimer's disease (Baran et al. 1999) and in schizophrenia (Schwarcz et al. 2001; Nilsson et al. 2005), playing a part in the cognitive decline. A number of experiments have demonstrated an elevated KYNA level or L-KYN exerts a neuroprotective effect in brain ischaemia (Nozaki and Beal 1992; Gigler et al. 2007; Robotka et al. 2008a; Sas et al. 2008).

4 Conclusion

Excitotoxicity is a major factor in the pathomechanism of several neurological disorders. The overstimulation of EAA receptors results in an excessive influx of Ca^{2+} into the cells, subsequently initiating downstream pathological processes, resulting in damage to the cell structure, mitochondrial dysfunction, and an energy impairment leading to apoptosis. The kynurenine pathway is the main route of the metabolism of tryptophan in the mammalian brain. This pathway generates various neuroactive compounds which play an important role in the regulation of neurotransmission. There is accumulating evidence that imbalances in the kynurenine metabolism are involved in the pathomechanism of several neurodegenerative disorders, thereby providing a valuable target for future therapeutic strategies.

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References

Arundine, M., & Tymianski, M. (2003). Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. *Cell Calcium, 34*, 325–337.

Arundine, M., & Tymianski, M. (2004). Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. *Cellular and Molecular Life Sciences, 61*, 657–658.

Baran, H., Jellinger, K., & Deecke, L. (1999). Kynurenine metabolism in Alzheimer's disease. *Journal of Neural Transmission, 106*, 165–181.

Beadle, G. W., Mitchell, H. K., & Nyc, J. F. (1947). Kynurenine as an intermediate in the formation of nicotinic acid from tryptophane by neurospora. *Proceedings of the National Academy of Sciences United States of America, 33*, 155–158.

Beal, M. F., Kowall, N. W., Ellison, D. W., Mazurek, M. F., Swartz, K. J., & Martin, J. B. (1986). Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature, 321*, 168–171.

Beal, M. F., Matson, W. R., Swartz, K. J., Gamache, P. H., & Bird, E. D. (1990). Kynurenine pathway measurements in Huntington's disease striatum: Evidence for reduced formation of kynurenic acid. *Journal of Neurochemistry, 55*(4), 1327–1339.

Beal, M. F., Matson, W. R., Storey, E., Milbury, P., Ryan, E. A., Ogawa, T., & Bird, E. D. (1992). Kynurenic acid concentrations are reduced in Huntington's disease cerebral cortex. *Journal of the Neurological Sciences, 108*(1), 80–87.

Behan, W. M., McDonald, M., Darlington, L. G., & Stone, T. W. (1999). Oxidative stress as a mechanism for quinolinic acid-induced hippocampal damage: Protection by melatonin and deprenyl. *British Journal of Pharmacology, 128*(8), 1754–1760.

Berdichevsky, E., Riveros, N., Sanches-Armass, S., & Orrego, F. (1983). Kainate, N-methylaspartate and other excitatory amino acids increase calcium influx into rat brain cortex cells in vitro. *Neuroscience Letter, 36*, 75–80.

Birch, P. J., Grossman, C. J., & Hayes, A. G. (1988). Kynurenic acid antagonises responses to NMDA via an action at the strychnine-insensitive glycine receptor. *European Journal of Pharmacology, 154*, 85–87.

Carpenedo, R., Chiarugi, A., Russi, P., et al. (1994). Inhibitors of kynurenine hydroxylase and kynureninase increase cerebral formation of kynurenate and have sedative and anticonvulsant activities. *Neuroscience, 61*, 237–243.

Carpenedo, R., Pittaluga, A., Cozzi, A., Attucci, S., Galli, A., Raiteri, M., & Moroni, F. (2001). Presynaptic kynurenate-sensitive receptors inhibit glutamate release. *European Journal of Neuroscience, 13*(11), 2141–2147.

Carvalho, A. L., Duarte, C. B., & Carvalho, A. P. (2000). Regulation of AMPA receptors by phosphorylation. *Neurochemical Research, 25*, 1245–1255.

Chess, A. C., & Bucci, D. J. (2006). Increased concentration of cerebral kynurenic acid alters stimulus processing and conditioned responding. *Behavioural Brain Research, 170*, 326–332.

Choi, D. W., Maulucci-Gedde, M., & Kriegstein, A. R. (1987). Glutamate neurotoxicity in cortical cell culture. *The Journal of Neuroscience, 7*, 357–368.

Ciabarra, A. M., Sullivan, J. M., Gahn, L. G., Pecht, G., Heinemann, S., & Sevarino, K. A. (1995). Cloning and characterization of chi-1: A developmentally regulated member of a novel class of the ionotropic glutamate receptor family. *The Journal of Neuroscience, 15*, 6498–6508.

Connick, J. H., & Stone, T. W. (1988). Quinolinic acid effects on amino acid release from the rat cerebral cortex in vitro and in vivo. *British Journal of Pharmacology, 93*(4), 868–876.

Cozzi, A., Carpenedo, R., & Moroni, F. (1999). Kynurenine hydroxylase inhibitors reduce ischemic brain damage: Studies with (m-nitrobenzoyl)-alanine (mNBA) and 3, 4-dimethoxy-[-N-4-(nitrophenyl) thiazol-2yl]- benzenesulfonamide (Ro 61–8048) in models of focal or global brain ischemia. *Journal of Cerebral Blood Flow & Metabolism, 19*, 771–777.

Croucher, M. J., Collins, J. F., & Meldrum, B. S. (1982). Anticonvulsant action of excitatory amino acid antagonists. *Science, 216*, 899–901.

de Carvalho, L.P., Bochet, P., & Rossier, J. (1996). The endogenous agonist quinolinic acid and the non endogenous homoquinolinic acid discriminate between NMDAR2 receptor subunits. *Neurochemistry International, 28*(4), 445–52.

Du, F., Schmidt, W., Okuno, E., Kido, R., Köhler, C., & Schwarz, R. (1992). Localization of kynurenine aminotransferase immunoreactivity in the rat hippocampus. *The Journal of Comparative Neurology, 321*, 477–487.

Erhardt, S., Schwieler, L., Emanuelsson, C., & Geyer, M. (2004). Endogenous kynurenic acid disrupts prepulse inhibition. *Biological Psychiatry, 56*, 255–260.

Fukui, S., Schwarz, R., Rapoport, S. I., Takada, Y., & Smith, Q. R. (1991). Blood-brain barrier transport of kynurenines: Implications for brain synthesis and metabolism. *Journal of Neurochemistry, 56*, 2007–2017.

Gigler, G., Szénási, G., Simó, A., Lévay, G., Hársing, L. G., Jr., Sas, K., Vécsei, L., & Toldi, J. (2007). Neuroprotective effect of L-kynurenine sulfate administered before focal cerebral ischemia in mice and global cerebral ischemia in gerbils. *European Journal of Pharmacology, 564*, 116–122.

Guidetti, P., Okuno, E., & Schwarz, R. (1997). Characterization of rat brain kynurenine aminotransferases. *Journal of Neuroscience Research, 50*, 457–465.

Guidetti, P., Luthi-Carter, R. E., Augood, S. J., & Schwarcz, R. (2004). Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. *Neurobiology of Disease, 17*(3), 455–461.

Guidetti, P., Bates, G. P., Graham, R. K., Hayden, M. R., Leavitt, B. R., MacDonald, M. E., et al. (2006). Elevated brain 3-hydroxykynurenine and quinolinate levels in Huntington's disease mice. *Neurobiology of Disease, 23*, 190–197.

Guidetti, P., Amori, L., Sapko, M. T., Okuno, E., & Schwarcz, R. (2007). Mitochondrial aspartate aminotransferase: A third kynurenate-producing enzyme in the mammalian brain. *Journal of Neurochemistry, 102*(1), 103–111.

Guillemin, G. J., Kerr, S. J., Smythe, G. A., et al. (2001). Kynurenine pathway metabolism in human astrocytes: A paradox for neuronal protection. *Journal of Neurochemistry, 78*, 842–853.

Han, Q., Li, J., & Li, J. (2004). pH dependence, substrate specificity and inhibition of human kynurenine aminotransferase I. *European Journal of Biochemistry, 271*, 4804–4814.

Han, Q., Cai, T., Tagle, D. A., & Li, J. (2010). Structure, expression, and function of kynurenine aminotransferases in human and rodent brains. *Cellular and Molecular Life Sciences, 67*(3), 353–368.

Hartai, Z., Klivenyi, P., Janaky, T., Penke, B., Dux, L., & Vecsei, L. (2005). Kynurenine metabolism in plasma and in red blood cells in Parkinson's disease. *Journal of the Neurological Sciences, 239*, 31–35.

Hilmas, C., Pereira, E. F. R., Alkondon, M., Rassoulpour, A., Sczwarz, R., & Albuquerque, E. X. (2001). The brain metabolite kynurenic acid inhibits α7-nicotinic receptor activity and increases non-α7-nicotinic receptor expression: Physiological implications. *Journal of Neuroscience, 21*, 7463–7473.

Hollmann, M., & Heinemann, S. (1994). Cloned glutamate receptors. *Annual Review of Neuroscience, 17*, 31–108.

Hollmann, M., Maron, C., & Heinemann, S. (1994). N-glycosylation site tagging suggests a three transmembrane domain topology for the glutamate receptor GluR1. *Neuron, 13*, 1331–1343.

Kapoor, R., Okuno, E., Kido, R., & Kapoor, V. (1997). Immunolocalization of kynurenine aminotransferase (KAT) in the rat medulla and spinal cord. *Neuroreport, 8*, 3619–3623.

Kessler, M., Terramani, T., Lynch, G., & Boundary, M. (1989). A glycine site associated with N-methyl-D-aspartic acid receptors characterization and identification of a new class of antagonists. *Journal of Neurochemistry, 52*, 1319–1328.

Kim, J. P., & Choi, D. W. (1987). Quinolinate neurotoxicity in cortical cell culture. *Neuroscience, 23*, 423–432.

Kiss, C., Ceresoli-Borroni, C., Guidetti, P., Zielke, C. L., Zielke, H. R., & Schwarz, R. (2003). Kynurenate production by cultured human astrocytes. *Journal of Neural Transmission, 110*, 1–14.

Kita, T., Morrison, P. F., Heyes, M. P., & Markey, S. P. (2002). Effects of systemic and central nervous system localized inflammation on the contributions of metabolic precursors of the L-kynurenine and quinolinic acid pools in brain. *Journal of Neurochemistry, 82*, 258–268.

Klivenyi, P., Toldi, J., & Vecsei, L. (2004). Kynurenines in neurodegenerative disorders: Therapeutic consideration. *Advances in Experimental Medicine and Biology, 541*, 169–183.

Knyihar-Csillik, E., Okuno, E., & Vécsei, L. (1999). Effects of in vivo sodium azide administration on the immunohistochemical localization of kynurenine aminotransferase in the rat brain. *Neuroscience, 94*, 269–277.

Krieglstein, J. (1997). Excitotoxicity and neuroprotection. *European Journal of Pharmaceutical Sciences, 5*, 181–187.

Lehrmann, E., Molinari, A., Speciale, C., & Schwarz, R. (2001). Immunohistochemical visualization of newly formed quinolinate in the normal and excitotoxically lesioned rat striatum. *Experimental Brain Research, 141*, 389–397.

Lucas, D. R., & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *A.M.A. Archives of ophthalmology, 58*(2), 193–201.

Madden, D. R. (2002). The structure and function of glutamate receptor ion channels. *Nature Reviews Neuroscience, 3*, 91–101.

Morita, T., Saito, K., Takemura, M., Maekawa, N., Fujigaki, S., Fujii, H., et al. (1999). L-tryptophan kynurenine pathway metabolite 3-hydroxyanthranilic acid induces apoptosis in macrophage-derived cells under pathophysiological conditions. *Advances in Experimental Medicine and Biology, 467*, 559–563.

Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N., & Nakanishi, S. (1991). Molecular cloning and characterization of the rat NMDA receptor. *Nature, 354*, 31–37.

Moroni, F. (1999). Tryptophan metabolism and brain function: Focus on kynurenine and other indole metabolites. *European Journal of Pharmacology, 375*, 87–100.

Moroni, F., Russi, G., Lombardi, G., Beni, M., & Carla, V. (1988). Presence of kynurenic acid in the mammalian brain. *Journal of Neurochemistry, 51*, 177–180.

Nilsson, L. K., Linderholm, K. R., Engberg, G., Paulson, L., Blennow, K., Lindstro¨m, L. H., Nordin, C., Karanti, A., Persson, P., & Erhardt, S. (2005). Elevated levels of kynurenic acid in the cerebrospinal fluid of male patients with schizophrenia. *Schizophrenia research, 80*, 315–322.

Novelli, A., Reilly, J. A., Lysko, P. G., & Henneberry, R. C. (1988). Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Research, 451*(1–2), 205–212.

Nozaki, K., & Beal, M. F. (1992). Neuroprotective effects of L-kynurenine on hypoxia-ischemia and NMDA lesions in neonatal rats. *Journal of Cerebral Blood Flow and Metabolism, 12*, 400–407.

Ogawa, T., Matson, W. R., Beal, M. F., Myers, R. H., Bird, E. D., Milbury, P., & Saso, S. (1992). Kynurenine pathway abnormalities in Parkinson's disease. *Neurology, 42*(9), 1702–1706.

Ohshiro, H., Tonai-Kachi, H., & Ichikawa, K. (2008). GPR35 is a functional receptor in rat dorsal root ganglion neurons. *Biochemical and Biophysical Research Communications, 365*, 344–348.

Okuda, S., Nishiyama, N., Saito, H., & Katsuki, H. (1998). 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity. *Journal of Neurochemistry, 70*, 299–307.

Okuno, E., Nakamura, M., & Schwarz, R. (1991). Two kynurenine aminotransferases in human brain. *Brain Research, 542*, 307–312.

Olney, J. W. (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science, 164*(3880), 719–721.

Olney, J. W., de Gubareff, T., & Labruyere, J. (1979). Alpha-aminoadipate blocks the neurotoxic action of N-methyl aspartate. *Life Sciences, 25*, 537–540.

Olney, J. W., Price, M. T., Samson, L., & Labruyere, J. (1986). The role of specific ions in glutamate neurotoxicity. *Neuroscience Letters, 65*, 65–71.

Ozawa, S., Kamiya, H., & Tsuzuki, K. (1998). Glutamate receptors in the mammalian central nervous system. *Progress in Neurobiology, 54*, 581–618.

Pearson, S. J., & Reynolds, G. P. (1991). Determination of 3-hydroxykynurenine in human brain and plasma by high-performance liquid chromatography with electrochemical detection. Increased concentrations in hepatic encephalopathy. *Journal of Chromatography, 565*, 436–440.

Pearson, S. J., & Reynolds, G. P. (1992). Increased brain concentrations of a neurotoxin, 3-hydroxykynurenine, in Huntington's disease. *Neuroscience Letters, 144*, 199–201.

Pellegrini-Giampietro, D. E., Gorter, J. A., Bennett, M. V. L., & Zukin, R. S. (1997). The GluR2 (GluR-B) hypothesis: Ca^{2+}-permeable AMPA receptors in neurological disorders. *Trends in Neurosciences, 20*, 464–470.

Pereira, E. F., Hilmas, C., Santos, M. D., Alkondon, M., Maelicke, A., & Albuquerque, E. X. (2002). Unconventional ligands and modulators of nicotinic receptors. *Journal of Neurobiology, 53*, 479–500.

Perkins, M. N., & Stone, T. W. (1985). Actions of kynurenic acid and quinolinic acid in the rat hippocampus in vivo. *Experimental Neurology, 88*, 570–579.

Prescott, C., Weeks, A. M., Staley, K. J., & Parton, K. M. (2006). Kynurenic acid has a dual action on AMPA receptor responses. *Neuroscience Letters, 402*, 108–112.

Ravikumar, A., Deepadevi, K. V., Arun, P., Manojkumar, V., & Kurup, P. A. (2000). Tryptophan and tyrosine catabolic pattern in neuropsychiatric disorders. *Neurology India, 48*(3), 231–238.

Rios, C., & Santamaria, A. (1991). Quinolinic acid is a potent lipid peroxidant in rat brain homogenates. *Neurochemical Research, 16*(10), 1139–1143.

Robotka, H., Sas, K., Ágoston, M., Rózsa, É., Szénási, G., Gigler, G., Vécsei, L., & Toldi, J. (2008a). Neuroprotection achieved in the ischaemic rat cortex with L-kynurenine sulphate. *Life Sciences, 82*, 915–919.

Robotka, H., Toldi, J., & Vécsei, L. (2008b). L-kynurenine: Metabolism and mechanism of neuroprotection. *Future Neurology, 3*(2), 169–188.

Rodriguez-Martinez, E., Camacho, A., Maldonado, P. D., Pedraza-Chaverri, J., Santamaria, D., Galvan-Arzate, S., & Santamaria, A. (2000). Effect of quinolinic acid on endogenous antioxidants in rat corpus striatum. *Brain Research, 858*(2), 436–439.

Rossi, F., Schwarcz, R., & Rizzi, M. (2008). Curiosity to kill the KAT (kynurenine aminotransferase): Structural insights into brain kynurenic acid synthesis. *Current Opinion in Structural Biology, 18*, 748–755.

Rothman, S. M. (1985). The neurotoxicity of excitatory amino acids is produced by passive chloride influx. *The Journal of Neuroscience, 5*, 1483–1489.

Rozsa, E., Robotka, H., Vecsei, L., & Toldi, J. (2008). The Janus-face kynurenic acid. *Journal of Neural Transmission, 115*, 1087–1091.

Sas, K., Robotka, H., Rózsa, É., Ágoston, M., Szénási, G., Gigler, G., Marosi, M., Kiss, Z., Farkas, T., Vécsei, L., & Toldi, J. (2008). Kynurenine diminishes the ischemia-induced histological and electrophysiological deficits in the rat hippocampus. *Neurobiology of Disease, 32*, 302–308.

Schwarcz, R. (2004). The kynurenine pathway of tryptophan degradation as a drug target. *Current Opinion in Pharmacology, 4*, 12–17.

Schwarcz, R., & Pellicciari, R. (2002). Manipulation of brain kynurenines: Glial targets, neuronal effects, and clinical opportunities. *The Journal of Pharmacology and Experimental Therapeutics, 303*, 1–10.

Schwarcz, R., Collins, J. F., & Parks, D. A. (1982). Alpha-amino-omega-phosphono carboxylates block ibotenate but not kainate neurotoxicity in rat hippocampus. *Neuroscience Letters, 33*, 85–90.

Schwarcz, R., Whetsell, W. O., Jr., & Mangano, R. M. (1983). Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. *Science, 219*, 316–318.

Schwarcz, R., Foster, A. C., French, E. D., Whetsell, W. O., Jr., & Kohler, C. (1984). Excitotoxic models for neurodegenerative disorders. *Life Science, 35*, 19–32.

Schwarcz, R., Rassoulpour, A., Wu, H.-Q., Medoff, D., Tamminga, C. A., & Roberts, R. C. (2001). Increased cortical kynurenate content in schizophrenia. *Biological Psychiatry, 50*, 521–530.

Shepard, P. D., Joy, B., Clerkin, L., & Schwarcz, R. (2003). Micromolar brain levels of kynurenic acid are associated with a disruption of auditory sensory gating in the rat. *Neuropsychopharmacology, 28*, 1454–1462.

Simon, R. P., Swan, J. H., Griffiths, T., & Meldrum, B. S. (1984). Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science, 226*, 850–852.

Sommer, B., Keinanen, K., Verdoorn, T. A., Wisden, W., Burnashev, N., Herb, A., Kohler, M., Takagi, T., Sakmann, B., & Seeburg, P. H. (1990). Flip and flop: A cell-specific functional switch in glutamate-operated channels of the CNS. *Science, 249*, 1580–1585.

Stone, T. W., & Connick, J. H. (1985). Quinolinic acid and other kynurenines in the central nervous system. *Neuroscience, 15*, 597–617.

Stone, T. W., & Perkins, M. N. (1981). Quinolinic acid: A potent endogenous excitant at amino acid receptors in CNS. *European Journal of Pharmacology, 72*(4), 411–412.

Stoy, N., Mackay, G. M., Forrest, C. M., Christofides, J., Egerton, M., Stone, T. W., et al. (2005). Tryptophan metabolism and oxidative stress in patients with Huntington's disease. *Journal of Neurochemistry, 3*, 611–623.

Swartz, K. J., During, M. J., Freese, A., & Beal, M. F. (1990). Cerebral synthesis and release of kynurenic acid: An endogenous antagonist of excitatory amino acid receptors. *The Journal of Neuroscience, 10*, 2965–2973.

Szydlowska, K., & Tymianski, M. (2010). Calcium, ischemia and excitotoxicity. *Cell Calcium, 47*, 122–129.

Tavares, R. G., Tasca, C. I., Santos, C. E., Alves, L. B., Porciuncula, L. O., Emanuelli, T., & Souza, D. O. (2002). Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochemistry International, 40*(7), 621–627.

Turski, W. A., Nakamura, M., Todd, W. P., Carpenter, B. K., Whetsell, W. O., & Schwarz, R. (1988). Identification and quantification of kynurenic acid in human brain tissue. *Brain Research, 454*, 164–169.

Vamos, E., Pardutz, A., Klivenyi, P., Toldi, J., & Vecsei, L. (2009). The role of kynurenines in disorders of the central nervous system: Possibilities for neuroprotection. *Journal of the Neurological Sciences, 283*, 21–27.

Wang, J., Simonavicius, N., Wu, X., Swaminath, G., Reagan, J., Tian, H., & Ling, L. (2006). Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *The Journal of Biological Chemistry, 281*, 22021–22028.

Yu, P., Li, Z., Zhang, L., Tagle, D. A., & Cai, T. (2006). Characterization of kynurenine aminotransferase III, a novel member of a phylogenetically conserved KAT family. *Gene, 365*, 111–118.

Zadori, D., Klivenyi, P., Plangar, I., Toldi, J., & Vecsei, L. (2011). Endogenous neuroprotection in chronic neurodegenerative disorders: With particular regard to the kynurenines. *Journal of Cellular and Molecular Medicine, 15*(4), 701–717.

Neuroprotective Strategies in Amyotrophic Lateral Sclerosis: Modulation of Neurotransmittory and Neurotrophic Input to Motor Neurons

Pierre-François Pradat and Luc Dupuis

Contents

Abstract

Amyotrophic lateral sclerosis (ALS) is an adult-onset degenerative disease characterized by the selective progressive death of lower and upper motor neurons. Although its primary cause remains unknown, multiple pathogenic pathways have been identified. The major pathological event in ALS is the degeneration of lower motor neurons (LMNs), and it is thought to be the ultimate cause of death. Thus, neuroprotection of LMNs is assumed to be a reasonable target for

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treating ALS. This assumption guided most of research effort in the last 20 years to develop neuroprotective strategies able to preserve the remaining LMNs. Three major possibilities have been explored and are presented in this review. First, it is thought that LMN survival and degeneration are regulated by neurotransmittory inputs that LMN integrates. Several experimental and imaging studies have shown an impairment of multiple neuronal types innervating motor neurons, in particular glycinergic interneurons, glutamatergic input from proprioceptive type Ia fibers, and serotonergic neurons. The abnormalities in glutamatergic input proved especially fruitful since they led to the discovery of riluzole. A second potential neuroprotective strategy would be to modulate neurotrophic input to motor neurons. Indeed, studies of motor neuron development have shown that their survival is governed by neurotrophic input from its muscle target or from surrounding glia. Thus, modulating neurotrophic support to LMN might be a therapeutic strategy, although the multiple clinical trials based on this possibility were up to now unsuccessful. A last possibility to provide neuroprotection in ALS would be to indirectly modulate the motor neuron survival through action on events occurring outside this cell type, in particular inflammation or abnormal energy metabolism.

Keywords

ALS • GABA • Glutamate • Glycin • Interneuron • Motor neuron • MRI • Neurotrophic factor • PET • Serotonin

List of Abbreviations

^{1}H-MRS	Primary motor area, M1, proton magnetic resonance spectroscopy
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
ALS	Amyotrophic lateral sclerosis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	Brain-derived neurotrophic factor
CNTF	Ciliary-derived neurotrophic factor
CSF	Cerebrospinal fluid
DTI	Diffusion tensor imaging
EAAT2	Excitatory amino acid transporter type 2
Exc.	Excitatory
GABA	Gamma-aminobutyric acid
GDNF	Glial cell line-derived neurotrophic factor
Glu	Glutamate
Gly	Glycine
IGF-1	Insulin-like growth factor-1
IN	Interneuron
inh.	Inhibitory
LMN	Lower motor neuron
mRNA	Messenger ribonucleic acid

MT	Magnetization transfer
NMDA	*N*-methyl-D-aspartate
PET	Positron emission tomography
PM	Premotor
SICI	Short-interval intracortical inhibition
SMA	Supplementary motor area
SOD1	Superoxide dismutase type 1
UMN	Upper motor neuron
VEGF	Vascular endothelial growth factor
VGLUT2	Vesicular glutamate transporter type 2

1 Introduction

Amyotrophic lateral sclerosis (ALS) is the major adult-onset motor neuron disease with an incidence of 2–3/100,000. ALS typically affects lower motor neurons (LMNs), which degenerate, leading to a progressively lethal paralysis. ALS symptoms might start in limbs (spinal onset, 2/3 of cases) or in muscles controlling speech and/or swallowing (bulbar onset, 1/3 of cases). Apart from LMN involvement, ALS is associated with degeneration of upper motor neurons (UMNs), and most patients develop UMN signs, in particular hyperreflexia and spasticity. A number of patients also display focal or more widespread frontal lobe disease (Schreiber et al. 2005). Other non-motor symptoms include major defects in energy metabolism, typified by weight loss, muscle wasting, and hypermetabolism (Dupuis et al. 2011). Death of ALS patients typically occurs within 2–5 years after diagnosis, but a huge heterogeneity among patients exists, with survival after diagnosis ranging from few weeks to more than 30 years (Chio et al. 2009).

The major pathological event in ALS being degeneration of LMNs, it is thought to be the ultimate cause of death, thus neuroprotection of LMNs is assumed to be a reasonable target for treating ALS. This assumption indeed guided most of research effort in the last 20 years to develop neuroprotective strategies able to preserve the remaining LMNs. Three major possibilities have been explored and will be developed sequentially in this chapter (Fig. 1). First, it is thought that LMN survival and degeneration is regulated by neurotransmittory inputs that LMN integrates, in particular glutamatergic input. This possibility proved especially fruitful since it culminated in the discovery of riluzole. Second, studies of LMN development have shown that LMN survival is governed by neurotrophic input from its muscle target or from surrounding glia. Thus, modulating neurotrophic support to LMN might be a therapeutic strategy. Third, most recent results in ALS field suggested that neuroprotection of LMN might be indirectly achieved through modulation of LMN independent events such as inflammation or nutrition.

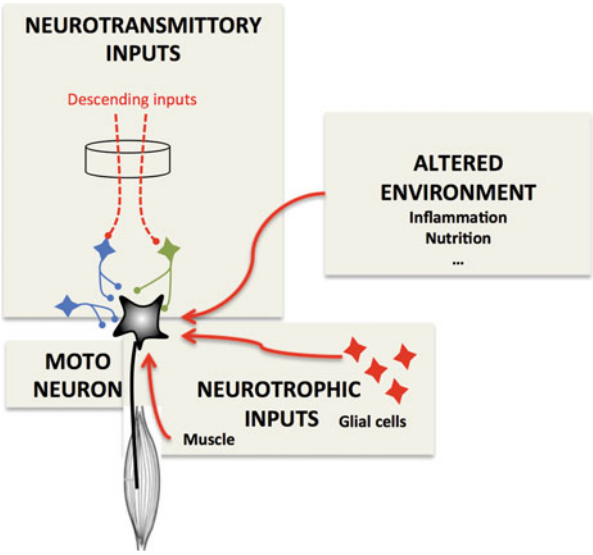

Fig. 1 Three ways to achieve neuroprotection in ALS. Lower motor neurons might be rescued by altering their neurotransmittory input, by improving their neurotrophic support, and/or by modifying their altered, potentially toxic environment

2 Neuroprotection Through Modulation of Neurotransmittory Inputs to Motor Neurons

In this first section, we will review how input to motor neurons might be modulated to achieve neuroprotection in ALS.

2.1 Major Inputs to Motor Neurons

Motor neuron activity is controlled by two types of inputs. First, activation of ionotropic receptors, either excitatory or inhibitory, will generate postsynaptic currents that summate at the soma. If the net resulting current is sufficient to elicit an action potential, then the motoneuron discharge, which triggers the muscle contraction. Second, neuromodulatory inputs, through metabotropic receptors, activate intracellular signalling pathways that modulate the responses to ionotropic receptors. Since electrical activity is critical in the survival of any neuron, it is likely that identifying the abnormalities of inputs to motor neurons and correct these through pharmacological means will provide therapeutic strategies.

Motor neurons receive inputs from the periphery (in particular proprioceptive neurons) and from central projections. A schematic drawing of inputs to LMNs is presented in Fig. 2. There are about 100,000 synaptic boutons per lumbar motor neurons in cat (Rekling et al. 2000). A majority of these boutons are inhibitory, either GABAergic or glycinergic. Such inhibitory connections come from Renshaw cells that mediate recurrent inhibition of motor neurons and from other spinal inhibitory interneurons, which integrate numerous descending and peripheral

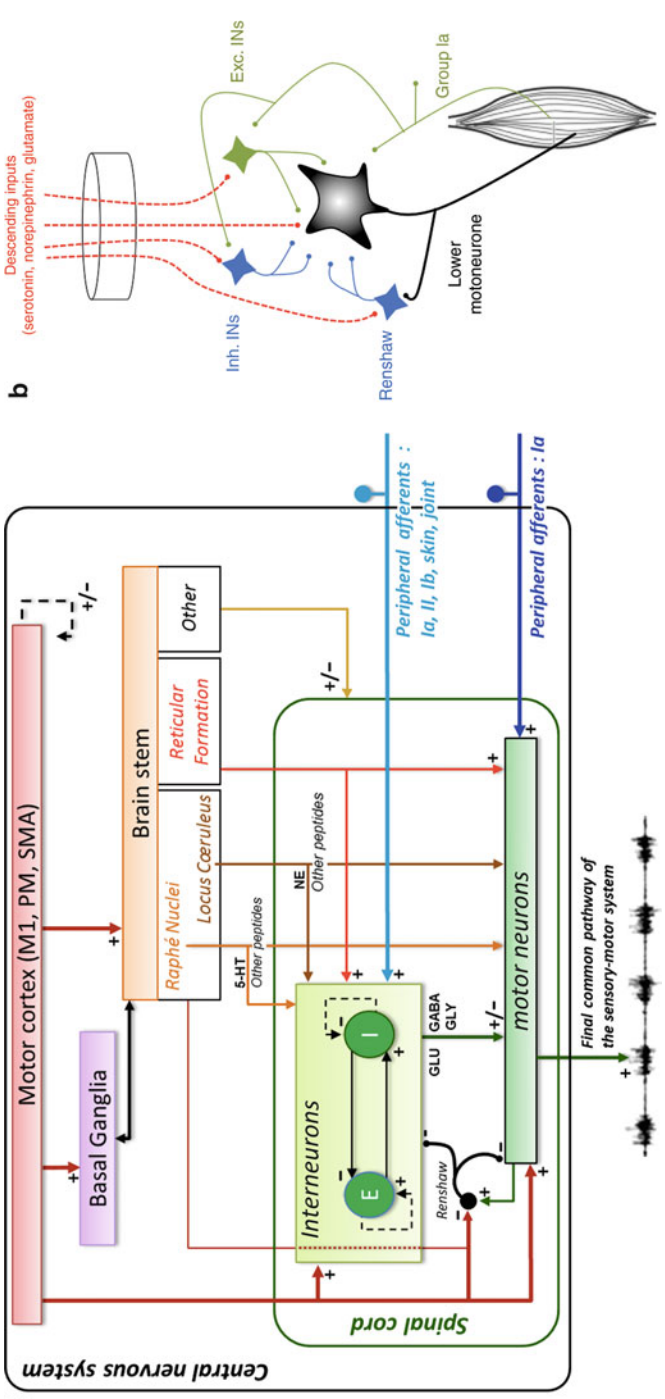

Fig. 2 Motor neurons receive afferences from the central and peripheral nervous system. These afferences project either directly to motor neurons (excitatory glutamatergic inputs) or indirectly through spinal interneurons. Inputs from interneurons are mainly inhibitory, either either GABAergic or glycinergic, or excitatory, glutamatergic. Inhibitory interneurons include the Renshaw cells, glycinergic, which are activated by upper motor neurons and the interneurons, either GABAergic or glycinergic, which are activated by peripheral sensory neurons from skin and muscle spindles. Type Ia fibers coming from muscle spindles are glutamatergic and directly innervate motor neurons.

inputs. Inhibitory projections from brainstem nuclei and nucleus tractus solitarius also innervate spinal motor neurons.

About one fifth of synaptic boutons are glutamatergic, and include projections from type Ia muscle spindles and rubrospinal neurons. In rodent, corticospinal neurons do not directly project to motor neurons, but to spinal cord interneurons, thus only indirectly modulating motor neuron activity. In contrast, corticospinal neurons directly innervate motor neurons in primates to control fine movements. Spinal motor neurons also receive extensive projections from monoaminergic neurons from the brainstem, mostly from noradrenergic and serotonergic neurons. Many peptides are co-localized with monoaminergic projections, suggesting that they might be co-released with either serotonin or norepinephrine.

2.2 Abnormalities of Inputs to Motor Neuron in ALS

A number of cell types innervating motor neurons have been shown to degenerate in ALS animal models and/or patients. In this section, we will present the abnormalities by neurotransmitter.

2.2.1 GABAergic Abnormalities

A number of GABAergic abnormalities have been documented in ALS.

Neuroimaging studies suggest that a loss of central nervous system inhibitory influence, in particular GABA, plays a role in ALS pathogenesis. Flumazenil binds to the benzodiazepine subunit of the GABA-A receptor present on neurons of the cerebral cortex. Using positron emission tomography (PET), a study showed a widespread loss of binding of [^{11}C]-flumazenil in sporadic ALS patients. The abnormalities were less extensive in patients with SOD1-linked familial ALS patients, suggesting that GABAergic neurotransmission may be less severely impaired in these cases (Lloyd et al. 2000). Recent advances in proton magnetic resonance spectroscopy (^{1}H-MRS) allow direct quantification of GABA in the cortex (Zhu et al. 2011). Using high resolution 3 Tesla edited ^{1}H-MRS, a study in a small number of subjects showed that decreased levels of GABA were present in the motor cortex of ALS patients compared to healthy controls (Foerster et al. 2012).

What could be the consequences of loss of cortical GABA? Studies with transcranial motor magnetic stimulation (TMS) suggest that the cortical hyperexcitability observed in ALS patients could be due to alterations in GABA-A signalling. In this technique, the intensity required to stimulate the motor cortex as well as the duration of the cortex silent period and the response to paired stimulation are measured, providing a good estimate of cortical excitability (Eisen and Weber 2001). The increased excitation observed in ALS patients may reflect a combination of an intrinsic hyperexcitability of the corticomotoneurons themselves and reduced inhibition due to dysfunction of cortical inhibitory neurons. Although there are some conflicting data (Attarian et al. 2009), a loss of inhibition is suggested by several studies using paired-pulse stimulation that showed a decreased short-interval intracortical inhibition (SICI)

attributed to GABA-A receptors (Zanette et al. 2002; Yokota et al. 1996; Vucic and Kiernan 2006; Desiato et al. 1999; Caramia et al. 2000).

The role of cortical hyperexcitability in ALS remains debated, in particular with respect to its timing. Some authors have suggested it is an early feature (Mills and Nithi 1997; Prout and Eisen 1994; Caramia et al. 2000) whether others argued that it was a late phenomenon (Zanette et al. 2002). At least in SOD1-linked familial ALS, results obtained using a threshold-tracking TMS technique suggested that reduction of SICI is an early phenomenon that may precede the development of clinical symptoms (Vucic et al. 2008). Indeed, reduction of SICI was observed in three asymptomatic SOD1-mutation carriers who subsequently developed typical clinical features of ALS. A question is whether changes in excitability have implications on clinical symptoms. A study using threshold-tracking TMS showed a correlation between the reduction of SICI and fatigue (Vucic et al. 2011). The authors concluded that maladaptation of cortical processes related to degeneration of inhibitory GABAergic intracortical circuits may have a role in the development of fatigue in ALS.

All the previous results were obtained in cortical regions. It would be of high interest to obtain PET imaging or MRS data on inhibitory activity at the level of the spinal cord in ALS patients. However, technical limitations render such data currently inaccessible.

The GABAergic system has been poorly studied in animal models of ALS. In the few studies existing, GABAergic projections to motor neurons appeared much less affected than glycine interneurons (Chang and Martin 2009; Petri et al. 2005). However, the response of motor neurons to GABA might be slightly abnormal (Carunchio et al. 2008).

2.2.2 Glycinergic Abnormalities

In patients, an impairment of Renshaw cells has been suggested by electrophysiological studies testing the mixed nerve silent period (Shefner and Logigian 1998) or using a conditioned H-reflex technique (Raynor and Shefner 1994). In contrast to spastic spinal cord-injured patients, spastic ALS patients showed strikingly reduced recurrent inhibition, suggesting that reduction of the recurrent inhibition suggested a different physiology for spasticity in ALS compared to spinal cord transection (Raynor and Shefner 1994). The role of Renshaw cells is however controversial in ALS since more distal muscles, more affected in ALS, lack recurrent inhibition (Mazzocchio and Rossi 2010).

The glycinergic system, and in particular the Renshaw cells, have been studied in more detail in experimental models of ALS. First, Renshaw cells lose connection with motor neurons in end-stage mice (Chang and Martin 2009; Wootz et al. 2012), and this might contribute to motor neuron hyperexcitability. Consistent with this, several authors described degeneration of glycinergic interneurons on spinal motor neurons of SOD1(G93A) mice and showed that glycinergic currents are decreased in SOD1(G93A) motor neurons (Chang and Martin 2009, 2011; Martin and Chang 2012). The pathology in glycinergic neurons appears however to follow the pathology of motor neurons, suggesting that it is either a very late event or a consequence of degeneration of motor neurons (Hossaini et al. 2011).

A recent study in worms however suggested that, at least in this model, glycinergic interneurons are early and strongly affected by the disease process (McGown et al. 2012). More research is needed to ascertain this point in vertebrates.

2.2.3 Glutamatergic Abnormalities

Glutamatergic abnormalities in ALS have been the subject of intense research in the last 30 years. Most research focused on abnormalities of glutamate release or reuptake by astrocytes as the source of increased glutamate in the cerebrospinal fluid (CSF). The contribution of neurons themselves has been scarcely studied. Direct glutamatergic innervation has been reported to be decreased in one study (Schutz 2005), but an opposite result has been recently published (Sunico et al. 2011). Consistent with an importance of glutamatergic innervation in motor neuron survival, decreasing vesicular glutamate transporter VGLUT2, responsible for a major part of glutamate release on motor neurons, decreased motor neuron degeneration, but did not have influence animals survival (Wootz et al. 2010). Although only indirectly innervating LMN in mice, it is clear that corticospinal motor neurons, a major glutamatergic population, degenerate in ALS mouse models (Ozdinler et al. 2011). It has been suggested a possible alteration of glutamatergic proprioceptive Ia afferents originating from muscle spindles primary endings, which have monosynaptic projections onto motor neurons. An impairment of the sensory pathways, with a degeneration of the dorsal funiculus, has been detected in animal model of SOD1-linked ALS (Guo et al. 2009). In a rat model of ALS, an impairment of dorsal roots was detected at an early and asymptomatic stage when motor neuron cell bodies did not appear to be compromised (Malaspina et al. 2010). Interestingly, sensory neurons that are functionally linked to muscle-spindle afferents by large-caliber myelinated fibers, corresponding to Ia fibers, were affected. The authors stated that these findings supported the pathogenic relevance in the development of the disease of sensory inputs coming from the muscles. Interestingly, two recent studies in a mouse model of another motoneuron degenerative disease, spinal muscular atrophy, demonstrated an early impairment of proprioceptive inputs to motor neurons. It was speculated that even a modest loss of proprioceptive function may have implications on motoneuron activity, since it has been estimated that one single Ia afferent can sprout collaterals and can develop over 2,700 synapses with up to 300 motoneurons in a motor neuron pool that innervates the medial gastrocnemius (Mendell and Henneman 1971).

Recently a spinal magnetic resonance imaging study showed that an impairment in the dorsal column, containing large myelinated sensory fibers, occurred at an early stage of the disease (Cohen-Adad et al. 2012) (Fig. 3). Together with findings in animal models, these in vivo findings in ALS patients suggest that the modifications glutamatergic input from type Ia muscle spindles to motoneurons may play a role in the pathogenesis of ALS.

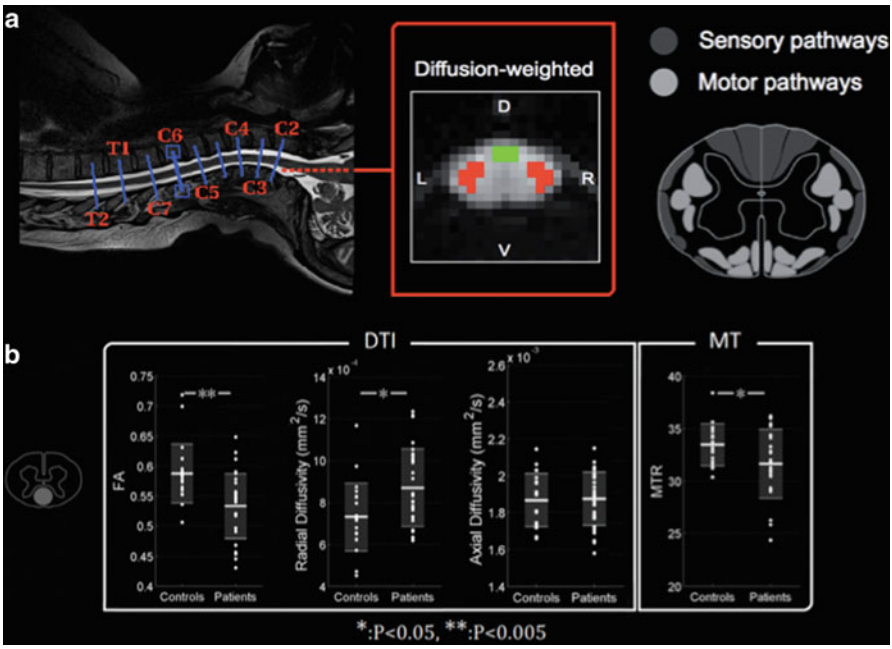

Fig. 3 Magnetic resonance imaging abnormalities of sensory pathways in ALS patients. (**a**) Regions of interest were selected in the lateral segment (turquoise blue) to include most of the lateral cortico-spinal tract and in the dorsal segment (purple) to include most of the dorsal columns (**b**) Individual plots of MRI metrics averaged in the dorsal columns. Differences were also detected in the lateral cortico-spinal tract (not shown). MTR stands for Magnetization Transfer Ratio.

2.2.4 Serotonergic Abnormalities

Besides glutamatergic neurons, other long projection neurons targeting the motor neurons are affected by the disease, although this area is still largely unexplored. In particular, it was recently shown that serotonergic neurons profoundly degenerate in both patients and animal models. This degeneration is variable between nuclei and is mostly neuritic, with some loss of cell bodies. Most importantly, this degeneration contributes to spasticity in animal models (Dentel et al. 2012). Other consequences of serotonergic loss in ALS are still unexplored.

2.3 Strategies Targeting Glutamate

Multiple therapeutic strategies have been designed to target glutamate in ALS (Van Den Bosch et al. 2006). Indeed, glutamate levels are increased in the CSF of ALS patients, and motor neurons are exquisitely sensitive to increased glutamate because they express calcium permeable AMPA receptors, due to the very low levels of the GluR2 subunit on motor neurons and to abnormal editing of its mRNA (Kawahara and Kwak 2005; Shaw and Eggett 2000). This high permeability to

calcium, combined with poor buffering capacity of calcium in the cytoplasm of motor neurons, likely causes high cytosolic calcium increases in motor neurons in response to excess of glutamate, leading to cell death through excitotoxicity (Van Den Bosch et al. 2006). Multiple antiglutamatergic strategies have been built on this framework.

2.3.1 Riluzole

Bensimon and collaborators demonstrated in 1994 that riluzole improved survival in ALS patients (Bensimon et al. 1994). A second larger confirmatory study published 2 years after this initial breakthrough confirmed this protective effect (Lacomblez et al. 1996a, b). Taking into account safety results, it was concluded from these two trials that the 100 mg dose of riluzole has the best benefit-to-risk ratio and that riluzole increases survival of patients with ALS. A meta-analysis of published riluzole trials in ALS has been recently performed and confirmed the increase in lifespan of ALS patients under riluzole (Miller et al. 2012). Taken together, interventional and population-based studies converge to demonstrate a consistent positive effect of riluzole on the survival of ALS patients. Apparently, this effect is stronger in patients treated early after symptom onset and in patients with bulbar onset. Although 3 months survival benefit is significant, there is no apparent slowing of disease progression. To our knowledge, riluzole is the only drug in the field of neurodegenerative diseases that proved increasing lifespan in multiple interventional trials. However, the underlying mechanism, long postulated to be glutamatergic, is extremely unclear, and multiple studies point to riluzole being rather an inhibitor of sodium persistent inward currents, rather than an antiglutamatergic drug (Bellingham 2011).

2.3.2 Other Antiglutamatergic Agents

Results of therapeutic trials testing other antiglutamatergic agents have been disappointing. Gabapentin, which presumably acts through inhibition of glutamate biosynthesis, showed promising preclinical effects in SOD1 mice (Gurney et al. 1996), but a randomized clinical trial was negative in ALS patients (Miller et al. 2001). Lamotrigine, which is thought to inhibit glutamate release, also failed to demonstrate a beneficial effect in ALS patients (Ryberg et al. 2003). A potential therapeutic strategy to target excitotoxicity consists in the blockade of selected glutamate receptors. However, phase III clinical trials were negative in ALS when testing AMPA receptors blockers such as talampanel or topiramate (Cudkowicz et al. 2003). Clinical trial with NMDA receptor antagonists, dextromethorphan (Blin et al. 1996) or memantine (de Carvalho et al. 2010), was unsuccessful as well. The excitatory amino acid transporter EAAT2 is one of the major glutamate transporters expressed predominantly in astroglial cells and is responsible for 90 % of total glutamate uptake. The beta-lactam antibiotic ceftriaxone was shown to increase EAAT2 levels and to prevent motor neuron death in an animal model (Rothstein et al. 2005). However, a therapeutic trial recently failed to show a therapeutic effect in ALS patients (Cudkowicz 2012).

2.4 Other Potential Strategies (Serotonin, Glycine)

To our knowledge, no drug based either on glycinergic transmission has been rigorously tested in an ALS clinical trial. Preclinical research is to be done. Concerning serotonergic transmission, serotonin levels are decreased in ALS blood (Dupuis et al. 2010), and decreased serotonin is responsible for the development of spasticity in ALS models (Dentel et al. 2012). Interestingly, providing ALS mice with serotonin precursor 5-hydroxytryptophan (5-HTP) increased their lifespan (Turner et al. 2003). However, here again, no drug targeting specific serotonin receptor subtype has been tested in models or patients.

3 Modulation of Motor Neuron Loss Through Increased Neurotrophic Activity

3.1 Neurotrophic Factors: A Potential Therapeutic Strategy

It is widely recognized that providing more trophic support to dying motor neurons might slow down their degeneration. This is supported by a number of observations suggesting that neurotrophic factor expression and/or activity might be abnormal in ALS. For instance, expression of glial cell line-derived neurotrophic factor (GDNF), a major neurotrophic factor, is increased in ALS muscle (Lie and Weis 1998), as a likely consequence of denervation. Indeed, there are reported abnormalities in c-Ret, the GDNF receptor, in ALS motor neurons (Mitsuma et al. 1999; Ryu et al. 2011). Last, overexpressing GDNF is protective in multiple ALS models, using various mode of administration (Li et al. 2003, 2007 Manabe et al. 2002, 2003; Suzuki et al. 2008).

A second example is vascular endothelial growth factor (VEGF): VEGF expression is decreased in ALS spinal cord (Anagnostou et al. 2010) and in mouse models, and such a decrease is on its own sufficient to trigger ALS-like disease in mouse. Moreover, overexpression of VEGF is able to provide strong protection to animal models (Azzouz et al. 2004; Devos et al. 2004; Lambrechts et al. 2003; Oosthuyse et al. 2001; Storkebaum et al. 2005; Van Den Bosch et al. 2004; Wang et al. 2007).

Besides documented alterations in ALS, trophic factors are in general of interest in the treatment of ALS. Indeed, during development, motor neurons are produced in excess, and those that are unable to contact efficiently skeletal muscles are eliminated (Kanning et al. 2010). It is thus possible that similar mechanisms of survival might slow down degeneration of LMNs in adults affected with ALS. During development, survival of motor neurons is strictly under the control of the innervated limb through the production of multiple trophic factors that include GDNF or ciliary-derived neurotrophic factor (CNTF). During the last 20 years, a plethora of motor neurotrophic factors have been identified which are able in vitro to provide protection to isolated cultured motor neurons. Each of these neurotrophins is thus a potential candidate for ALS therapy.

3.2 Treatment with Neurotrophic Factors in ALS Patients

Despite promising preclinical studies, direct administration of neurotrophic factor in human ALS patients failed to show a therapeutic effect in ALS patients. The major agent studied were brain-derived neurotrophic factor (BDNF) (BDNF Study Group 1999), CNTF (Miller et al. 1996), and insulin-like growth factor-1 (IGF-1) (Mitchell et al. 2007; Sorenson et al. 2008; Borasio et al. 1998; Lai et al. 1997). Clinical trial with xaliproden, an orally administered non-peptide compound that promotes the secretion of several endogenous motor neuron growth factors, failed to demonstrate a statistically significant therapeutic effect (Meininger et al. 2004). A human study with intraventricular injection of VEGF is currently in a phase I/II clinical trial.

4 Conclusion

The last 20 years of research in the field of neuroprotection in ALS have been very successful for identifying potential strategies to improve the survival of motor neurons in ALS mouse models. However, none of these potential treatments successfully translated to the clinics. This failure might be due to multiple causes, and we would like to highlight two of these potential causes.

First, research efforts were almost exclusively devoted to identify compounds and/or strategies to provide protection to motor neuron cell body. However, such a protection is now known to be largely insufficient (Dupuis and Loeffler 2009). For instance, we and others have shown that providing a complete protection of motor neuron cell bodies does not ameliorate profoundly the phenotype of ALS mice because the remaining LMNs are still denervated and do not reinnervate affected muscles (Dewil et al. 2007; Gould et al. 2006; Rouaux et al. 2007). Thus, it is critical to identify the mechanisms that cause synapse loss, rather than motor neuron loss.

Second, despite it is largely assumed that ALS patients die of motor neuron degeneration, multiple events, many of them not occurring in motor neurons, strongly contribute to disease pathogenesis. In particular, despite weight loss is a strong predictor of survival, its causes remain unknown (Dupuis et al. 2011). The recent examples of microglial (Boillee et al. 2006) or serotonergic (Dentel et al. 2012) contributions to clinical picture should illuminate our need to understand how non-motoneuronal cell types could be targeted by specific treatments to achieve neuroprotection.

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References

Anagnostou, G., Akbar, M. T., Paul, P., Angelinetta, C., Steiner, T. J., & de Belleroche, J. (2010). Vesicle associated membrane protein B (VAPB) is decreased in ALS spinal cord. *Neurobiology of Aging, 31*, 969–985.

Attarian, S., Pouget, J., & Schmied, A. (2009). Changes in cortically induced inhibition in amyotrophic lateral sclerosis with time. *Muscle & Nerve, 39*, 310–317.

Azzouz, M., Ralph, G. S., Storkebaum, E., Walmsley, L. E., Mitrophanous, K. A., Kingsman, S. M., Carmeliet, P., & Mazarakis, N. D. (2004). VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature, 429*, 413–417.

BDNF Study Group. (1999). A controlled trial of recombinant methionyl human BDNF in ALS: The BDNF Study Group (Phase III). *Neurology, 52*, 1427–1433.

Bellingham, M. C. (2011). A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis: What have we learned in the last decade? *CNS Neuroscience and Therapeutics, 17*, 4–31.

Bensimon, G., Lacomblez, L., & Meininger, V. (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *The New England Journal of Medicine, 330*, 585–591.

Blin, O., Azulay, J. P., Desnuelle, C., Bille-Turc, F., Braguer, D., Besse, D., Branger, E., Crevat, A., Serratrice, G., & Pouget, J. Y. (1996). A controlled one-year trial of dextromethorphan in amyotrophic lateral sclerosis. *Clinical Neuropharmacology, 19*, 189–192.

Boillee, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., Kollias, G., & Cleveland, D. W. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science, 312*, 1389–1392.

Borasio, G. D., Robberecht, W., Leigh, P. N., Emile, J., Guiloff, R. J., Jerusalem, F., Silani, V., Vos, P. E., Wokke, J. H., & Dobbins, T. (1998). A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. European ALS/IGF-I Study Group. *Neurology, 51*, 583–586.

Caramia, M. D., Palmieri, M. G., Desiato, M. T., Iani, C., Scalise, A., Telera, S., & Bernardi, G. (2000). Pharmacologic reversal of cortical hyperexcitability in patients with ALS. *Neurology, 54*, 58–64.

Carunchio, I., Mollinari, C., Pieri, M., Merlo, D., & Zona, C. (2008). GAB(A) receptors present higher affinity and modified subunit composition in spinal motor neurons from a genetic model of amyotrophic lateral sclerosis. *European Journal of Neuroscience, 28*, 1275–1285.

Chang, Q., & Martin, L. J. (2009). Glycinergic innervation of motoneurons is deficient in amyotrophic lateral sclerosis mice: a quantitative confocal analysis. *American Journal of Pathology, 174*, 574–585.

Chang, Q., & Martin, L. J. (2011). Glycine receptor channels in spinal motoneurons are abnormal in a transgenic mouse model of amyotrophic lateral sclerosis. *Journal of Neuroscience, 31*, 2815–2827.

Chio, A., Logroscino, G., Hardiman, O., Swingler, R., Mitchell, D., Beghi, E., & Traynor, B. G. (2009). Prognostic factors in ALS: A critical review. *Amyotrophic Lateral Sclerosis, 10*, 310–323.

Cohen-Adad, J., Mendili, M. M., Morizot-Koutlidis, R., Lehericy, S., Meininger, V., Blancho, S., Rossignol, S., Benali, H., & Pradat, P. F. (2012). Involvement of spinal sensory pathway in ALS and specificity of cord atrophy to lower motor neuron degeneration. *Amyotrophic Lateral Sclerosis, 14*(1), 30–38.

Cudkowicz, M. (2012). Clinical trial of ceftriaxone in ALS: Final results. In *23rd international symposium on ALS/MND, Chicago*, 5–7 December 2012.

Cudkowicz, M. E., Shefner, J. M., Schoenfeld, D. A., Brown, J. R., Jr., Johnson, H., Qureshi, M., Jacobs, M., Rothstein, J. D., Appel, S. H., Pascuzzi, R. M., Heiman-Patterson, T. D., Donofrio,

P. D., David, W. S., Russell, J. A., Tandan, R., Pioro, E. P., Felice, K. J., Rosenfeld, J., Mandler, R. N., Sachs, G. M., Bradley, W. G., Raynor, E. M., Baquis, G. D., Belsh, J. M., Novella, S., Goldstein, J., & Hulihan, J. (2003). A randomized, placebo-controlled trial of topiramate in amyotrophic lateral sclerosis. *Neurology, 61*, 456–464.

de Carvalho, M., Pinto, S., Costa, J., Evangelista, T., Ohana, B., & Pinto, A. (2010). A randomized, placebo-controlled trial of memantine for functional disability in amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis, 11*, 456–460.

Dentel, C., Palamiuc, L., Henriques, A., Lannes, B., Spreux-Varoquaux, O., Gutknecht, L., Rene, F., Echaniz-Laguna, A., Gonzalez de Aguilar, J. L., Lesch, K. P., Meininger, V., Loeffler, J. P., & Dupuis, L. (2012). Degeneration of serotonergic neurons in amyotrophic lateral sclerosis: A link to spasticity. *Brain, 136*(Pt 2), 483–493.

Desiato, M. T., Palmieri, M. G., Giacomini, P., Scalise, A., Arciprete, F., & Caramia, M. D. (1999). The effect of riluzole in amyotrophic lateral sclerosis: A study with cortical stimulation. *Journal of Neurological Sciences, 169*, 98–107.

Devos, D., Moreau, C., Lassalle, P., Perez, T., De Seze, J., Brunaud-Danel, V., Destee, A., Tonnel, A. B., & Just, N. (2004). Low levels of the vascular endothelial growth factor in CSF from early ALS patients. *Neurology, 62*, 2127–2129.

Dewil, M., dela Cruz, V. F., Van Den Bosch, L., & Robberecht, W. (2007). Inhibition of p38 mitogen activated protein kinase activation and mutant SOD1(G93A)-induced motor neuron death. *Neurobiology of Disease, 26*, 332–341.

Dupuis, L., & Loeffler, J. P. (2009). Neuromuscular junction destruction during amyotrophic lateral sclerosis: Insights from transgenic models. *Current Opinion in Pharmacology, 9*, 341–346.

Dupuis, L., Spreux-Varoquaux, O., Bensimon, G., Jullien, P., Lacomblez, L., Salachas, F., Bruneteau, G., Pradat, P. F., Loeffler, J. P., & Meininger, V. (2010). Platelet serotonin level predicts survival in amyotrophic lateral sclerosis. *PLoS One, 5*, e13346.

Dupuis, L., Pradat, P. F., Ludolph, A. C., & Loeffler, J. P. (2011). Energy metabolism in amyotrophic lateral sclerosis. *Lancet Neurology, 10*, 75–82.

Eisen, A., & Weber, M. (2001). The motor cortex and amyotrophic lateral sclerosis. *Muscle & Nerve, 24*, 564–573.

Foerster, B. R., Callaghan, B. C., Petrou, M., Edden, R. A., Chenevert, T. L., & Feldman, E. L. (2012). Decreased motor cortex gamma-aminobutyric acid in amyotrophic lateral sclerosis. *Neurology, 78*, 1596–1600.

Gould, T. W., Buss, R. R., Vinsant, S., Prevette, D., Sun, W., Knudson, C. M., Milligan, C. E., & Oppenheim, R. W. (2006). Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *Journal of Neuroscience, 26*, 8774–8786.

Guo, Y. S., Wu, D. X., Wu, H. R., Wu, S. Y., Yang, C., Li, B., Bu, H., Zhang, Y. S., & Li, C. Y. (2009). Sensory involvement in the SOD1-G93A mouse model of amyotrophic lateral sclerosis. *Experimental & Molecular Medicine, 41*, 140–150.

Gurney, M. E., Cutting, F. B., Zhai, P., Doble, A., Taylor, C. P., Andrus, P. K., & Hall, E. D. (1996). Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. *Annals of Neurology, 39*, 147–157.

Hossaini, M., Cardona Cano, S., van Dis, V., Haasdijk, E. D., Hoogenraad, C. C., Holstege, J. C., & Jaarsma, D. (2011). Spinal inhibitory interneuron pathology follows motor neuron degeneration independent of glial mutant superoxide dismutase 1 expression in SOD1-ALS mice. *Journal of Neuropathology and Experimental Neurology, 70*, 662–677.

Kanning, K. C., Kaplan, A., & Henderson, C. E. (2010). Motor neuron diversity in development and disease. *Annual Review of Neuroscience, 33*, 409–440.

Kawahara, Y., & Kwak, S. (2005). Excitotoxicity and ALS: What is unique about the AMPA receptors expressed on spinal motor neurons? *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 6*, 131–144.

Lacomblez, L., Bensimon, G., Leigh, P. N., Guillet, P., & Meininger, V. (1996a). Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet, 347*, 1425–1431.

Lacomblez, L., Bensimon, G., Leigh, P. N., Guillet, P., Powe, L., Durrleman, S., Delumeau, J. C., & Meininger, V. (1996b). A confirmatory dose-ranging study of riluzole in ALS. ALS/Riluzole Study Group-II. *Neurology, 47*, S242–S250.

Lai, E. C., Felice, K. J., Festoff, B. W., Gawel, M. J., Gelinas, D. F., Kratz, R., Murphy, M. F., Natter, H. M., Norris, F. H., & Rudnicki, S. A. (1997). Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. The North America ALS/IGF-I Study Group. *Neurology, 49*, 1621–1630.

Lambrechts, D., Storkebaum, E., Morimoto, M., Del-Favero, J., Desmet, F., Marklund, S. L., Wyns, S., Thijs, V., Andersson, J., van Marion, I., Al-Chalabi, A., Bornes, S., Musson, R., Hansen, V., Beckman, L., Adolfsson, R., Pall, H. S., Prats, H., Vermeire, S., Rutgeerts, P., Katayama, S., Awata, T., Leigh, N., Lang-Lazdunski, L., Dewerchin, M., Shaw, C., Moons, L., Vlietinck, R., Morrison, K. E., Robberecht, W., Van Broeckhoven, C., Collen, D., Andersen, P. M., & Carmeliet, P. (2003). VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. *Nature Genetics, 34*, 383–394.

Li, W., Brakefield, D., Pan, Y., Hunter, D., Myckatyn, T. M., & Parsadanian, A. (2007). Muscle-derived but not centrally derived transgene GDNF is neuroprotective in G93A-SOD1 mouse model of ALS. *Experimental Neurology, 203*, 457–471.

Lie, D. C., & Weis, J. (1998). GDNF expression is increased in denervated human skeletal muscle. *Neuroscience Letters, 250*, 87–90.

Lloyd, C. M., Richardson, M. P., Brooks, D. J., al Chalabi, A., & Leigh, P. N. (2000). Extramotor involvement in ALS: PET studies with the GABA(A) ligand [(11)C]flumazenil. *Brain, 123*(Pt 11), 2289–2296.

Lu, Y. Y., Wang, L. J., Muramatsu, S., Ikeguchi, K., Fujimoto, K., Okada, T., Mizukami, H., Matsushita, T., Hanazono, Y., Kume, A., Nagatsu, T., Ozawa, K., & Nakano, I. (2003). Intramuscular injection of AAV-GDNF results in sustained expression of transgenic GDNF, and its delivery to spinal motoneurons by retrograde transport. *Neuroscience Research, 45*, 33–40.

Malaspina, A., Ngoh, S. F., Ward, R. E., Hall, J. C., Tai, F. W., Yip, P. K., Jones, C., Jokic, N., Averill, S. A., Michael-Titus, A. T., & Priestley, J. V. (2010). Activation transcription factor-3 activation and the development of spinal cord degeneration in a rat model of amyotrophic lateral sclerosis. *Neuroscience, 169*, 812–827.

Manabe, Y., Nagano, I., Gazi, M. S., Murakami, T., Shiote, M., Shoji, M., Kitagawa, H., Setoguchi, Y., & Abe, K. (2002). Adenovirus-mediated gene transfer of glial cell line-derived neurotrophic factor prevents motor neuron loss of transgenic model mice for amyotrophic lateral sclerosis. *Apoptosis, 7*, 329–334.

Manabe, Y., Nagano, I., Gazi, M. S., Murakami, T., Shiote, M., Shoji, M., Kitagawa, H., & Abe, K. (2003). Glial cell line-derived neurotrophic factor protein prevents motor neuron loss of transgenic model mice for amyotrophic lateral sclerosis. *Neurological Research, 25*, 195–200.

Martin, L. J., & Chang, Q. (2012). Inhibitory synaptic regulation of motoneurons: A new target of disease mechanisms in amyotrophic lateral sclerosis. *Molecular Neurobiology, 45*, 30–42.

Mazzocchio, R., & Rossi, A. (2010). Role of Renshaw cells in amyotrophic lateral sclerosis. *Muscle & Nerve, 41*, 441–443.

McGown, A., McDearmid, J. R., Panagiotaki, N., Tong, H., Al Mashhadi, S., Redhead, N., Lyon, A. N., Beattie, C. E., Shaw, P. J., & Ramesh, T. M. (2012). Early interneuron dysfunction in ALS: Insights from a mutant sod1 zebrafish model. *Annals of Neurology, 73*(2), 246–258.

Meininger, V., Bensimon, G., Bradley, W. R., Brooks, B., Douillet, P., Eisen, A. A., Lacomblez, L., Leigh, P. N., & Robberecht, W. (2004). Efficacy and safety of xaliproden in amyotrophic lateral sclerosis: Results of two phase III trials. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 5*, 107–117.

Mendell, L. M., & Henneman, E. (1971). Terminals of single Ia fibers: Location, density, and distribution within a pool of 300 homonymous motoneurons. *Journal of Neurophysiology, 34*, 171–187.

Miller, R. G., Petajan, J. H., Bryan, W. W., Armon, C., Barohn, R. J., Goodpasture, J. C., Hoagland, R. J., Parry, G. J., Ross, M. A., & Stromatt, S. C. (1996). A placebo-controlled trial of recombinant human ciliary neurotrophic (rhCNTF) factor in amyotrophic lateral sclerosis. rhCNTF ALS Study Group. *Annals of Neurology, 39*, 256–260.

Miller, R. G., Moore, D. H., Gelinas, D. F., Dronsky, V., Mendoza, M., Barohn, R. J., Bryan, W., Ravits, J., Yuen, E., Neville, H., Ringel, S., Bromberg, M., Petajan, J., Amato, A. A., Jackson, C., Johnson, W., Mandler, R., Bosch, P., Smith, B., Graves, M., Ross, M., Sorenson, E. J., Kelkar, P., Parry, G., & Olney, R. (2001). Phase III randomized trial of gabapentin in patients with amyotrophic lateral sclerosis. *Neurology, 56*, 843–848.

Miller, R. G., Mitchell, J. D., & Moore, D. H. (2012). Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database of Systematic Reviews, 3*, CD001447.

Mills, K. R., & Nithi, K. A. (1997). Corticomotor threshold is reduced in early sporadic amyotrophic lateral sclerosis. *Muscle & Nerve, 20*, 1137–1141.

Mitchell, J. D., Wokke, J. H., & Borasio, G. D. (2007). Recombinant human insulin-like growth factor I (rhIGF-I) for amyotrophic lateral sclerosis/motor neuron disease. *Cochrane Database of Systematic Reviews*, CD002064.

Mitsuma, N., Yamamoto, M., Li, M., Ito, Y., Mitsuma, T., Mutoh, T., Takahashi, M., & Sobue, G. (1999). Expression of GDNF receptor (RET and GDNFR-alpha) mRNAs in the spinal cord of patients with amyotrophic lateral sclerosis. *Brain Research, 820*, 77–85.

Oosthuyse, B., Moons, L., Storkebaum, E., Beck, H., Nuyens, D., Brusselmans, K., Van Dorpe, J., Hellings, P., Gorselink, M., Heymans, S., Theilmeier, G., Dewerchin, M., Laudenbach, V., Vermylen, P., Raat, H., Acker, T., Vleminckx, V., Van Den Bosch, L., Cashman, N., Fujisawa, H., Drost, M. R., Sciot, R., Bruyninckx, F., Hicklin, D. J., Ince, C., Gressens, P., Lupu, F., Plate, K. H., Robberecht, W., Herbert, J. M., Collen, D., & Carmeliet, P. (2001). Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. *Nature Genetics, 28*, 131–138.

Ozdinler, P. H., Benn, S., Yamamoto, T. H., Guzel, M., Brown, R. H., Jr., & Macklis, J. D. (2011). Corticospinal motor neurons and related subcerebral projection neurons undergo early and specific neurodegeneration in hSOD1G(9)(3)A transgenic ALS mice. *Journal of Neuroscience, 31*, 4166–4177.

Petri, S., Schmalbach, S., Grosskreutz, J., Krampfl, K., Grothe, C., Dengler, R., Van Den Bosch, L., Robberecht, W., & Bufler, J. (2005). The cellular mRNA expression of GABA and glutamate receptors in spinal motor neurons of SOD1 mice. *Journal of Neurological Sciences, 238*, 25–30.

Prout, A. J., & Eisen, A. A. (1994). The cortical silent period and amyotrophic lateral sclerosis. *Muscle & Nerve, 17*, 217–223.

Raynor, E. M., & Shefner, J. M. (1994). Recurrent inhibition is decreased in patients with amyotrophic lateral sclerosis. *Neurology, 44*, 2148–2153.

Rekling, J. C., Funk, G. D., Bayliss, D. A., Dong, X. W., & Feldman, J. L. (2000). Synaptic control of motoneuronal excitability. *Physiological Reviews, 80*, 767–852.

Rothstein, J. D., Patel, S., Regan, M. R., Haenggeli, C., Huang, Y. H., Bergles, D. E., Jin, L., Dykes, H. M., Vidensky, S., Chung, D. S., Toan, S. V., Bruijn, L. I., Su, Z. Z., Gupta, P., & Fisher, P. B. (2005). Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature, 433*, 73–77.

Rouaux, C., Panteleeva, I., Rene, F., Gonzalez de Aguilar, J. L., Echaniz-Laguna, A., Dupuis, L., Menger, Y., Boutillier, A. L., & Loeffler, J. P. (2007). Sodium valproate exerts neuroprotective effects in vivo through CREB-binding protein-dependent mechanisms but does not improve survival in an amyotrophic lateral sclerosis mouse model. *Journal of Neuroscience, 27*, 5535–5545.

Ryberg, H., Askmark, H., & Persson, L. I. (2003). A double-blind randomized clinical trial in amyotrophic lateral sclerosis using lamotrigine: effects on CSF glutamate, aspartate, branched-chain amino acid levels and clinical parameters. *Acta Neurologica Scandinavica, 108*, 1–8.

Ryu, H., Jeon, G. S., Cashman, N. R., Kowall, N. W., & Lee, J. (2011). Differential expression of c-Ret in motor neurons versus non-neuronal cells is linked to the pathogenesis of ALS. *Laboratory Investigation, 91*, 342–352.

Schreiber, H., Gaigalat, T., Wiedemuth-Catrinescu, U., Graf, M., Uttner, I., Muche, R., & Ludolph, A. C. (2005). Cognitive function in bulbar- and spinal-onset amyotrophic lateral sclerosis. A longitudinal study in 52 patients. *Journal of Neurology, 252*, 772–781.

Schutz, B. (2005). Imbalanced excitatory to inhibitory synaptic input precedes motor neuron degeneration in an animal model of amyotrophic lateral sclerosis. *Neurobiology of Disease, 20*, 131–140.

Shaw, P. J., & Eggett, C. J. (2000). Molecular factors underlying selective vulnerability of motor neurons to neurodegeneration in amyotrophic lateral sclerosis. *Journal of Neurology, 247* (Suppl 1), I17–I27.

Shefner, J. M., & Logigian, E. L. (1998). The mixed nerve silent period in normal subjects and patients with amyotrophic lateral sclerosis. *Electromyography and Clinical Neurophysiology, 38*, 505–510.

Sorenson, E. J., Windbank, A. J., Mandrekar, J. N., Bamlet, W. R., Appel, S. H., Armon, C., Barkhaus, P. E., Bosch, P., Boylan, K., David, W. S., Feldman, E., Glass, J., Gutmann, L., Katz, J., King, W., Luciano, C. A., McCluskey, L. F., Nash, S., Newman, D. S., Pascuzzi, R. M., Pioro, E., Sams, L. J., Scelsa, S., Simpson, E. P., Subramony, S. H., Tiryaki, E., & Thornton, C. A. (2008). Subcutaneous IGF-1 is not beneficial in 2-year ALS trial. *Neurology, 71*, 1770–1775.

Storkebaum, E., Lambrechts, D., Dewerchin, M., Moreno-Murciano, M. P., Appelmans, S., Oh, H., Van Damme, P., Rutten, B., Man, W. Y., De Mol, M., Wyns, S., Manka, D., Vermeulen, K., Van Den Bosch, L., Mertens, N., Schmitz, C., Robberecht, W., Conway, E. M., Collen, D., Moons, L., & Carmeliet, P. (2005). Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. *Nature Neuroscience, 8*, 85–92.

Sunico, C. R., Dominguez, G., Garcia-Verdugo, J. M., Osta, R., Montero, F., & Moreno-Lopez, B. (2011). Reduction in the motoneuron inhibitory/excitatory synaptic ratio in an early-symptomatic mouse model of amyotrophic lateral sclerosis. *Brain Pathology, 21*, 1–15.

Suzuki, M., McHugh, J., Tork, C., Shelley, B., Hayes, A., Bellantuono, I., Aebischer, P., & Svendsen, C. N. (2008). Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS. *Molecular Therapy, 16*, 2002–2010.

Turner, B. J., Lopes, E. C., & Cheema, S. S. (2003). The serotonin precursor 5-hydroxytryptophan delays neuromuscular disease in murine familial amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 4*, 171–176.

Van Den Bosch, L., Storkebaum, E., Vleminckx, V., Moons, L., Vanopdenbosch, L., Scheveneels, W., Carmeliet, P., & Robberecht, W. (2004). Effects of vascular endothelial growth factor (VEGF) on motor neuron degeneration. *Neurobiology of Disease, 17*, 21–28.

Van Den Bosch, L., Van Damme, P., Bogaert, E., & Robberecht, W. (2006). The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochimica et Biophysica Acta, 1762*, 1068–1082.

Vucic, S., & Kiernan, M. C. (2006). Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. *Brain, 129*, 2436–2446.

Vucic, S., Nicholson, G. A., & Kiernan, M. C. (2008). Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. *Brain, 131*, 1540–1550.

Vucic, S., Cheah, B. C., & Kiernan, M. C. (2011). Maladaptation of cortical circuits underlies fatigue and weakness in ALS. *Amyotrophic Lateral Sclerosis, 12*, 414–420.

Wang, Y., Mao, X. O., Xie, L., Banwait, S., Marti, H. H., Greenberg, D. A., & Jin, K. (2007). Vascular endothelial growth factor overexpression delays neurodegeneration and prolongs survival in amyotrophic lateral sclerosis mice. *Journal of Neuroscience, 27*, 304–307.

Wootz, H., Enjin, A., Wallen-Mackenzie, A., Lindholm, D., & Kullander, K. (2010). Reduced VGLUT2 expression increases motor neuron viability in Sod1(G93A) mice. *Neurobiology of Disease, 37*, 58–66.

Wootz, H., Fitzsimons-Kantamneni, E., Larhammar, M., Rotterman, T. M., Enjin, A., Patra, K., Andre, E., van Zundert, B., Kullander, K., & Alvarez, F. J. (2012). Alterations in the motor neuron-renshaw cell circuit in the Sod1(G93A) mouse model. *Journal of Comparative Neurology, 37*(1), 58–66.

Yokota, T., Yoshino, A., Inaba, A., & Saito, Y. (1996). Double cortical stimulation in amyotrophic lateral sclerosis. *Journal of Neurology, Neurosurgery, and Psychiatry, 61*, 596–600.

Zanette, G., Tamburin, S., Manganotti, P., Refatti, N., Forgione, A., & Rizzuto, N. (2002). Changes in motor cortex inhibition over time in patients with amyotrophic lateral sclerosis. *Journal of Neurology, 249*, 1723–1728.

Zhu, H., Edden, R. A., Ouwerkerk, R., & Barker, P. B. (2011). High resolution spectroscopic imaging of GABA at 3 Tesla. *Magnetic Resonance in Medicine, 65*, 603–609.

Neurotoxicity and ALS: Insights into Pathogenesis

Steve Vucic and Matthew C. Kiernan

Contents

Abstract

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder of the motor neurons in the spinal cord, brainstem, and motor cortex. While the mechanisms underlying the development of ALS remain to be fully elucidated, evidence is now emerging to suggest that the pathophysiological mechanisms underlying ALS are multifactorial, reflecting a complex interaction between causal genes and local environment. In particular, dysfunction of metabolic pathways including excessive oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction, and defective axonal transport

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systems along with abnormalities of nonneuronal supporting cells may cause critical injury to target proteins and organelles within the motor neuron, thereby leading to neurotoxicity and neurodegeneration in ALS. The clinical effectiveness afforded by anti-glutamatergic agents such as riluzole underscores the importance of glutamate excitotoxicity in the development of neurodegeneration in ALS, with anterior horn cell degeneration mediated by corticomotoneuronal hyperexcitability via an anterograde transsynaptic process. This chapter will review current understanding of the pathophysiological mechanisms underlying the development of ALS, with a particular focus on the role of neurotoxicity in mediating neurodegeneration in ALS.

Keywords
ALS • Glutamate excitotoxicity • Neurodegeneration • Neurotoxicity

1 Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder of motor neurons in the spinal cord, brainstem, and motor cortex (Kiernan et al. 2011). The ALS phenotype is clinically characterized by the presence of upper and lower motor neuron abnormalities (Kiernan et al. 2011; Vucic et al. 2007; Winhammar et al. 2005), whereby lower motor neuron (LMN) features include fasciculations, muscle wasting, and weakness, while upper motor neuron features include weakness, slowness of movement, increased tone, and hyperreflexia. This unique combination of upper and lower motor neuron abnormalities, not encountered in other neurodegenerative diseases, led Charcot to postulate the mechanism of neuronal involvement in ALS pathogenesis (Charcot and Joffroy 1869). Despite Charcot's initial observations, the precise pathophysiological mechanisms, and even the site of disease onset, remain the subject of ongoing debate (Ravits et al. 2007).

Evidence is now emerging to suggest that the pathogenic mechanisms underlying ALS are multifactorial, reflecting interaction between causal genes and local environment (Boillee et al. 2006a; Kiernan et al. 2011; Vucic and Kiernan 2009; Winhammar et al. 2005). These complex pathways, including oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction, and defective axonal transport systems (Fig. 1), combined with abnormalities of nonneuronal supporting cells such as the astrocytes, may cause injury of critical target proteins and organelles within the motor neuron, thereby resulting in neurotoxicity and degeneration in ALS (Gonzalez de Aguilar et al. 2007; Gros-Louis et al. 2006; Haidet-Phillips et al. 2011; Neusch et al. 2007; Pasinelli and Brown 2006; Patel and Maragakis 2002; Vucic and Kiernan 2009). As such, this chapter will review current understanding of the pathophysiological mechanisms underlying the development of ALS, with a particular focus on the role of neurotoxicity.

1.1 Glutamate-Mediated Excitotoxicity

Glutamate-mediated excitotoxicity appears to be an important mechanism in ALS pathogenesis (Boillee et al. 2006; Kiernan et al. 2011). Glutamate is the major excitatory neurotransmitter in the central nervous system (Heath and Shaw 2002; Watkins and Evans 1981), synthesized from reductive deamination of alpha-ketoglutarate or from the action of amino acids of aminotransferases (Heath and Shaw 2002). Approximately 20% of the total glutamate pool is stored in presynaptic nerve terminals, and during impulse transmission, glutamate is released from presynaptic neurons through the effects of depolarization, diffusing across the synaptic cleft to activate postsynaptic receptors. Their excitatory signal is terminated upon removal of glutamate from the synaptic cleft by specific glutamate reuptake transporters located on both neurons and astrocytes (Dong et al. 1999; Vandenberg 1998). Within presynaptic astrocytes, glutamate is converted into glutamine by the enzyme glutamine synthetase and then returned to the neuron for resynthesis of glutamate (Laake et al. 1995).

Glutamate receptors are broadly classified into ionotropic or metabotropic receptors (Heath and Shaw 2002). Binding of glutamate to its ionotropic receptors results in a conformational change within the receptor, thereby enabling a passage of Na^+ and Ca^{2+} ions through a central pore. Metabotropic glutamate receptors are linked via G-proteins to second-messenger enzymes, which in turn can regulate a host of cellular activities (Simeone et al. 2004). Based on pharmacological studies, glutamate ionotropic receptors are further classified as (i) *N*-methyl-D-aspartate (NMDA), (ii) α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA), and (iii) kainite receptors. This pharmacological classification is supported by cloning studies that have identified six different families of glutamate ionotropic receptors that conform to the original agonist studies (Simeone et al. 2004). *N*-methyl-D-aspartate receptors are permeable to influx of Na^+ and Ca^{2+} and efflux of K^+ (Simeone et al. 2004). An essential feature of NMDA receptors is their voltage-dependent blockade by Mg^{2+} binding within the channel pore, which can be alleviated by depolarization (MacDermott et al. 1986). NMDA receptors are involved in excitatory neurotransmission, which is characterized by a slow rise time and decay. As such, the NMDA receptors are involved in complex physiological processes, such as generation of rhythmic motor activity (Traven et al. 1993), regulation of neuronal migration during embryogenesis (Komuro and Rakic 1993), and memory (Bliss and Collingridge 1993).

The NMDA receptor complex is composed of different subunits derived from 6 genes: NMDAR1 (eight splice variants described), NMDAR2 (A–D), and NMDAR3 (A,B) (Heath and Shaw 2002; Simeone et al. 2004). While the NMDAR1 subunit forms the basic structure of the receptor (Heath and Shaw 2002), the NMDAR2 subunit determines ion channel properties and forms ligand-binding sites (Kutsuwada et al. 1992; Meguro et al. 1992; Michaelis 1998). Functional and pharmacological properties of NMDA receptors are determined through specific combination of NMDAR1 and NMDAR2 subunits (Kutsuwada et al. 1992;

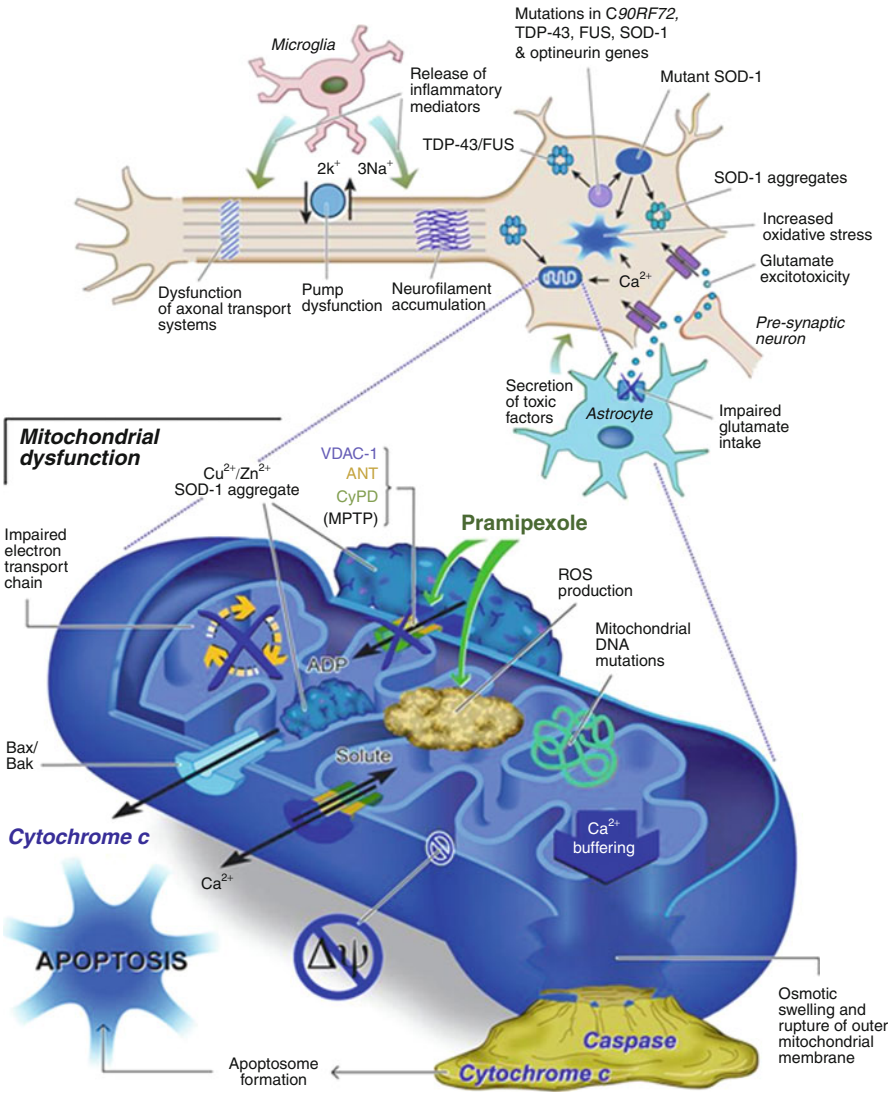

Fig. 1 Multiple interacting pathophysiological mechanisms appear to underlie the development of amyotrophic lateral sclerosis (*ALS*). Glutamate-mediated excitotoxicity is an important mechanism ALS progression and seems to precede the clinical development of ALS. Dysfunction of the excitatory amino acid transporter type 2 (EAAT2), located on astrocytes, in part induces the development of glutamate excitotoxicity via excessive extracellular accumulation of glutamate. In addition, activation of nonneuronal cells (astrocytes and microglia) in ALS results in secretion of proinflammatory cytokines and other cytotoxic factors that ultimately results in further neurotoxicity and degeneration. In conjunction with glutamate-mediated excitotoxicity, other molecular processes induce neurotoxicity via multifactorial mechanisms. Within the neuron, mutations in a host of ALS-related genes, including C9orf72, superoxide dismutase 1 (*SOD1*), TDP-43, and FUS, result in ALS via multiple mechanisms. Specifically, mutations in the SOD-1

Monyer et al. 1992). In addition, there are regional variations in the expression of NMDA receptor subtypes (Ciabarra and Sevarino 1997; Ishii et al. 1993; Kutsuwada et al. 1992; Monyer et al. 1992; Watanabe et al. 1993a, b, 1994a, b), with the NMDAR3B subunit heavily expressed in somatic motoneurons (Chatterton et al. 2002; Nishi et al. 2001).

AMPA receptors mediate a rapid influx of monovalent ions, such as Na^+, K^+, and chloride (Cl^-) but unlike NMDA receptors are impermeable to Ca^{2+} (Heath and Shaw 2002). Four AMPA receptor subtypes have been cloned (*Glu*R1-4) and are composed of three transmembrane domains (M1, M3, M4) and a fourth cytoplasmic hairpin loop (M2), which contributes to the pore-lining region (Dingledine et al. 1999; Simeone et al. 2004). The AMPA receptor exists as a pentameric structure in vivo, which is formed by the arrangement of subunits to create receptor diversity (Heath and Shaw 2002). The GluR2 subunit influences the Ca^{2+} permeability of AMPA receptors, whereby those receptors expressing an immature GluR2 subunit are more permeable to Ca^{2+} ions. Following activation of these AMPA receptors, excessive influx of Ca^{2+} results in neurodegeneration through activation of Ca^{2+}-dependent pathways (Heath and Shaw 2002; Simeone et al. 2004).

1.2 The Role of Glutamate in ALS Pathogenesis

As discussed, glutamate excitotoxicity is mediated by excessive activation of postsynaptic glutamate receptors (Heath and Shaw 2002). In ALS, glutamate excitotoxicity has been postulated to induce anterior horn cell degeneration via a transsynaptic anterograde process mediated by corticomotoneurons (Eisen et al. 1992). Support for such a mechanism has been provided by transcranial magnetic stimulation studies (TMS) which have demonstrated that cortical hyperexcitability, a biomarker of glutamate excitotoxicity, is an early feature in sporadic and familial ALS, linked to motor neuron degeneration (Blair et al. 2010; Caramia et al. 1991; Desiato et al. 2002; Eisen et al. 1993; Prout and Eisen 1994; Vucic and Kiernan 2006, 2009, 2010; Vucic et al. 2008). In addition, longitudinal studies in asymptomatic SOD-1 mutation carriers revealed that cortical hyperexcitability developed prior to the clinical onset of ALS (Vucic et al. 2008), a feature also evident in the G93A SOD-1 mouse model (Browne et al. 2006). Of relevance, loss of γ-aminobutyric acid (GABA) secreting parvalbumin-positive inhibitory interneurons in the motor cortex of ALS patients may further contribute

Fig. 1 (continued) gene result in toxic gain of function of the SOD1 enzyme which affects a host of critical cellular organelles, such as DNA/RNA metabolism. In addition, mitochondrial dysfunction is a feature of ALS, linked to glutamate excitotoxicity and SOD-1 gene mutations, resulting in a reduced production of ATP and calcium-sequestering ability, as well as an increase in free radical formation. Of further relevance, mitochondrial dysfunction may contribute to glutamate excitotoxicity. Ultimately, these multiple pathogenic processes result in critical cell dysfunction and motor neuron degeneration

to the development of cortical hyperexcitability (Nihei et al. 1993), a finding underscored by recent neuroradiological studies reporting a significant reduction of GABA within the motor cortex of ALS patients (Foerster et al. 2012).

Molecular-based studies have provided further evidence for glutamate-mediated excitotoxicity in ALS. Specifically, molecular studies established significant reduction in the expression and function of the astrocytic glutamate transporter (EAAT2), which mediates glutamate reuptake at synapses thereby reducing glutamate excitotoxicity, in the superoxide dismutase-1 (SOD-1) mouse model and the motor cortex and spinal cord of ALS patients (Boillee et al. 2006; Ionov 2007; Rothstein et al. 1993, 1995, Trotti et al. 1999). Of further relevance, dysfunction of the EAAT2 transporter appears to be a preclinical feature in the SOD-1 mouse model (Boston-Howes et al. 2006; Gibb et al. 2007), and an increase in the expression and transporter activity of EAAT2 increases the life span of mutant SOD-1 mice (Rothstein et al. 2005).

At a postsynaptic level, increased expression of AMPA receptors with the unedited GluR2 subunit has been reported in ALS (Kawahara et al. 2004; Kwak and Kawahara 2005; Takuma et al. 1999; Van Damme et al. 2002, 2005). This editing defect appears to be specific for ALS, thereby rendering the motor neurons more permeable to Ca^{2+}, potentially explaining the increased sensitivity of motor neurons to excitotoxicity (Cox et al. 2007; Heath and Shaw 2002). Further support for glutamate excitotoxicity has been provided by the clinical benefit of riluzole in ALS patients (Bensimon et al. 1994; Cheah et al. 2010; Gurney et al. 1996, 1998; Lacomblez et al. 1996). Specifically, riluzole is a glutamate antagonist that exerts effects in the central nervous system by reducing the release of glutamate from presynaptic nerve terminals and enhancing the reuptake of glutamate (Azbill et al. 2000; Cheah et al. 2010; Quinlan 2011; Wang et al. 2004).

For the glutamate hypothesis to be a plausible mechanism of motor neuron degeneration in ALS, it must explain how motor neurons became selectively damaged through overactivity of the glutaminergic system and provide a mechanism by which degeneration occurs. A number of cell-specific molecular features possessed by the motor neurons render them vulnerable to glutamate toxicity in ALS. Motor neurons affected in ALS preferentially express AMPA receptors lacking the functional GluR2 subunit, thereby rendering the motor neurons more permeable to Ca^{2+} (Kawahara et al. 2004; Kwak and Kawahara 2005; Van Damme et al. 2002, 2005). In addition, motor neurons vulnerable to degeneration lack the intracellular expression of proteins parvalbumin and calbindin D28k which are required to buffer intracellular Ca^{2+} (Ince et al. 1993). Of further relevance, increased expression of the inositol 1,4,5-triphosphate receptor 2 (ITPR2) gene was reported in ALS (van Es et al. 2007). The ITPR2 is involved in glutamate-mediated neurotransmission, whereby stimulation of glutamate receptors results in binding of inositol 1,4, 5-triphosphate to ITPR2, which subsequently increases intracellular calcium (Choe and Ehrlich 2006; van Es et al. 2007). Aberrant activity of ITPR2 results in higher intracellular concentration of Ca^{2+} leading ultimately to neurodegeneration (Gutstein and Marks 1997). Of further relevance, motor neurons in ALS, at least in animal

models, appear to be larger, with an increase in distal dendritic branching (Amendola and Durand 2008). Consequently, the input conductance of the motor neurons is increased rendering the motor neuron more vulnerable to electrical and metabolic stresses, in particular those imparted by glutamate excitotoxicity (Quinlan 2011).

Although details of the molecular mechanisms by which glutamate exerts neurotoxicity are still to be fully elucidated, several pathways have been defined. Initially, an influx of Na^+ and Cl^- ions occurs along with water molecules, resulting in acute neuronal swelling that is reversible with removal of agonist (Choi 1987; Shaw and Kuncl 2002; Stys 1998). Subsequently, an influx of Ca^{2+} ions occurs via activation of ionotropic receptors such as the NMDA and Ca^{2+}-permeable AMPA receptors, as well activation of voltage-gated Ca^{2+} channels (Choi 1987; Miller et al. 1989). Ultimately, activation of these ionic pathways results in increased intracellular Ca^{2+} concentration and activation of Ca^{2+}-dependent enzymatic pathways leading to neuronal death (Cox et al. 2007; Meldrum and Garthwaite 1990; Regan et al. 1995; Shaw and Kuncl 2002). Further, glutamate excitotoxicity results in production of free radicals that can further damage the intracellular organelles to thereby cause cell death (Bondy and Lee 1993; Lees 1993; Maher and Davis 1996).

In his original manuscript, Charcot concluded that ALS was a disorder of the brain and that lower motor neuron degeneration resulted from downstream effects (Charcot and Joffroy 1869). This view was not universal, and some of Charcot's contemporaries, such as Gower's, argued that upper and lower motor neuron degeneration were independent. Over the past two decades the site of ALS onset has been revisited to a large extent precipitated by the advent of modern noninvasive technologies such as TMS. Three schools of thought have developed pertaining to the role of the UMN and related pathophysiological processes in ALS: (i) "the *dying forward*" hypothesis; (ii) "the *dying back*" hypothesis, and (iii) "the *independent degeneration*" hypothesis (Fig. 2).

The dying forward hypothesis proposed that ALS was primarily a disorder of the corticomotoneurons, which connect monosynaptically with anterior horn cells (Eisen et al. 1992). Corticomotoneuronal hyperexcitability was postulated to induce anterior horn cell degeneration transsynaptically via an anterograde glutamate-mediated excitotoxic process (Eisen et al. 1992; Vucic and Kiernan 2009). This dying forward hypothesis was based on a number of poignant clinical observations including (i) relative preservation of extraocular and sphincter muscles in ALS, postulated to be due to a paucity of corticomotoneuronal projections onto the motor nuclei innervating these muscles; (ii) absence of an animal model of ALS, ascribed to a lack of direct corticomotoneuronal-anterior horn cell connections (Armand 1982; Lemon and Griffiths 2005); (iii) rarity of pure lower motor neuron forms of ALS, with subclinical upper motor neuron dysfunction invariably detected with TMS studies (Eisen and Shtybel 1990); and (iv) the specificity of dissociated muscle atrophy (Eisen and Kuwabara 2012; Kuwabara et al. 2008; Menon et al. 2011; Wilbourn 2000), termed the split hand phenomenon in ALS remains best explained by a dying forward mechanism (Eisen and Kuwabara 2012; Menon et al. 2011).

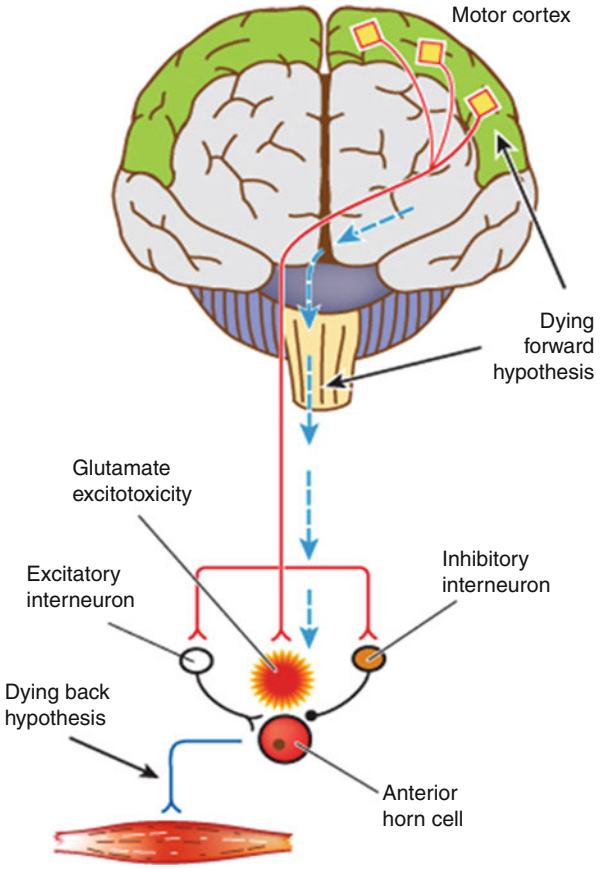

Fig. 2 The dying forward and dying back hypothesis of amyotrophic lateral sclerosis (*ALS*). The "dying forward" hypothesis proposed that ALS was primarily a disorder of the corticomotoneurons (highlighted in *red*), with anterior horn cell degeneration mediated via a transsynaptic anterograde glutamate-mediated excitotoxic process. In contrast, the dying back hypothesis proposed that ALS was primarily a disorder of the lower motor neurons with pathogens retrogradely transported from the neuromuscular junction to the cell body where these pathogens may exert their deleterious effects

Utilizing TMS technology, it is becoming increasingly apparent that cortical hyperexcitability develops as an early feature in sporadic and familial ALS, linked to the process of motor neuron degeneration (Caramia et al. 1991; Desiato et al. 2002; Eisen et al. 1993; Prout and Eisen 1994; Vucic and Kiernan 2006, 2009, 2010; Vucic et al. 2008). Furthermore, longitudinal studies in asymptomatic SOD-1 mutation carriers established that cortical hyperexcitability developed prior to the clinical onset of ALS (Vucic et al. 2008), a feature also reported in the G93A SOD-1 mouse model (Browne et al. 2006). In keeping with a cortical origin of ALS is the now accepted view that ALS and frontotemporal dementia (FTD) represent an overlapping continuum of the same disorder (Lillo and Hodges 2009; Neumann et al. 2006), an observation further underscored by recent genetic discoveries that increased hexanucleotide repeat expansions in the first intron of C9ORF72 gene (9p21) was associated with both ALS and FTD (DeJesus-Hernandez et al. 2011; Renton et al. 2011; Traynor 2012).

The dying back hypothesis proposed that ALS is primarily a disorder of the lower motor neurons, with pathogens retrogradely transported from the

neuromuscular junction to the cell body where they exert their deleterious effects (Chou and Norris 1993). Although some pathological studies have indirectly supported a dying back process (Gould et al. 2006; Pagani et al. 2006; Pun et al. 2006), no pathogens of any type has been identified in relation to ALS. The presence of widespread dysfunction within the frontal cortex, including the primary, supplementary, and prefrontal motor cortices in ALS, remains difficult to reconcile with any dying back process (Miller et al. 2009; Turner et al. 2009; Vucic et al. 2007). In addition, the absence of central pathology in other lower motor neuron disorders such as Kennedy's disease or poliomyelitis provides a further argument against a dying back process (Eisen and Weber 2001; Vucic and Kiernan 2008).

The independent degeneration hypothesis suggested that the upper and lower motor neurons degenerated independently (Gowers 1886–1888). Limited neuropathological studies provided indirect support for independent degeneration, whereby the degeneration of upper and lower motor neurons appeared to be independent (Kiernan and Hudson 1991; Pamphlett et al. 1995). These correlative morphological techniques, however, were significantly confounded by the anatomical and functional complexity of the corticomotoneuronal system (Flament et al. 1993). In particular, there remains considerable variability in the corticomotoneuronal to anterior horn cell ratio, due to synaptic changes, and as such, attempts to correlate upper and lower motor neurons as a "one-off" on autopsy studies are divorced from clinical and in vivo reality (Eisen and Weber 2001).

1.3 Neurotoxicity and the Role of SOD-1 Gene

Mutations in the SOD-1 gene, the first ALS gene reported and mapped to the long arm of chromosome 21 [21 q22.1] (Siddique et al. 1991), was postulated to exert pathogenic effects by resulting in acquisition of aberrant cytotoxic enzyme activity (Andersen 2006a; Dewil et al. 2004; Robberecht 2002). The SOD-1 gene spans 11 kilobases of genomic DNA, comprises five exons and four introns (Levanon et al. 1985), and encodes a highly conserved 153-amino acid long protein, which together with a catalytic copper (Cu) ion and a stabilizing zinc (Zn) ion form a subunit (Andersen 2006; Levanon et al. 1985). A disulfide bridge stabilizes each subunit, and the two identical subunits combine through non-covalent bonds to form the Cu-Zn SOD-1 enzyme. The main function of the SOD-1 enzyme involves free radical scavenging whereby the enzyme catalyses the conversion of the superoxide anion to molecular oxygen and hydrogen peroxide, which in turn is reduced to water by glutathione peroxidase and catalase (Andersen 2006; Fridovich 1986; Shaw and Kuncl 2002). The Cu-Zn SOD enzyme constitutes 0.5–1% of soluble protein in the brain and spinal cord and is located within the cytosol and nucleus and between two mitochondrial membranes (Andersen 2006; Bowling et al. 1995; Pardo et al. 1995).

To date, over 150 different mutations have been reported in the SOD-1 gene, with the majority of being missense mutations, resulting in changes in single amino acids but preserving the SOD-1 protein length. The remaining mutations are either nonsense or deletion mutations that either introduce novel nucleotides or remove existing

nucleotides resulting in alteration of the polypeptide length (Andersen 2006; Dewil et al. 2004; Gros-Louis et al. 2006). An autosomal dominant pattern of inheritance is evident with most mutations, except for the D90A mutation, which may be transmitted in an autosomal recessive manner. Compound heterozygosity has also been reported with SOD1 FALS, whereby two siblings with a slowly progressive ALS phenotype may be carriers of both the D90A and D96N mutations (Hand et al. 2001). The mutations are widely distributed throughout the gene with preponderance for exon 4 and 5 (Andersen 2006; Andersen et al. 2003; Cudkowicz et al. 1997; Radunovic and Leigh 1996). Globally, the most frequent mutation is the substitution of aspartate for alanine (D90A), followed by alanine to valine (A4V) and isoleucine for threonine (I113T) (Andersen 2006; Dewil et al. 2004).

Evidence for a neurotoxic gain of function of the SOD-1 enzyme has been provided by several lines of evidence. Specifically, increased activity of the SOD-1 enzyme was reported in the transgenic SOD-1 mouse models, supporting a toxic gain of function mechanism (Bruijn et al. 1997; Gurney et al. 1994; Ripps et al. 1995; Wong et al. 1995). Of further relevance, SOD-1 knockout mice failed to develop the ALS phenotype, thereby suggesting the importance of SOD-1 enzyme activity in ALS pathogenesis (Reaume et al. 1996). Aberrant biochemical activity of the SOD-1 enzyme has been reported to underlie this toxic gain of function (Andrus et al. 1998; Beckman et al. 1993; Bruijn et al. 1997, 1998, 2004). Specifically, SOD-1 gene mutations reportedly induced structural changes in the SOD-1 enzyme, enabling substrates other than the superoxide anion to gain access to the active center, thereby resulting in increased production of hydroxyl and free radicals (Bogdanov et al. 1998; Liu et al. 1998). In addition, the mutated SOD-1 enzyme also accepts peroxynitrate as a substrate, resulting in nitration of tyrosine residues on critical cellular proteins (Beckman et al. 1993; Beckman and Koppenol 1996; Crow et al. 1997). Ultimately, this aberrant biochemical activity of the SOD-1 enzyme resulted in cell injury and death (Beckman et al. 1993). Importantly, upregulation of protein-tyrosine nitration has been reported in anterior horn cells (Abe et al. 1997; Chou et al. 1996) as well as elevation of 3-nitortyrosine in the spinal cord of ALS patients and transgenic SOD-1 mice (Beal et al. 1997; Ferrante et al. 1997).

Mutations in the SOD-1 gene may also result in improper binding of zinc to the mutated SOD-1 peptide, thereby allowing reduction of SOD-1 bound copper, which in turn results in formation of superoxide anions and cell injury (Estevez et al. 1999). Diminished metal ion binding by the mutated SOD-1 peptide may also release zinc and copper ions, thereby further contributing to neurotoxicity and degeneration (Pasinelli and Brown 2006). Of further relevance, aberrant SOD-1 enzyme activity may result in oxidative stress via upregulation of proinflammatory cytokines (Hensley et al. 2006). Specifically, increased expression of proinflammatory mediators such as nitric oxide, interleukins 1, 6, and 12 has been reported in the SOD-1 mouse model with resultant neurotoxicity to motor neurons in spinal cord preparations (Kim et al. 2006). Importantly, antagonism of these agents with neutralizing antibodies resulted in increased motor neuron survival (Kim et al. 2006).

Alternatively, mutations in the SOD-1 gene may lead to conformational instability and misfolding of the SOD-1 peptide, resulting in formation of toxic

intracellular aggregates. In the transgenic SOD-1 mouse model and human ALS cases, immunoreactive SOD-1 aggregates were reported in motor neurons and glial cells (Bruijn et al. 1998; Jonsson et al. 2006; Zetterstrom et al. 2007). Whether the intracellular aggregates were neurotoxic to motor neurons remains unknown, although a number of possible cytotoxic mechanisms have been proposed, including (i) co-aggregating with vital cellular constitutes, (ii) inhibiting normal proteosome function, and (iii) exerting mechanical or biochemical effects on the cell, such as disruption of axonal transport systems (Bruijn et al. 1997, 1998; Pasinelli and Brown 2006; Williamson and Cleveland 1999).

1.4 Mitochondrial Dysfunction

In conjunction with glutamate excitotoxicity and oxidative stress, there is mounting evidence that mitochondrial dysfunction exerts an important role in the pathophysiology of ALS (Boillee et al. 2006; Chung and Suh 2002; Higgins et al. 2003; Kirkinezos et al. 2005; Lederer et al. 2007; Pasinelli and Brown 2006; Xu et al. 2004). Mitochondria are intracellular organelles whose main function is to generate energy for the cell in the form of ATP. Under conditions of excessive Ca^{2+} load, as may be evident with glutamate excitotoxicity (Dugan and Choi 1994), mitochondrial production of free radicals induces injury of critical neuronal cellular proteins and DNA. In addition, mitochondria remain sensitive to free radical damage at both the protein and DNA level, resulting in further mitochondrial dysfunction (Bowling and Beal 1995). Mitochondrial damage may in turn enhance glutamate excitotoxicity by disrupting the normal resting membrane potential, thereby resulting in a loss of the normal voltage-dependent Mg^{2+}-mediated block of NMDA receptor channels (Heath and Shaw 2002; Shaw and Kuncl 2002).

Mitochondrial degeneration and dysfunction has been reported in ALS patients and in the transgenic SOD-1 mouse model (Higgins et al. 2003; Kong and Xu 1998; Xu et al. 2004). Ultrastructural abnormalities of muscle mitochondria, paracrystalline inclusions, and abnormal cristae have been reported in ALS (Chung and Suh 2002; Comi et al. 1998; Lederer et al. 2007). Dysfunction of mitochondrial enzymes involved in energy generation, such as cytochrome C oxidase and respiratory chain complexes I and IV, as well as downregulation of nuclear genes encoding mitochondrial components within the motor cortex has been reported in ALS (Comi et al. 1998; Fujita et al. 1996; Jung et al. 2002; Kirkinezos et al. 2005; Lederer et al. 2007). Of further relevance, mitochondrial dysfunction including reduction in protein import, impairment in Ca^{2+} sequestering ability, and an exaggerated depolarizing response of the inner mitochondrial membrane to Ca^{2+} stimulation may occur in the presymptomatic stages of ALS (Bilsland et al. 2008; Damiano et al. 2006; Jaiswal et al. 2009; Li et al. 2010; Nguyen et al. 2009). Ultimately, severe damage to the mitochondrial membrane potential, respiration, and electron transfer chain ensues, resulting in reduced ATP synthesis and neurodegeneration (Quinlan 2011).

The transportation and distribution of mitochondria within the neurons appears to be impaired in ALS (Quinlan 2011). Mitochondria are normally highly mobile organelles, evident in both the axons and dendrites (MacAskill et al. 2010). The movement of mitochondria is regulated through Ca^{2+} signalling and synaptic activity (MacAskill et al. 2010). An increase in intracellular Ca^{2+} concentration, as occurs with glutamate excitotoxicity, interrupts the movement of mitochondria within the cell, in particular at the level of the synapse (MacAskill et al. 2009). Abnormalities of mitochondrial distribution and transport have been reported in ALS, with evidence of reduced distribution in the axons and more frequent pauses in mitochondrial movements (Bilsland et al. 2010). Importantly, the slow and fast axonal transport systems, vital for mitochondrial transport, seem to be impaired in ALS and have been linked to glutamate excitotoxicity (Bilsland et al. 2010; De Vos et al. 2007; Quinlan 2011). Ultimately, this interruption in mitochondrial mobility may result in depletion of energy supply in critical neuronal segments, essential for the maintenance of the resting membrane potential and generation of action potentials, with resultant neuronal degeneration.

From a therapeutic perspective, a recent phase II trial of dexpramipexole, a pharmacological agent that enhances mitochondrial function (Cheah and Kiernan 2010), was shown to be effective in slowing ALS disease progression and reducing mortality over a 24-week period (Cudkowicz et al. 2011). Currently, a phase III, multicenter international trial is underway to assess the clinical efficacy of dexpramipexole as add-on therapy to riluzole in ALS (ClinicalTrials.gov-NCT01281189).

2 Non-neuronal Cells and Neurotoxicity in ALS

Dysfunction of nonneuronal cells, astrocytes, and microglia also appears to be important in ALS pathogenesis (Boillee et al. 2006a, b; Haidet-Phillips et al. 2011; Neusch et al. 2007). Molecular studies in the SOD-1 mouse model have established that expression of the mutant SOD-1 protein in either the motor neurons or astrocytes in isolation failed to induce degeneration, thereby suggesting that the pathogenic process in ALS may involve a complex interaction between motor neurons interacting with nonneuronal cells (Gong et al. 2000; Lino et al. 2002; Pramatarova et al. 2001). Underscoring the importance of nonneuronal cells in ALS pathogenesis are findings that motor neuron toxicity appears to be modulated by expression of mutant SOD-1 in nonneuronal cells (Clement et al. 2003). However, mutant SOD-1 expressing nonneuronal cells seem to be more involved in the regulation of disease progression rather than onset of ALS (Beers et al. 2006; Boillee et al. 2006).

Similar to animal models, nonneuronal cells seem also to be an important pathogenic factor in the human disease. Specifically, astrocytes derived from postmortem spinal cord neural progenitor cells (NPCs) of sporadic and familial (SOD1) patients were selectively toxic to motor neurons (Haidet-Phillips et al. 2011). Upregulation of inflammatory genes encompassing chemokines, proinflammatory cytokines and components of the complement cascade, was evident in the neurotoxic astrocytes, further suggesting that neurotoxicity may in

part be mediated by an inflammatory mechanism. In addition, downregulation of mutant and wild-type SOD-1 expression in astrocytes derived from familial and sporadic ALS, respectively, was neuroprotective (Haidet-Phillips et al. 2011). Taken together, these findings suggest that astrocyte-mediated neurotoxicity contributed to neurodegeneration in familial (SOD-1) and sporadic ALS, thereby suggesting that novel cell-based approaches, particularly focusing on nonneuronal supportive cells, may be therapeutic in ALS.

3 Conclusion

Multiple interacting molecular and genetic mechanisms appear to underlie the development of motor neurodegeneration in ALS. Studies in animal models and human ALS have suggested an important role for glutamate neurotoxicity in ALS pathogenesis. Specifically, neurophysiological studies have suggested that cortical hyperexcitability, a biomarker of glutamate neurotoxicity, appears as a primary event in ALS, with motor neuron degeneration mediated via transsynaptic anterograde mechanisms. In conjunction with glutamate excitotoxicity, other molecular processes including mitochondrial dysfunction and abnormalities of axonal transport, together with neurotoxicity of the mutant SOD-1 enzyme, oxidative stress, and dysfunction of nonneuronal supporting cells, such as astrocytes, appear to contribute to ALS pathogenesis. From a therapeutic perspective, further insights into ALS pathogenesis will undoubtedly result in development of novel therapeutic strategies.

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References

Abe, K., Pan, L. H., Watanabe, M., Konno, H., Kato, T., & Itoyama, Y. (1997). Upregulation of protein-tyrosine nitration in the anterior horn cells of amyotrophic lateral sclerosis. *Neurological Research, 19*, 124–128.

Amendola, J., & Durand, J. (2008). Morphological differences between wild-type and transgenic superoxide dismutase 1 lumbar motoneurons in postnatal mice. *The Journal of Comparative Neurology, 511*, 329–341.

Andersen, P. (2006a). Amyotrophic lateral sclerosis genetics with Mendelian inheritance. In *Amyotrophic lateral sclerosis* (2nd ed., pp. 187–207). London: Informa Healthcare.

Andersen, P. M. (2006b). Amyotrophic lateral sclerosis associated with mutations in the CuZn superoxide dismutase gene. *Current Neurology and Neuroscience Reports, 6*, 37–46.

Andersen, P. M., Sims, K. B., Xin, W. W., Kiely, R., O'Neill, G., Ravits, J., et al. (2003). Sixteen novel mutations in the Cu/Zn superoxide dismutase gene in amyotrophic lateral sclerosis: A decade of discoveries, defects and disputes. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 4*, 62–73.

Andrus, P. K., Fleck, T. J., Gurney, M. E., & Hall, E. D. (1998). Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Journal of Neurochemistry, 71*, 2041–2048.

Armand, J. (1982). The origin, course and terminations of corticospinal fibers in various mammals. *Progress in Brain Research, 57*, 329–360.

Azbill, R. D., Mu, X., & Springer, J. E. (2000). Riluzole increases high-affinity glutamate uptake in rat spinal cord synaptosomes. *Brain Research, 871*, 175–180.

Beal, M. F., Ferrante, R. J., Browne, S. E., Matthews, R. T., Kowall, N. W., & Brown, R. H., Jr. (1997). Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis. *Annals of Neurology, 42*, 644–654.

Beckman, J. S., Carson, M., Smith, C. D., & Koppenol, W. H. (1993). ALS, SOD and peroxynitrite. *Nature, 364*, 584.

Beckman, J. S., & Koppenol, W. H. (1996). Nitric oxide, superoxide, and peroxynitrite: The good, the bad, and ugly. *The American Journal of Physiology, 271*, 1424–1437.

Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., et al. (2006). Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 16021–16026.

Bensimon, G., Lacomblez, L., & Meininger, V. (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *The New England Journal of Medicine, 330*, 585–591.

Bilsland, L. G., Nirmalananthan, N., Yip, J., Greensmith, L., & Duchen, M. R. (2008). Expression of mutant SOD1G93A in astrocytes induces functional deficits in motoneuron mitochondria. *Journal of Neurochemistry, 107*, 1271–1283.

Bilsland, L. G., Sahai, E., Kelly, G., Golding, M., Greensmith, L., & Schiavo, G. (2010). Deficits in axonal transport precede ALS symptoms in vivo. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 20523–20528.

Blair, I. P., Williams, K. L., Warraich, S. T., Durnall, J. C., Thoeng, A. D., Manavis, J., et al. (2010). FUS mutations in amyotrophic lateral sclerosis: clinical, pathological, neurophysiological and genetic analysis. *J Neurol Neurosurg Psychiatry, 81*, 1286–1288.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature, 361*, 31–39.

Bogdanov, M. B., Ramos, L. E., Xu, Z., & Beal, M. F. (1998). Elevated "hydroxyl radical" generation in vivo in an animal model of amyotrophic lateral sclerosis. *Journal of Neurochemistry, 71*, 1321–1324.

Boillee, S., Vande Velde, C., & Cleveland, D. W. (2006a). ALS: A disease of motor neurons and their nonneuronal neighbors. *Neuron, 52*, 39–59.

Boillee, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., et al. (2006b). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science, 312*, 1389–1392.

Bondy, S. C., & Lee, D. K. (1993). Oxidative stress induced by glutamate receptor agonists. *Brain Research, 610*, 229–233.

Boston-Howes, W., Gibb, S. L., Williams, E. O., Pasinelli, P., Brown, R. H., Jr., & Trotti, D. (2006). Caspase-3 cleaves and inactivates the glutamate transporter EAAT2. *The Journal of Biological Chemistry, 281*, 14076–14084.

Bowling, A. C., Barkowski, E. E., McKenna-Yasek, D., Sapp, P., Horvitz, H. R., Beal, M. F., et al. (1995). Superoxide dismutase concentration and activity in familial amyotrophic lateral sclerosis. *Journal of Neurochemistry, 64*, 2366–2369.

Bowling, A. C., & Beal, M. F. (1995). Bioenergetic and oxidative stress in neurodegenerative diseases. *Life Sciences, 56*, 1151–1171.

Browne, S. E., Yang, L., DiMauro, J. P., Fuller, S. W., Licata, S. C., & Beal, M. F. (2006). Bioenergetic abnormalities in discrete cerebral motor pathways presage spinal cord pathology in the G93A SOD1 mouse model of ALS. *Neurobiology of Disease, 22*, 599–610.

Bruijn, L. I., Beal, M. F., Becher, M. W., Schulz, J. B., Wong, P. C., Price, D. L., et al. (1997). Elevated free nitrotyrosine levels, but not protein-bound nitrotyrosine or hydroxyl radicals, throughout amyotrophic lateral sclerosis (ALS)-like disease implicate tyrosine nitration as an aberrant in vivo property of one familial ALS-linked superoxide dismutase 1 mutant. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 7606–7611.

Bruijn, L. I., Houseweart, M. K., Kato, S., Anderson, K. L., Anderson, S. D., Ohama, E., et al. (1998). Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science, 281*, 1851–1854.

Bruijn, L. I., Miller, T. M., & Cleveland, D. W. (2004). Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annual Review of Neuroscience, 27*, 723–749.

Caramia, M. D., Cicinelli, P., Paradiso, C., Mariorenzi, R., Zarola, F., Bernardi, G., et al. (1991). Excitability changes of muscular responses to magnetic brain stimulation in patients with central motor disorders. *Electroencephalography and Clinical Neurophysiology, 81*, 243–250.

Charcot, J., & Joffroy, A. (1869). Deux cas d'atrophie musculaire progressive avec lesion de la substance grise et des faisceaux antero-lateraux de la moelle epiniere. *Archives of Physiology, Neurology and Pathology, 2*, 744–754.

Chatterton, J. E., Awobuluyi, M., Premkumar, L. S., Takahashi, H., Talantova, M., Shin, Y., et al. (2002). Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature, 415*, 793–798.

Cheah, B., Vucic, S., Krishnan, A., & Kiernan, M. (2010). Riluzole, neuroprotection and amyotrophic lateral sclerosis. *Current Medicinal Chemistry, 17*, 1942–1959.

Cheah, B. C., & Kiernan, M. C. (2010). Dexpramipexole, the R(+) enantiomer of pramipexole, for the potential treatment of amyotrophic lateral sclerosis. *IDrugs, 13*, 911–920.

Choe, C. U., & Ehrlich, B. E. (2006). The inositol 1,4,5-trisphosphate receptor (IP3R) and its regulators: Sometimes good and sometimes bad teamwork. *Science's STKE, 2006*, re15.

Choi, D. W. (1987). Ionic dependence of glutamate neurotoxicity. *The Journal of Neuroscience, 7*, 369–379.

Chou, S. M., & Norris, F. H. (1993). Amyotrophic lateral sclerosis: Lower motor neuron disease spreading to upper motor neurons. *Muscle & Nerve, 16*, 864–869.

Chou, S. M., Wang, H. S., & Taniguchi, A. (1996). Role of SOD-1 and nitric oxide/cyclic GMP cascade on neurofilament aggregation in ALS/MND. *Journal of the Neurological Sciences, 139*(Suppl), 16–26.

Chung, M. J., & Suh, Y. L. (2002). Ultrastructural changes of mitochondria in the skeletal muscle of patients with amyotrophic lateral sclerosis. *Ultrastructural Pathology, 26*, 3–7.

Ciabarra, A. M., & Sevarino, K. A. (1997). An anti-chi-1 antibody recognizes a heavily glycosylated protein in rat brain. *Brain Research. Molecular Brain Research, 46*, 85–90.

Clement, A. M., Nguyen, M. D., Roberts, E. A., Garcia, M. L., Boillee, S., Rule, M., et al. (2003). Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science, 302*, 113–117.

Comi, G. P., Bordoni, A., Salani, S., Franceschina, L., Sciacco, M., Prelle, A., et al. (1998). Cytochrome c oxidase subunit I microdeletion in a patient with motor neuron disease. *Annals of Neurology, 43*, 110–116.

Cox, L., Kirby, J., & Shaw, P. (2007). Pathogenesis of motor neurone disease. In *The motor neurone disease handbook* (pp. 26–55). Sydney: Australasian Medical.

Crow, J. P., Sampson, J. B., Zhuang, Y., Thompson, J. A., & Beckman, J. S. (1997). Decreased zinc affinity of amyotrophic lateral sclerosis-associated superoxide dismutase mutants leads to enhanced catalysis of tyrosine nitration by peroxynitrite. *Journal of Neurochemistry, 69*, 1936–1944.

Cudkowicz, M., Bozik, M. E., Ingersoll, E. W., Miller, R., Mitsumoto, H., Shefner, J., et al. (2011). The effects of dexpramipexole (KNS-760704) in individuals with amyotrophic lateral sclerosis. *Nature Medicine, 17*, 1652–1656.

Cudkowicz, M. E., McKenna-Yasek, D., Sapp, P. E., Chin, W., Geller, B., Hayden, D. L., et al. (1997). Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. *Annals of Neurology, 41*, 210–221.

Damiano, M., Starkov, A. A., Petri, S., Kipiani, K., Kiaei, M., Mattiazzi, M., et al. (2006). Neural mitochondrial Ca^{2+} capacity impairment precedes the onset of motor symptoms in G93A Cu/Zn-superoxide dismutase mutant mice. *Journal of Neurochemistry, 96*, 1349–1361.

De Vos, K. J., Chapman, A. L., Tennant, M. E., Manser, C., Tudor, E. L., Lau, K.-F., et al. (2007). Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. *Human Molecular Genetics, 16*, 2720–2728.

DeJesus-Hernandez, M., Mackenzie Ian, R., Boeve Bradley, F., Boxer Adam, L., Baker, M., Rutherford Nicola, J., et al. (2011). Expanded GGGGCC hexanucleotide repeat in non-coding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron, 72*, 245–256.

Desiato, M., Bernardi, G., Hagi, A. H., Boffa, L., & Caramia, M. D. (2002). Transcranial magnetic stimulation of motor pathways directed to muscles supplied by cranial nerves in ALS. *Clinical Neurophysiology, 113*, 132–140.

Dewil, M., Andersen, P., Van Den Bosch, L., & Robberecht, W. (2004). Genetics of amyotrophic lateral sclerosis. In *Clinical neurophysiology of motor neuron diseases* (pp. 169–186). Amsterdam: Elsevier.

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacological Reviews, 51*, 7–61.

Dong, H., Zhang, P., Song, I., Petralia, R. S., Liao, D., & Huganir, R. L. (1999). Characterization of the glutamate receptor-interacting proteins GRIP1 and GRIP2. *The Journal of Neuroscience, 19*, 6930–6941.

Dugan, L. L., & Choi, D. W. (1994). Excitotoxicity, free radicals, and cell membrane changes. *Annals of Neurology, 35*, S17–S21.

Eisen, A., Kim, S., & Pant, B. (1992). Amyotrophic lateral sclerosis (ALS): A phylogenetic disease of the corticomotoneuron? *Muscle & Nerve, 15*, 219–224.

Eisen, A., & Kuwabara, S. (2012). The split hand syndrome in amyotrophic lateral sclerosis. *Journal of Neurology, Neurosurgery, and Psychiatry, 83*, 399–403.

Eisen, A., Pant, B., & Stewart, H. (1993). Cortical excitability in amyotrophic lateral sclerosis: A clue to pathogenesis. *The Canadian Journal of Neurological Sciences, 20*, 11–16.

Eisen, A., & Weber, M. (2001). The motor cortex and amyotrophic lateral sclerosis. *Muscle & Nerve, 24*, 564–573.

Eisen, A. A., & Shtybel, W. (1990). AAEM minimonograph #35: Clinical experience with transcranial magnetic stimulation. *Muscle & Nerve, 13*, 995–1011.

Estevez, A. G., Crow, J. P., Sampson, J. B., Reiter, C., Zhuang, Y., Richardson, G. J., et al. (1999). Induction of nitric oxide-dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. *Science, 286*, 2498–2500.

Ferrante, R. J., Shinobu, L. A., Schulz, J. B., Matthews, R. T., Thomas, C. E., Kowall, N. W., et al. (1997). Increased 3-nitrotyrosine and oxidative damage in mice with a human copper/zinc superoxide dismutase mutation. *Annals of Neurology, 42*, 326–334.

Flament, D., Goldsmith, P., Buckley, C. J., & Lemon, R. N. (1993). Task dependence of responses in first dorsal interosseous muscle to magnetic brain stimulation in man. *The Journal of Physiology, 464*, 361–378.

Foerster, B. R., Callaghan, B. C., Petrou, M., Edden, R. A., Chenevert, T. L., & Feldman, E. L. (2012). Decreased motor cortex gamma-aminobutyric acid in amyotrophic lateral sclerosis. *Neurology, 78*, 1596–1600.

Fridovich, I. (1986). Superoxide dismutases. *Advances in Enzymology and Related Areas of Molecular Biology, 58*, 61–97.

Fujita, K., Yamauchi, M., Shibayama, K., Ando, M., Honda, M., & Nagata, Y. (1996). Decreased cytochrome c oxidase activity but unchanged superoxide dismutase and glutathione peroxidase

activities in the spinal cords of patients with amyotrophic lateral sclerosis. *Journal of Neuroscience Research, 45*, 276–281.

Gibb, S. L., Boston-Howes, W., Lavina, Z. S., Gustincich, S., Brown, R. H., Jr., Pasinelli, P., et al. (2007). A caspase-3-cleaved fragment of the glial glutamate transporter EAAT2 is sumoylated and targeted to promyelocytic leukemia nuclear bodies in mutant SOD1-linked amyotrophic lateral sclerosis. *The Journal of Biological Chemistry, 282*, 32480–32490.

Gong, Y. H., Parsadanian, A. S., Andreeva, A., Snider, W. D., & Elliott, J. L. (2000). Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. *The Journal of Neuroscience, 20*, 660–665.

Gonzalez de Aguilar, J. L., Echaniz-Laguna, A., Fergani, A., Rene, F., Meininger, V., Loeffler, J. P., et al. (2007). Amyotrophic lateral sclerosis: All roads lead to Rome. *Journal of Neurochemistry, 101*, 1153–1160.

Gould, T. W., Buss, R. R., Vinsant, S., Prevette, D., Sun, W., Knudson, C. M., et al. (2006). Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *The Journal of Neuroscience, 26*, 8774–8786.

Gowers, W. R. (1886). *Manual of diseases of the nervous system*. London: Churchill.

Gros-Louis, F., Gaspar, C., & Rouleau, G. A. (2006). Genetics of familial and sporadic amyotrophic lateral sclerosis. *Biochimica et Biophysica Acta, 1762*, 956–972.

Gurney, M. E., Cutting, F. B., Zhai, P., Doble, A., Taylor, C. P., Andrus, P. K., et al. (1996). Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. *Annals of Neurology, 39*, 147–157.

Gurney, M. E., Fleck, T. J., Himes, C. S., & Hall, E. D. (1998). Riluzole preserves motor function in a transgenic model of familial amyotrophic lateral sclerosis. *Neurology, 50*, 62–66.

Gurney, M. E., Pu, H., Chiu, A. Y., Dal Canto, M. C., Polchow, C. Y., Alexander, D. D., et al. (1994). Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science, 264*, 1772–1775.

Gutstein, D. E., & Marks, A. R. (1997). Role of inositol 1,4,5-trisphosphate receptors in regulating apoptotic signaling and heart failure. *Heart and Vessels, Suppl 12*, 53–57.

Haidet-Phillips, A. M., Hester, M. E., Miranda, C. J., Meyer, K., Braun, L., Frakes, A., et al. (2011). Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. *Nature Biotechnology, 29*, 824–828.

Hand, C. K., Mayeux-Portas, V., Khoris, J., Briolotti, V., Clavelou, P., Camu, W., et al. (2001). Compound heterozygous D90A and D96N SOD1 mutations in a recessive amyotrophic lateral sclerosis family. *Annals of Neurology, 49*, 267–271.

Heath, P. R., & Shaw, P. J. (2002). Update on the glutamatergic neurotransmitter system and the role of excitotoxicity in amyotrophic lateral sclerosis. *Muscle & Nerve, 26*, 438–458.

Hensley, K., Mhatre, M., Mou, S., Pye, Q. N., Stewart, C., West, M., et al. (2006). On the relation of oxidative stress to neuroinflammation: Lessons learned from the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. *Antioxidants & Redox Signaling, 8*, 2075–2087.

Higgins, C. M., Jung, C., & Xu, Z. (2003). ALS-associated mutant SOD1G93A causes mitochondrial vacuolation by expansion of the intermembrane space and by involvement of SOD1 aggregation and peroxisomes. *BMC Neuroscience, 4*, 16.

Ince, P., Stout, N., Shaw, P., Slade, J., Hunziker, W., Heizmann, C. W., et al. (1993). Parvalbumin and calbindin D-28k in the human motor system and in motor neuron disease. *Neuropathology and Applied Neurobiology, 19*, 291–299.

Ionov, I. D. (2007). Survey of ALS-associated factors potentially promoting Ca(2+) overload of motor neurons. *Amyotrophic Lateral Sclerosis, 8*, 260–265.

Ishii, T., Moriyoshi, K., Sugihara, H., Sakurada, K., Kadotani, H., Yokoi, M., et al. (1993). Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. *The Journal of Biological Chemistry, 268*, 2836–2843.

Jaiswal, M., Zech, W.-D., Goos, M., Leutbecher, C., Ferri, A., Zippelius, A., et al. (2009). Impairment of mitochondrial calcium handling in a mtSOD1 cell culture model of motoneuron disease. *BMC Neuroscience, 10*, 64.

Jonsson, P. A., Graffmo, K. S., Brannstrom, T., Nilsson, P., Andersen, P. M., & Marklund, S. L. (2006). Motor neuron disease in mice expressing the wild type-like D90A mutant superoxide dismutase-1. *Journal of Neuropathology and Experimental Neurology, 65*, 1126–1136.

Jung, C., Higgins, C. M., & Xu, Z. (2002). Mitochondrial electron transport chain complex dysfunction in a transgenic mouse model for amyotrophic lateral sclerosis. *Journal of Neurochemistry, 83*, 535–545.

Kawahara, Y., Ito, K., Sun, H., Aizawa, H., Kanazawa, I., & Kwak, S. (2004). Glutamate receptors: RNA editing and death of motor neurons. *Nature, 427*, 801.

Kiernan, J., & Hudson, A. (1991). Changes in sizes of cortical and lower motor neurons in amyotrophic lateral sclerosis. *Brain, 114*, 843–853.

Kiernan, M. C., Vucic, S., Cheah, B. C., Turner, M. R., Eisen, A., Hardiman, O., et al. (2011). Amyotrophic lateral sclerosis. *Lancet, 377*, 942–955.

Kim, Y. S., Martinez, T., Deshpande, D. M., Drummond, J., Provost-Javier, K., Williams, A., et al. (2006). Correction of humoral derangements from mutant superoxide dismutase 1 spinal cord. *Annals of Neurology, 60*, 716–728.

Kirkinezos, I. G., Bacman, S. R., Hernandez, D., Oca-Cossio, J., Arias, L. J., Perez-Pinzon, M. A., et al. (2005). Cytochrome c association with the inner mitochondrial membrane is impaired in the CNS of G93A-SOD1 mice. *The Journal of Neuroscience, 25*, 164–172.

Komuro, H., & Rakic, P. (1993). Modulation of neuronal migration by NMDA receptors. *Science, 260*, 95–97.

Kong, J., & Xu, Z. (1998). Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *The Journal of Neuroscience, 18*, 3241–3250.

Kutsuwada, T., Kashiwabuchi, N., Mori, H., Sakimura, K., Kushiya, E., Araki, K., et al. (1992). Molecular diversity of the NMDA receptor channel. *Nature, 358*, 36–41.

Kuwabara, S., Sonoo, M., Komori, T., Shimizu, T., Hirashima, F., Inaba, A., et al. (2008). Dissociated small hand muscle atrophy in amyotrophic lateral sclerosis: Frequency, extent, and specificity. *Muscle & Nerve, 37*, 426–430.

Kwak, S., & Kawahara, Y. (2005). Deficient RNA editing of GluR2 and neuronal death in amyotropic lateral sclerosis. *Journal of Molecular Medicine, 83*, 110–120.

Laake, J. H., Slyngstad, T. A., Haug, F. M., & Ottersen, O. P. (1995). Glutamine from glial cells is essential for the maintenance of the nerve terminal pool of glutamate: Immunogold evidence from hippocampal slice cultures. *Journal of Neurochemistry, 65*, 871–881.

Lacomblez, L., Bensimon, G., Leigh, P. N., Guillet, P., & Meininger, V. (1996). Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet, 347*, 1425–1431.

Lederer, C. W., Torrisi, A., Pantelidou, M., Santama, N., & Cavallaro, S. (2007). Pathways and genes differentially expressed in the motor cortex of patients with sporadic amyotrophic lateral sclerosis. *BMC Genomics, 8*, 26.

Lees, G. J. (1993). Contributory mechanisms in the causation of neurodegenerative disorders. *Neuroscience, 54*, 287–322.

Lemon, R. N., & Griffiths, J. (2005). Comparing the function of the corticospinal system in different species: Organizational differences for motor specialization? *Muscle & Nerve, 32*, 261–279.

Levanon, D., Lieman-Hurwitz, J., Dafni, N., Wigderson, M., Sherman, L., Bernstein, Y., et al. (1985). Architecture and anatomy of the chromosomal locus in human chromosome 21 encoding the Cu/Zn superoxide dismutase. *The EMBO Journal, 4*, 77–84.

Li, Q., Vande Velde, C., Israelson, A., Xie, J., Bailey, A. O., Dong, M.-Q., et al. (2010). ALS-linked mutant superoxide dismutase 1 (SOD1) alters mitochondrial protein composition and decreases protein import. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 21146–21151.

Lillo, P., & Hodges, J. R. (2009). Frontotemporal dementia and motor neurone disease: Overlapping clinic-pathological disorders. *Journal of Clinical Neuroscience, 16*, 1131–1135.

Lino, M. M., Schneider, C., & Caroni, P. (2002). Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *The Journal of Neuroscience, 22*, 4825–4832.

Liu, R., Althaus, J. S., Ellerbrock, B. R., Becker, D. A., & Gurney, M. E. (1998). Enhanced oxygen radical production in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Annals of Neurology, 44*, 763–770.

MacAskill, A. F., Atkin, T. A., & Kittler, J. T. (2010). Mitochondrial trafficking and the provision of energy and calcium buffering at excitatory synapses. *The European Journal of Neuroscience, 32*, 231–240.

MacAskill, A. F., Rinholm, J. E., Twelvetrees, A. E., Arancibia-Carcamo, I. L., Muir, J., Fransson, A., et al. (2009). Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. *Neuron, 61*, 541–555.

MacDermott, A. B., Mayer, M. L., Westbrook, G. L., Smith, S. J., & Barker, J. L. (1986). NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature, 321*, 519–522.

Maher, P., & Davis, J. B. (1996). The role of monoamine metabolism in oxidative glutamate toxicity. *The Journal of Neuroscience, 16*, 6394–6401.

Meguro, H., Mori, H., Araki, K., Kushiya, E., Kutsuwada, T., Yamazaki, M., et al. (1992). Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature, 357*, 70–74.

Meldrum, B., & Garthwaite, J. (1990). Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends in Pharmacological Sciences, 11*, 379–387.

Menon, P., Kiernan, M. C., & Vucic, S. (2011). Appearance, phenomenology and diagnostic utility of the split hand in amyotrophic lateral sclerosis. *Neurodegenerative Disease Management, 1*, 457–462.

Michaelis, E. K. (1998). Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. *Progress in Neurobiology, 54*, 369–415.

Miller, R. G., Jackson, C. E., Kasarskis, E. J., England, J. D., Forshew, D., Johnston, W., et al. (2009). Practice parameter update: The care of the patient with amyotrophic lateral sclerosis: multidisciplinary care, symptom management, and cognitive/behavioral impairment (an evidence-based review): Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology, 73*, 1227–1233.

Miller, R. J., Murphy, S. N., & Glaum, S. R. (1989). Neuronal Ca^{2+} channels and their regulation by excitatory amino acids. *Annals of the New York Academy of Sciences, 568*, 149–158.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., et al. (1992). Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science, 256*, 1217–1221.

Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Chou, T. T., et al. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science, 314*, 130–133.

Neusch, C., Bahr, M., & Schneider-Gold, C. (2007). Glia cells in amyotrophic lateral sclerosis: New clues to understanding an old disease? *Muscle & Nerve, 35*, 712–724.

Nguyen, K. T., García-Chacón, L. E., Barrett, J. N., Barrett, E. F., & David, G. (2009). The Psi(m) depolarization that accompanies mitochondrial Ca^{2+} uptake is greater in mutant SOD1 than in wild-type mouse motor terminals. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 2007–2011.

Nihei, K., McKee, A. C., & Kowall, N. W. (1993). Patterns of neuronal degeneration in the motor cortex of amyotrophic lateral sclerosis patients. *Acta Neuropathologica, 86*, 55–64.

Nishi, M., Hinds, H., Lu, H. P., Kawata, M., & Hayashi, Y. (2001). Motoneuron-specific expression of NR3B, a novel NMDA-type glutamate receptor subunit that works in a dominant-negative manner. *The Journal of Neuroscience, 21*, RC185.

Pagani, M. R., Reisin, R. C., & Uchitel, O. D. (2006). Calcium signaling pathways mediating synaptic potentiation triggered by amyotrophic lateral sclerosis IgG in motor nerve terminals. *The Journal of Neuroscience, 26*, 2661–2672.

Pamphlett, R., Kril, J., & Hng, T. (1995). Motor neuron disease: A primary disorder of corticomotoneurons? *Muscle & Nerve, 18*, 314–318.

Pardo, C. A., Xu, Z., Borchelt, D. R., Price, D. L., Sisodia, S. S., & Cleveland, D. W. (1995). Superoxide dismutase is an abundant component in cell bodies, dendrites, and axons of motor neurons and in a subset of other neurons. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 954–958.

Pasinelli, P., & Brown, R. H. (2006). Molecular biology of amyotrophic lateral sclerosis: Insights from genetics. *Nature Reviews Neuroscience, 7*, 710–723.

Patel, S. A., & Maragakis, N. J. (2002). Amyotrophic lateral sclerosis: Pathogenesis, differential diagnoses, and potential interventions. *The Journal of Spinal Cord Medicine, 25*, 262–273.

Pramatarova, A., Laganiere, J., Roussel, J., Brisebois, K., & Rouleau, G. A. (2001). Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *The Journal of Neuroscience, 21*, 3369–3374.

Prout, A. J., & Eisen, A. (1994). The cortical silent period and ALS. *Muscle & Nerve, 17*, 217–223.

Pun, S., Santos, A. F., Saxena, S., Xu, L., & Caroni, P. (2006). Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. *Nature Neuroscience, 9*, 408–419.

Quinlan, K. A. (2011). Links between electrophysiological and molecular pathology of amyotrophic lateral sclerosis. *Integrative and Comparative Biology, 51*, 913–925.

Radunovic, A., & Leigh, P. N. (1996). Cu/Zn superoxide dismutase gene mutations in amyotrophic lateral sclerosis: correlation between genotype and clinical features. *Journal of Neurology, Neurosurgery, and Psychiatry, 61*, 565–572.

Ravits, J., Paul, P., & Jorg, C. (2007). Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. *Neurology, 68*, 1571–1575.

Reaume, A. G., Elliott, J. L., Hoffman, E. K., Kowall, N. W., Ferrante, R. J., Siwek, D. F., et al. (1996). Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nature Genetics, 13*, 43–47.

Regan, R. F., Panter, S. S., Witz, A., Tilly, J. L., & Giffard, R. G. (1995). Ultrastructure of excitotoxic neuronal death in murine cortical culture. *Brain Research, 705*, 188–198.

Renton, A. E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J. R., et al. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron, 72*, 257–268.

Ripps, M. E., Huntley, G. W., Hof, P. R., Morrison, J. H., & Gordon, J. W. (1995). Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 689–693.

Robberecht, W. (2002). Genetics of familial amyotrophic lateral sclerosis and ethical aspects. In *Motor neuron disease* (pp. 75–95). London: W.B. Saunders.

Rothstein, J. D., Jin, L., Dykes-Hoberg, M., & Kuncl, R. W. (1993). Chronic inhibition of glutamate uptake produces a model of slow neurotoxicity. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 6591–6595.

Rothstein, J. D., Patel, S., Regan, M. R., Haenggeli, C., Huang, Y. H., Bergles, D. E., et al. (2005). Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature, 433*, 73–77.

Rothstein, J. D., Van Kammen, M., Levey, A. I., Martin, L. J., & Kuncl, R. W. (1995). Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Annals of Neurology, 38*, 73–84.

Shaw, P., & Kuncl, R. (2002). Current concepts in the pathogenesis of ALS. In *Motor neuron disease* (pp. 37–73). Lodon: WB Saunders.

Siddique, T., Figlewicz, D. A., Pericak-Vance, M. A., Haines, J. L., Rouleau, G., Jeffers, A. J., et al. (1991). Linkage of a gene causing familial amyotrophic lateral sclerosis to chromosome 21 and evidence of genetic-locus heterogeneity. *The New England Journal of Medicine, 324*, 1381–1384.

Simeone, T. A., Sanchez, R. M., & Rho, J. M. (2004). Molecular biology and ontogeny of glutamate receptors in the mammalian central nervous system. *Journal of Child Neurology, 19*, 343–360.

Stys, P. K. (1998). Anoxic and ischemic injury of myelinated axons in CNS white matter: From mechanistic concepts to therapeutics. *Journal of Cerebral Blood Flow and Metabolism, 18*, 2–25.

Takuma, H., Kwak, S., Yoshizawa, T., & Kanazawa, I. (1999). Reduction of GluR2 RNA editing, a molecular change that increases calcium influx through AMPA receptors, selective in the spinal ventral gray of patients with amyotrophic lateral sclerosis. *Annals of Neurology, 46*, 806–815.

Traven, H. G., Brodin, L., Lansner, A., Ekeberg, O., Wallen, P., & Grillner, S. (1993). Computer simulations of NMDA and non-NMDA receptor-mediated synaptic drive: Sensory and supraspinal modulation of neurons and small networks. *Journal of Neurophysiology, 70*, 695–709.

Traynor, B. J. (2012). Road to the chromosome 9p-linked ALS/FTD locus. *Journal of Neurology, Neurosurgery, and Psychiatry, 83*, 356–357.

Trotti, D., Rolfs, A., Danbolt, N. C., Brown, R. H., Jr., & Hediger, M. A. (1999). SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. *Nature Neuroscience, 2*, 848.

Turner, M. R., Kiernan, M. C., Leigh, P. N., & Talbot, K. (2009). Biomarkers in amyotrophic lateral sclerosis. *Lancet Neurology, 8*, 94–109.

Van Damme, P., Braeken, D., Callewaert, G., Robberecht, W., & Van Den Bosch, L. (2005). GluR2 deficiency accelerates motor neuron degeneration in a mouse model of amyotrophic lateral sclerosis. *Journal of Neuropathology and Experimental Neurology, 64*, 605–612.

Van Damme, P., Van Den Bosch, L., Van Houtte, E., Callewaert, G., & Robberecht, W. (2002). GluR2-dependent properties of AMPA receptors determine the selective vulnerability of motor neurons to excitotoxicity. *Journal of Neurophysiology, 88*, 1279–1287.

van Es, M. A., Van Vught, P. W., Blauw, H. M., Franke, L., Saris, C. G., Andersen, P. M., et al. (2007). ITPR2 as a susceptibility gene in sporadic amyotrophic lateral sclerosis: A genome-wide association study. *Lancet Neurology, 6*, 869.

Vandenberg, R. J. (1998). Molecular pharmacology and physiology of glutamate transporters in the central nervous system. *Clinical and Experimental Pharmacology & Physiology, 25*, 393–400.

Vucic, S., Burke, D., & Kiernan, M. C. (2007). Diagnosis of motor neuron disease. In *The motor neuron disease handbook* (pp. 89–115). Sydney: Australasian Medical.

Vucic, S., & Kiernan, M. (2009). Pathophysiology of degeneration in familial amyotrophic lateral sclerosis. *Current Molecular Medicine, 9*, 255–272.

Vucic, S., & Kiernan, M. C. (2006). Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. *Brain, 129*, 2436–2446.

Vucic, S., & Kiernan, M. C. (2008). Cortical excitability testing distinguishes Kennedy's disease from amyotrophic lateral sclerosis. *Clinical Neurophysiology, 119*, 1088–1096.

Vucic, S., & Kiernan, M. C. (2010). Upregulation of persistent sodium conductances in familial ALS. *Journal of Neurology, Neurosurgery, and Psychiatry, 81*, 222–227.

Vucic, S., Nicholson, G. A., & Kiernan, M. C. (2008). Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. *Brain, 131*, 1540–1550.

Wang, S. J., Wang, K. Y., & Wang, W. C. (2004). Mechanisms underlying the riluzole inhibition of glutamate release from rat cerebral cortex nerve terminals (synaptosomes). *Neuroscience, 125*, 191–201.

Watanabe, M., Inoue, Y., Sakimura, K., & Mishina, M. (1993a). Distinct distributions of five *N*-methyl-D-aspartate receptor channel subunit mRNAs in the forebrain. *The Journal of Comparative Neurology, 338*, 377–390.

Watanabe, M., Inoue, Y., Sakimura, K., & Mishina, M. (1993b). Distinct spatio-temporal distributions of the NMDA receptor channel subunit mRNAs in the brain. *Annals of the New York Academy of Sciences, 707*, 463–466.

Watanabe, M., Mishina, M., & Inoue, Y. (1994a). Distinct distributions of five NMDA receptor channel subunit mRNAs in the brainstem. *The Journal of Comparative Neurology, 343*, 520–531.

Watanabe, M., Mishina, M., & Inoue, Y. (1994b). Distinct spatiotemporal expressions of five NMDA receptor channel subunit mRNAs in the cerebellum. *The Journal of Comparative Neurology, 343*, 513–519.

Watkins, J. C., & Evans, R. H. (1981). Excitatory amino acid transmitters. *Annual Review of Pharmacology and Toxicology, 21*, 165–204.

Wilbourn, A. J. (2000). The "split hand syndrome". *Muscle & Nerve, 23*, 138.

Williamson, T. L., & Cleveland, D. W. (1999). Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nature Neuroscience, 2*, 50–56.

Winhammar, J. M., Rowe, D. B., Henderson, R. D., & Kiernan, M. C. (2005). Assessment of disease progression in motor neuron disease. *Lancet Neurology, 4*, 229–238.

Wong, P. C., Pardo, C. A., Borchelt, D. R., Lee, M. K., Copeland, N. G., Jenkins, N. A., et al. (1995). An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron, 14*, 1105–1116.

Xu, Z., Jung, C., Higgins, C., Levine, J., & Kong, J. (2004). Mitochondrial degeneration in amyotrophic lateral sclerosis. *Journal of Bioenergetics and Biomembranes, 36*, 395–399.

Zetterstrom, P., Stewart, H. G., Bergemalm, D., Jonsson, P. A., Graffmo, K. S., Andersen, P. M., et al. (2007). Soluble misfolded subfractions of mutant superoxide dismutase-1s are enriched in spinal cords throughout life in murine ALS models. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 14157–14162.

Neurotoxicity and Neuroprotection in Spinal Cord Injury

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Contents

Abstract

In the present chapter, the members of the neurotrophin family of neurotrophic factors and their receptors are first presented. This first section is then followed by a description of the neurotrophin-mediated signalling pathways that regulate cell survival, with particular emphasis on the "signalling endosome model" of intracellular signalling. The main functions of these neurotrophin-mediated signalling pathways are then described, and evidence that neurotrophic factors have the capacity to protect spinally projecting neurons against cell death and atrophy is subsequently examined. In this context, the neurotrophin-associated molecular

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cascades of events that lead to the regulation of cell survival are presented. The protective role of the different neurotrophic factors for injured spinal cord-projecting neurons and their capacity to upregulate regeneration-associated genes is subsequently examined. The regenerative property of neurotrophins on lesioned sensory and motor axons is then introduced, and evidence that the delivery of neurotrophic factors, if available in sufficient concentration, can outweigh the effect of inhibitory molecules present in the scar tissue is reviewed. Finally, the different strategies currently under investigation to deliver neurotrophin to the injured spinal cord are reviewed. Here, special attention is given to the evidence for and against a potential role for neurotrophins in the recovery of motor function.

Keywords
Motor function • Neurotrophic factors • Neurotrophin • Regeneration • Spinal cord injury • Spinal cord-projecting neurons

1 Introduction

The propensity of the adult spinal cord to regenerate is only limited, and damage that disrupts the long myelinated fiber pathways within the cord results in permanent functional deficits. Over the last three decades, research in neuroscience has identified a set of pathophysiological events that occur within the injured spinal cord tissue and that contribute to halt axonal regeneration. One of the obstacles to axonal growth through the site of injury is the presence, in the glial scar, of potent growth inhibitory extracellular matrix molecules such as chondroitin and keratan sulfate proteoglycans (for a review, see Bradbury and Carter 2011). Another major impediment to axonal regeneration through traumatic damage to the cord is the existence of the myelin-associated axon growth inhibitory molecules Nogo-A, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp) (recently reviewed by Sharma et al. 2012). However, the presence of these inhibitory molecules within the damaged tissue is only part of the reason why injured axons fail to regenerate through a spinal cord injury. Indeed, a large population of spinal cord-projecting neurons that are axotomized after a spinal cord injury have been shown to exhibit a downregulation of genes that are directly involved in axonal regeneration (e.g., GAP-43 and Tα1 Tubulin), and this change in gene expression coincides with neuronal death and atrophy (Tetzlaff et al. 1991).

In the developing central nervous system (CNS), where they abound, neurotrophins contribute to neuronal growth and survival and to the formation of synaptic pathways. In the mature CNS, however, neurotrophin expression is downregulated, with the exception of brain regions that retain high levels of functional plasticity (Maisonpierre et al. 1990b). It has been suggested that the powerful role played by neurotrophins in development could be harnessed for the treatment of several neurological conditions including injury to the adult spinal cord. This present chapter will first present an overview of the neurotrophin family of neurotrophic factors and their receptors as

well as a brief description of the diverse neurotrophin-mediated signalling pathways that regulate cell survival. This chapter will also examine work that shows that neurotrophins have the capacity to protect spinally projecting neurons against cell death and atrophy as well as to revert the downregulation of regeneration-associated genes that occurs following a spinal cord injury. In this chapter, evidence that the delivery of neurotrophins to the injured spinal cord can outweigh the effect of growth inhibitory molecules present at the injury site will be subsequently reviewed. Moreover, data that demonstrate that the delivery of neurotrophins in the injured cord can promote axonal regeneration through scar tissue will be considered. Finally, this chapter will discuss issues related to recovery of function (or lack thereof) that is reported to accompany axonal regeneration after neurotrophin treatment of the injured spinal cord.

2 Members of the Neurotrophin Family of Neurotrophic Factors and Their Receptors

Some of the pro-survival signals that specifically act on neuronal cells belong to a family of target-derived regulatory proteins called neurotrophins, whose main members include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). Nerve growth factor (NGF), isolated from snake venom, was the first neurotrophin to be identified (Cohen et al. 1954). Decades later, brain-derived neurotrophic factor (BDNF) was subsequently purified from pig brain tissue (Barde et al. 1982). Years later, neurotrophin-3 (NT-3) was simultaneously characterized by several groups of researchers with the use of molecular cloning techniques (Enfors et al. 1990; Hohn et al. 1990; Rosenthal et al. 1990; Jones and Reichardt 1990; Kaisho et al. 1990; Maisonpierre et al. 1990a). With these techniques, another member of the neurotrophin family, neurotrophin-4/5 (NT-4/5), was identified the following year (Berkemeier et al. 1991; Hallböök et al. 1991). More recently, neurotrophic factor-6 (NT-6) and neurotrophic factor-7 (NT-7), both satisfying the structural criteria that characterize the neurotrophin family, were cloned from a genomic DNA of the platyfish *Xiphophorus maculatus* and the fish *Cyprinus carpio*, respectively (Götz et al. 1994; Lai et al. 1998).

Each of these small proteins exhibits a unique molecular profile that determines its particular affinity with one subtype of transmembrane tyrosine kinase (Trk) receptor (recently reviewed in Skaper, 2012a). For instance, TrkA is the specific receptor for NGF (Kaplan et al. 1991; Klein et al. 1991a), while TrkB binds BDNF and NT-4/5 (Klein et al. 1991b; Soppet et al. 1991). NT-3 preferentially interacts with TrkC (Lamballe et al. 1991), but it also bind to TrkA and TrkB although with considerably less efficiency (Glass et al. 1991; Squinto et al. 1991; Klein et al. 1991b; Ip et al. 1993). Thus, the neurotrophins exert their trophic activity through the interaction with transmembrane tyrosine kinase (Trk) receptors to which they bind with distinct as well as with some overlapping degree of specificity (see Ip et al. 1993). The biological action of the different members of the neurotrophin family can also be modulated by the low-affinity nerve growth factor receptor or $p75^{NTR}$. The $p75^{NTR}$ can bind neurotrophins either alone or as a co-receptor that

regulates ligand binding to Trk receptors (Rodriguez-Tébar et al. 1990; Hempstead et al. 1991; Frade and Barde 1998; Bibel et al. 1999).

3 Neurotrophin/Trk Receptor Intracellular Signalling

The binding of target-derived molecules of neurotrophins to the extracellular region of two Trk receptor molecules at nerve terminals causes the receptors to associate into active dimers (Ullrich and Schlessinger 1990). In this process, the two Trk dimers are cross-linked through the dimeric ligand but they do not make contact with each other directly (Wehrman et al. 2007). Ligand-induced dimerization of the receptor subsequently leads to phosphorylation of several tyrosine kinase residues within the intracellular domain of the receptor. This process results in the recruitment and activation of a number of downstream signalling effector molecules that initiate the intracellular signalling cascade leading to gene expression and activation (Stephens et al. 1994; Cunningham and Greene 1998).

How the neurotrophin/Trk signal triggered at distal nerve terminals is forwarded to the cell body to elicit neuronal responses has been the focus of much investigation. Although at least two alternative models have been proposed (e.g., Ginty and Segal 2002; Ibáñez 2007), there is mounting evidence in support of the "signalling endosome model" (for a recent review, see Chowdary et al. 2012). This model envisions that the activated receptors are internalized through an endocytosic pathway involving clathrin (Howe et al. 2001; Howe and Mobley 2005; Zheng et al. 2008). These ligand/receptor complexes are then packaged with their downstream signalling molecules into neurotrophic factor receptor-associated protein (NTRAP)-mediated specialized membrane-bound vesicles called signalling endosomes (Delcroix et al. 2003; Fu et al. 2010; for related recent articles, see Philippidou et al. 2010; Harrington 2011). These signalling endosomes, which serve as signalling platforms, are subsequently translocated in a retrograde fashion, i.e., from the distal end of axons to the cell soma by dynein-dependent motors along the microtubule tracks (Bhattacharrya et al. 2002; Ha et al. 2008). A similar endosome signalling mechanism has been proposed for the anterograde transport of BDNF from the dendrite to the nucleus (Cohen et al. 2010).

Endosome-bound neurotrophin/Trk receptors complexes mediate several signalling pathways. For instance, they activate the Ras/Erk pathway through the phosphorylation of the cytoplasmic "switch" G-protein Ras that subsequently binds and promotes the translocation of the Raf protein to the plasma membrane (Wennerberg et al. 2005; Reichardt 2006). Raf then activates MEK, which in turn recruits Erk (see Lemmon and Schlessinger 2010, Reichardt 2006 and Huang and Reichardt 2003, for detailed reviews on the Trk-activated signalling pathways). The PI3K/Akt signalling pathway is recruited by the activation of the cytoplasmic "switch" Ras and/or the adaptor protein Gab1 that in turn activates Pl3K (Rodriguez-Viciana et al. 1994; Holgado-Madruga et al. 1997). Activated Pl3K subsequently activates Akt (Franke et al. 1995). Neurotrophin-dependent Trk activation can also initiate other

intracellular signalling cascades such as phospholipase C (PLC-γ1). A downstream effect of the PLC-γ1 pathway is the release of Ca^{2+} from reticulum endoplasmic stores, therefore providing a point of convergence between neurotrophin and depolarization-induced cell signalling (Mizoguchi et al. 2002; Blanquet and Lamour 1997; Vaillant et al. 1999). On the other hand, cyclic AMP/protein kinase A (cAMP/PKA) signalling can also be activated by neurotrophin-recruited Trk receptors.

4 Neurotrophins as Cell Survival Regulators

Some of the critical functions of these neurotrophin-mediated signalling pathways are to mediate neuronal survival (Levi-Montalcini 1987; Gosh et al. 1994; Fagan et al. 1996), neurite outgrowth and differentiation (Segal et al. 1995; Barnabé-Heider and Miller 2003), and synaptic plasticity (Canossa et al. 2001; Yamada et al. 2002). A potent role of the PI3K/Akt pathway in neuronal survival (Barnabé-Heider and Miller 2003; Atwal et al. 2000; Vaillant et al. 1999; Holgado-Madruga et al. 1997; Dudek et al. 1997) and in synaptic plasticity (Lin et al. 2001) is well established. The Ras/ERK pathway also contributes to neuronal survival (Impey et al. 1998; Watson et al. 2001), although it seems to be involved mainly in neurogenesis, neuronal growth, and differentiation as well as in axonal elongation (Krishna and Narang 2008; Barnabé-Heider and Miller 2003; Markus et al. 2002; Atwal et al. 2000). The PLC-γ1 signalling pathway is not completely understood but there is evidence that it is involved in synaptic plasticity and in the release of neurotrophin-mediated neurotrophin (Canossa et al. 2001; Yamada et al. 2002; Choi et al. 2001; Stephens et al. 1994). As to the cAMP/PKA and Ca^{2+}/CaM signalling cascades, there is evidence to suggest that they play a role in neurite extension and outgrowth (Cai et al. 1999; Aglash et al. 2008; Spencer et al. 2008; for a recent review, see Tedeschi 2011).

How does neurotrophin signalling regulate cell survival? The neurotrophin-activated signalling pathways discussed above reach a point of convergence in the cell nucleus where they are potent activators of the nuclear transcription factor cyclic AMP response element-binding protein (CREB). Indeed, neurotrophin-activated Ras/Erk, PI3K/AKT, PLC-γ, cAMP/PKA, and Ca^{2+}/CaM pathways all mediate the phosphorylation of CREB at Serine 133 (Gonzalez and Montminy 1989; Du and Montminy 1998; Perkinton et al. 2002; reviewed by Lonze and Ginty 2002; Arthur et al. 2004; Spencer et al. 2008). Phosphorylation of CREB subsequently regulates the transcription of the antiapoptotic protein Bcl-2 (Riccio et al. 1999; Bonni et al. 1999; Saini et al. 2004) which, in turn, inhibits apoptosis (reviewed by Yuan and Yankner 2000 and Benn and Woolf 2004). Furthermore, survival is mediated by the activation of Akt, a key component of the PI3K/AKT pathway that leads to the phosphorylation of BAD and Forkhead, two potent effectors that inhibit the proapoptotic pathway (Datta et al. 1997; Brunet et al. 1999). Conversely, activation of the Ras/Erk pathway recruits protein kinase Rsk, the phosphorylation of which also results in the deactivation of the proapoptotic protein Bad (Nebrada and Gavin 1999).

Through their different intracellular signalling pathways, neurotrophins are able to promote cell survival in both a transcription-dependent (e.g., through the recruitment of the transcription factor CREB) and an independent (e.g., through the inhibition of the cell's intrinsic death machinery) manner, therefore shifting the survival/death equilibrium towards survival during ontogenesis as well as throughout adulthood (Finkbeiner et al. 1997).

5 Neurotrophins Confer Neuroprotection to Injured Spinal Cord-Projecting Neurons

Corticospinal neurons are located in the sensorimotor cortex and give rise to the corticospinal tract (CST). On the other hand, rubrospinal neurons are found in the red nucleus, a midbrain structure from which the rubrospinal tract (RST) originates. Injury to these long descending fiber tracts results in motor dysfunction below the lesion site. In the adult spinal cord, there is little evidence to suggest that spontaneous regeneration of these motor pathways occurs and that axotomized corticospinal and rubrospinal neurons reinnervate their former target fields (but see Hiebert et al. 2000). What are the response of corticospinal and rubrospinal neurons to axonal transection? It has been long been put forward that the neurons of origin of the rubrospinal tract undergo chromatolysis in reaction to axotomy (Egan et al. 1977). It has also been observed that, in both the sensorimotor cortex and in the red nucleus, the severity of the pathological response is distance specific with more severe pathological reaction, e.g., atrophy and death observed when axonal damage is closer to the cell soma (Egan et al. 1977; Kobayashi et al. 1997).

Giehl and Tetzlaff (1996) showed that axotomy at the level of the internal capsule (i.e., short distance from cell soma) induces death in nearly half the corticospinal neurons, with the surviving neurons displaying severe atrophy (see also Fernandes et al. 1999). However, there is now mounting evidence that the death of axotomized corticospinal neurons can be fully prevented by intraparenchymal delivery of BDNF and NT-3 during the first week after the injury. Indeed, application of BDNF completely alleviates atrophy of corticospinal, whereas the delivery of NT-3 only partially reverses it (Giehl and Tetzlaff 1996). In the same experimental paradigm, the delivery of BDNF, but not NT-3, during the first 2 weeks after the injury promotes the survival of corticospinal neurons for up to 42 days without further exogenous supply of neurotrophins (Hammond et al. 1999). These data suggest that early treatment with BDNF can trigger long-term downstream mechanisms that protect neurons from axotomy-induced death (Hammond et al. 1999).

Infusion of BDNF or NT-4/5 applied on transected rubrospinal fibers (Kobayashi et al. 1997; Bregman et al. 1998) or in the red nucleus (Bretzner et al. 2008) also prevents atrophy of rat rubrospinal neurons. Interestingly, the same group of co-workers have provided evidence that BDNF applied to the cell soma of axotomized rubrospinal neurons promotes their survival up to 1 year after the injury

(Kwon et al. 2002), whereas delayed application of BDNF on truncated rubrospinal fibers (i.e., 2 months after axotomy) failed to reverse atrophy (Kwon et al. 2004). The latter immunohistochemical analysis by Kwon and colleagues revealed that TrkB receptors remain present on the cell bodies of rubrospinal neurons, but not on their axons after axotomy, therefore providing one explanation as to why delayed application of BDNF on truncated rubrospinal axons failed to reverse atrophy (Kwon et al. 2004). The post-axotomy delivery of BDNF, either to the red nucleus or to its transected fibers, has also been reported to reverse neuronal atrophy (Liu et al. 2002; Tobias et al. 2003; Ruitenberg et al. 2004).

6 Neurotrophins Upregulate Regeneration-Associated Genes in the Injured Spinal Cord

Axotomy of rubrospinal and corticospinal neurons is accompanied by changes in expression of several genes that are directly involved in regeneration (for a recent review, see Gerin et al. 2011). These include the genes encoding for growth-associated proteins GAP-43 and SCG10, which are known to be involved in the regulation of actin (Frey et al. 2000) and microtubule (Reiderer et al. 1997) dynamics in growth cones during axonal regeneration. A study by Mason and co-workers has demonstrated by means of in situ hybridization that both Gap-43 and SCG10 were upregulated after intracortical lesions that axotomized corticospinal neurons (Mason et al. 2003). Increased GAP-43 mRNA expression in rubrospinal neurons was also reported shortly after axotomy performed at cervical level 3 (C3) (Tetzlaff et al. 1991; Fernandes et al. 1999; Storer and Houle 2003). GAP-43 upregulation is accompanied by an increase in the mRNA encoding both Tα1- and βII-tubulin (Tetzlaff et al. 1991; Storer and Houle 2003), two microtubule proteins that are essential for neurite growth in both developing and regenerating neurons (Hoffman 1989; Gloster et al. 1994; Fausett and Goldman 2006). In the second week after axotomy, however, rubrospinal neurons exhibit a decline in GAP-43 and Tα1-tubulin expression that coincides with neuronal atrophy (Tetzlaff et al. 1991). Interestingly, the delivery of BDNF and NT-4/5 can maintain the upregulation of GAP-43 and Tα1-tubulin mRNAs expressed by axotomized rubrospinal neurons (Kobayashi et al. 1997; Kwon et al. 2007). The upregulation of GAP-43 and βII- tubulin mRNAs in rubrospinal neurons has also been observed after the application of GDNF, but not BDNF, directly on the truncated axons (Storer et al. 2003). Together, these results show that corticospinal and rubrospinal neurons have the intrinsic capacity to upregulate genes that encode growth-associated proteins in a manner that is similar to that exhibited by neurons capable of regeneration (Hoffman and Cleveland 1988; Miller et al. 1989). These data also suggest that the application of neurotrophins to injured spinally projecting neurons can maintain the axotomy-induced expression of growth-associated genes beyond the second week after the injury.

7 Neurotrophins Outweigh the Effects of Inhibitory Molecules in Spinal Cord Injury

The inability of axotomized spinally projecting neurons to regenerate has been attributed, at least partly, to myelin-derived axonal growth inhibitory factors present at the injury site (for reviews, see Silver and Miller 2004; Chaudhry and Filbin 2007; Sharma et al. 2012). Indeed, several myelin-associated inhibitory molecules are able to limit neurite outgrowth and to mediate growth cone collapse. These include Nogo-A (Chen et al. 2000), myelin-associated glycoprotein (MAG) (McKerrecher et al. 1994; Mukhopadhyay et al. 1994), and oligodendrocyte myelin glycoprotein (OMgp) (Mikol and Stefansson 1988; Mikol et al. 1990; recently reviewed in Skaper 2012b). On the other hand, glial scar tissue represents a powerful obstacle to axonal elongation through the lesion, with potent inhibitory extracellular matrix molecules such as chondroitin- and keratan-sulfate proteoglycans produced by reactive astrocytes (Gallo et al. 1987; Gallo and Bertolotto 1990; but see Rolls et al. 2009) and the repellent proteins semaphorin 3 (Pasterkamp et al. 1999; Pasterkamp and Verhaagen 2006; for a recent review, see Pastekamp 2012) and ephrins (Xu et al. 1999; Mellitzer et al. 1999). Since the initial identification of these above-mentioned inhibitory molecules, a wealth of effort has been deployed to reverse the effect of myelin- and scar-derived inhibitory molecule, with strategies such as blocking the effect of Nogo with antibodies (Merkler et al. 2001; GrandPré et al. 2002; Li and Strittmatter 2003; Weinmann et al. 2006; for a review, see Onose et al. 2006), delivering MAG (Vyas et al. 2005) and ephrin (Goldshmit et al. 2004) antagonists and enzymatically digesting chondroitin-sulfate proteoglycans with chondroitinase ABC (Brückner et al. 1999; Starkey et al. 2012; for a recent review, see Bradbury and Carter 2011).

Once thought to be an impenetrable barrier to axonal regeneration, the action of the different inhibitory molecules above mentioned can be counteracted by enhancing the intrinsic growth capacity of axotomized neurons (for recent reviews, see Giger et al. 2010; Hollis and Tuszynski 2011). The regenerative effect of neurotrophins has been extensively studied in rat models of spinal cord injury (for a recent review, see Bo et al. 2011). In the majority of these studies, gene therapy is selected as the method of choice for the delivery of neurotrophins. Briefly, the gene encoding the neurotrophin of choice is cloned into replication-deficient viral vectors that can be directly delivered at the site of injury (Schnell et al. 1994; Houweling et al. 1998; Namiki et al. 2000; Blits et al. 2003; Koda et al. 2004). In most cases, however, vehicle cells such as fibroblasts (Grill et al., 1997a, b; McTigue et al. 1998; Tobias et al. 2003; Tuszynski et al. 2003; Zhou et al. 2003), permissive autologous bone marrow stromal cells (Koda et al. 2007; Lu et al. 2012), Schwann cells (Xu et al. 1995), neuronal and glial progenitors (Bonner et al. 2010), and olfactory ensheathing glial cells (e.g., Ruitenberg et al. 2003) are modified by viral-mediated transfer of the gene sequence for BDNF. Such virally transduced cells are known to produce biologically active neurotrophins in vitro (e.g., Liu et al. 1999;

Jin et al. 2002; Ruitenberg et al. 2003). These genetically engineered cells are then implanted acutely into the cavity created by the spinal cord injury where they are known to release the therapeutic agent (e.g., Lu et al. 2005). Such BDNF-expressing cell implants have been reported to fill the lesion cavity and to survive in the spinal cord and express the transgene for up to 4 months (Ruitenberg et al. 2003; Tobias et al. 2003).

8 Regenerative Property of Neurotrophins on Lesioned Motor Axons

Several groups of researchers have reported that grafts of cells genetically modified to express BDNF create a permissive environment for the injured spinal cord and in particular for the rubrospinal tract. The regenerative properties of engineered cell grafts can be observed even if they are transplanted several weeks after the injury (Tobias et al. 2003). However, the extent to which these BDNF-secreting cell grafts promote axonal growth is somewhat inconsistent. For example, extensive axonal growth has been reported (Lu et al. 2005), sometime extending well outside the boundaries of the BDNF-producing cell implant (Liu et al. 1999, 2002), whereas only modest axonal growth (i.e., within the limits of the implant) has been observed by others (Namiki et al. 2000; Jin et al. 2002; Tobias et al. 2003, 2005; Ruitenberg et al. 2003; Koda et al. 2007). Axons found within the cellular grafts have been shown to be positive for GAP-43 (Tobias et al. 2003, 2005; Koda et al. 2007) and for the marker for axonal neurofilaments TR-97 (Tobias et al. 2003, 2005), therefore providing evidence of motor axonal regeneration. It is worth mentioning that BDNF as well as NT-3-induced axonal regeneration has been associated with oligodendrocyte proliferation and enhanced myelination of ingrowing motor axons (McTigue et al. 1998).

Nearly two decades ago, Schnell and co-workers have reported that injections of NT-3, but not BDNF, result in modest regenerative sprouting of the CST (Schnell et al. 1994). Similar results were obtained a few years later with NT-3 dissolved into a collagen matrix that was implanted in the lesioned spinal cord (Houweling et al. 1998). As is the case for BDNF, viral-mediated gene therapy involving NT-3 as the transgene has been widely used in acute and chronic animal models of spinal cord injury. Grill and co-workers (1997b) implanted primary rat fibroblasts genetically modified ex vivo to express NT-3 in dorsal spinal cord hemisections that include the CST. Three months later, significant CST growth was reported at, as well as for up to 8 mm distal to, the lesion site, showing the therapeutic benefits of NT-3 in this descending motor fiber pathway (Grill et al. 1997b). Grafts of NT-3-expressing fibroblasts in the injured cord also induce proliferation of oligodendrocytes and encourage myelinogenesis (McTigue et al. 1998). In a study performed by Tuszynsky and co-workers (2003), primary fibroblasts genetically modified to express human NT-3 were implanted in and distal to the lesion cavity after chronic

spinal cord injury. With this dual implantation protocol (e.g., in and distal to the lesion), the elongation and arborization of CST axons was observed up to 15 mm distal to the injury site. Multiple combinational therapy, with cAMP injections into brainstem reticular motor neuron area to encourage their growth state, implants of bone marrow stromal cells to provide a cellular bridge through the spinal cord lesions, and the delivery of BDNF-expressing viral vectors caudal to the lesion sites to attract axonal growth, has recently been performed (Lu et al. 2012). This complex combinational treatment leads to motor axon regeneration through and beyond the limits of the implant, with some evidence of synapse formation with BDNF-expressing neurons caudal to the implant.

Retrograde neural tracers are known to be internalized by nerve endings and transported in a retrograde fashion to the corresponding spinal cord motor neurons (Tosolini and Morris 2012). The retrograde machinery of neurons can also be used to deliver different transgenes to spinal cord motor neurons (Morris et al. 2004). Intramuscular injections of viral vectors containing the gene sequence for diverse neurotrophins have been used as an alternative method to cell-mediated delivery of neurotrophic genes in the spinal cord (Ghage et al. 1995; Baumgartner and Shine 1998a, b; Nakajima et al. 2007; Uchida et al. 2008, 2012; Zhang et al. 2010). Interestingly, it is not necessary to target all motor neurons to achieve sustainable levels of chemoattractant, as non-transduced motor neurons in the vicinity have been shown to internalize neurotrophins through a paracrine mechanism (Baumgartner and Shine 1997). This "bystander" effect greatly enhances the efficacy of neurotrophin gene interventions that take advantage of the retrograde machinery of neurons (e.g., Aharoni et al. 2005). Virally mediated retrograde delivery of the gene sequence for BDNF has been reported to protect motor neurons from apoptosis after a spinal cord injury in rats (Nakajima et al. 2007, 2010) and mice (Uchida et al. 2008, 2012). However, although the delivery and expression of BDNF in motor neurons near a spinal cord lesion should presumably encourage axonal regeneration, no evidence supporting this assumption is currently available. On the other hand, adenoviral vector carrying the gene sequence for NT-3 has been delivered to level motor neurons at lumbar levels via injections in the sciatic nerve in a rat model of unilateral CST lesion at the level of the hindbrain (Zhou et al. 2003). In this study, sprouting from the intact CST (i.e., contralateral to the injection of NT-3-expressing viral vector) was reported to cross the midline at lumbar levels L3/6, suggesting that this particular observation is an instance of NT-3-mediated axonal plasticity rather than true regeneration. The effect of implant-mediated transgenic delivery of NGF, GDNF, and NT-4/5 has been less investigated. However, cellular implants secreting NGF (Grill et al. 1997a; McTigue et al. 1998; Jones et al. 2003), GDNF (Blesch and Tuszynski 2003), and NT-4/5 (Blesch et al. 2004) have been shown to produce significant regeneration of specific motor pathways through the implants. Taken together, these data provide evidence that the delivery of growth-promoting agents such as neurotrophins, if available in sufficient concentration, can outweigh the effect of inhibitory molecules present in the scar tissue.

9 Regenerative Properties of Neurotrophins for Lesioned Sensory Axons

Rhizotomy, or crush lesions of the dorsal roots, has been used extensively to study sensory axon regeneration within the spinal cord (for a recent review, see Smith et al. 2012). In these injury models, centrally projecting sensory axons regenerate rapidly until they reach the transition between the peripheral and central nervous system, an area called the dorsal root entry zone (DREZ). Several neurotrophin therapies have been conducted in an effort to encourage sensory axons to reenter the spinal cord after dorsal root crush. After such lesions, intraspinal infusion of NGF (Odega and Hagg 1996; Ramer et al. 2000), NT-3 (Zhang et al. 1998; Ramer et al. 2000, 2001), and GDNF, but not BDNF (Ramer et al. 2000), has been shown to promote extensive regeneration of sensory axons through the DREZ and in the spinal cord. Dorsal horn injections of adenovirus carrying the gene sequence for NGF have been reported to induce robust regeneration of dorsal root sensory axons through the DREZ and into former target laminae II and IV in the dorsal horn of the cord (Romero et al. 2001). In this study, however, sensory afferent axons were also observed to innervate laminae III and VI in the dorsal horn as well as ventral horn laminae VII–X. In a follow-up study, the same group of researchers was able to confine the regenerating sensory axons to the superficial layers of the dorsal horn by coupling the injection of the NGF-expressing vector with that of a vector encoding the gene for the negative guidance molecule semaphorin 3A in the ventral horn (Tang et al. 2007).

Another model to study regeneration of sensory axons is to create a partial spinal cord lesion that disrupts the ascending dorsal column. With this model, Taylor and colleagues (2006) have shown that a continuous gradient of lentivirus-mediated neurotrophin-3 (NT-3) in and immediately rostral to a lesion elicit modest axonal growth beyond the lesion. However, the sensory axons were found to stop elongating when they reach the rostral limit of the NT-3 gradient (Taylor et al. 2006). The same group of researchers has further demonstrated that axons have the potential to reach and reconnect with their former target if the distance between the lesion and the target is very small (2 mm) and is filled with a continuous gradient of NT-3 (Taylor Alto et al. 2009). Together, these findings show that damaged axons in the adult animal not only have the potential to elongate but also can form new synapses with former targets and that neurotrophins play a critical role in this process.

10 Functional Recovery After Neurotrophin Treatment of the Injured Spinal Cord

In the previous sections of this chapter, evidence that severed motor and sensory fiber pathways can regenerate through scar tissue, if neurotrophins are made available, has been reviewed. Is neurotrophin-mediated axonal regeneration associated with the recovery of function? Unfortunately, the answer is not

straightforward. Of the various experimental models of spinal cord injury, dorsal rhizotomy seems to be particularly amenable to recovery of function after neurotrophin treatment. In particular, the delivery of NGF after dorsal rhizotomy at lumbar levels 4 and 5 (L4/5) has been associated with near complete recovery of thermal nociception (Romero et al. 2001; Tang et al. 2007). Particularly, the report by Ramer et al. (2000) that deafferented dorsal horn sensory neurons can be synaptically driven by peripheral nerve stimulation after NGF, NT-3, and GDNF treatment is strong evidence of functional recovery. A therapeutic effect for BDNF on the recovery of thermal sensitivity has also been reported (Shumsky et al. 2003) (see Table 1).

Several teams of scientists have provided evidence that BDNF treatment after cervical lesions that involve the RST is accompanied by various degrees of recovery of function. After such lesions, BDNF delivery has been reported by different teams of researchers to enhance performance on the rope walking (Kim et al. 2001; Ruitenberg et al. 2003; Tobias et al. 2005) and cylinder tests (Liu et al. 1999; Tobias et al. 2005). Open-field testing with the BBB locomotor rating scale (Basso et al. 1995), on the other hand, has led to conflicting results. Indeed, significant improvement on the BBB open-field locomotor test has been reported in BDNF-treated animals (Koda et al. 2004), whereas complete recovery on this test has been shown to be independently of the nature of the treatment, i.e., fibroblasts expressing BDNF or control fibroblasts (Kim et al. 2001; Tobias et al. 2005). Together, these findings therefore suggest that BBB score is not useful to assess recovery of motor function after cervical lesions that target the RST. It is worthwhile to point out that the rope walking and the narrow beam tests as well as the BBB open-field locomotor test assess motor performance involving whole limb movement per se, or in relation to the intact limbs. However, evidence that lesions restricted to the RST do not lead to deficits in whole limb movement (Whishaw et al. 1998; Morris et al. 2011) suggests that these above-mentioned behavioral tasks are not particularly stringent to evaluate functional recovery of the RST. Moreover, the return of performance to, or near, preoperative values on some of these tests may therefore reflect compensation of function mediated by other motor pathways, particularly when recovery of function is not accompanied by evidence of RST growth in and through the BDNF-secreting implant.

Significant recovery on the gridwalk test has been reported after dorsal thoracic hemisections that include the main component of the CST, followed by treatment with NT-3-expressing cells (Grill et al. 1997b; Houweling et al. 1998). In both studies, amelioration of locomotor capacity on this test was accompanied by growth of CST axons in the cellular implants. However, the report by Grill and co-workers (1997) that smaller lesions that still abolish the CST are not sufficient to create a deficit on the gridwalk test (Grill et al. 1997b) indicates that the observed CST growth and recovery on the gridwalk test are two independent events. Furthermore, this observation also suggests that multiple pathways, possibly including the lateral aspect of the CST and the RST, contribute to locomotor function as measured by the gridwalk test.

Table 1 Compilation of several scientific reports that have addressed recovery of function after diverse neurotrophin treatments

Authors	Type of injury	Treatment	Anatomical outcome	Behavioral testing	Functional recovery after treatment
Tang et al. (2007)	Dorsal rhizotomy at L4/5	Ad-mediated NGF gene and semaphorin3A transfer in dorsal and ventral horns, respectively	Sensory axon growth across DREZ and into superficial dorsal horn laminae I and II	Response to noxious thermal stimulation	Functional restoration of nociception
Ramer et al. (2000)	Dorsal rhizotomy between C4 and T2	Delivery of NGF, NT-3, BDNF, and GDNF	Sensory axon growth across DREZ and into the spinal cord after treatment with NGF, NT-3, GDNF but not BDNF	Peripheral nerve stimulation	Dorsal horn neurons found to be synaptically driven with NGF, NT-3, GDNF but not BDNF
Romero et al. (2001)	Dorsal rhizotomy at L4/5	Delayed Ad-mediated NGF gene transfer in dorsal horn	Robust axonal growth in normal and aberrant locations with the dorsal horn	Plantar heat test and grid runway	Near complete recovery of hindlimb thermal nociception
Shumsky et al. (2003)	Unilateral hemisection at C3/4	Delayed delivery of BDNF and NT-3-expressing fibroblasts	Not reported	Battery of motor and sensorimotor tasks	No recovery on motor tasks but modest recovery of thermal sensitivity
Ruitenberg et al. (2003)	Unilateral DLF lesion involving the RST at C4	Acute delivery of BDNF-expressing OECs	Sprouting of RST axons in graft	Rope walking	Improved hindlimb performance
Liu et al. (1999)	Unilateral LF lesion involving the RST at C3/4	Acute delivery of BDNF-expressing fibroblasts	Growth of RST axons 3–4 segments below grafts	Cylinder test	Significant recovery
Tobias et al. (2005)	Unilateral DLF lesion involving the RST at C4	Acute delivery of BDNF-expressing fibroblasts	No evidence of growth of RST fibers in the implant	Rope walking test, cylinder test, BBB locomotor test	Complete recovery on BBB in treated and control rats, partial recovery in treated rats on cylinder test, partial recovery of rope walking in treated and control rats

(*continued*)

Table 1 (continued)

Authors	Type of injury	Treatment	Anatomical outcome	Behavioral testing	Functional recovery after treatment
Koda et al. (2004)	Complete transection at T8	Ad-mediated BDNF gene transfer in both stumps	Growth of RST axons through the lesion site	BBB locomotor test (Basso et al. 1995)	Significant improvement for at least 6 weeks
Kim et al. (2001)	Unilateral LF lesion at C3/4	Acute delivery of BDNF-expressing fibroblasts	Not reported	Swim test, patch-removal test, rope walking test, BBB locomotor test, narrow beam test,	No recovery on swim test, shorter latency to touch the patch, significant recovery on the rope walking test, complete recovery on BBB locomotor, and narrow beam tests in treated and control rats
Blits et al. (2003)	T9/10 excision	Schwann cell bridge and acute delivery of AAV-mediated BDNF and NT-3 gene transfer 5 mm caudal to bridge	Axonal growth in the cellular bridge but not beyond its caudal limits	BBB locomotor scale	Modest improvement of hindlimb function
Namiki et al. (2000)	Compression injury at T3	Infusion of BDNF and NT-3 for 14 days after injury	Smaller lesion size and regeneration through the injury	Inclined plane technique	Significant recovery at 1 week after injury. No significant recovery at later times
Houweling et al. (1998)	Dorsal hemisection between T8 and T10	NT-3 in a collagen matrix applied at the site of the injury	Growth of CST axons into, but not caudal to, NT-3-containing collagen matrix	Gridwalk test	Significant recovery
Grill et al. (1997b)	Dorsal hemisection at T7	Acute delivery of NT-3-expressing fibroblasts	Significant growth of CST axons in cellular implant	Gridwalk test	Significant recovery

(*continued*)

Table 1 (continued)

Authors	Type of injury	Treatment	Anatomical outcome	Behavioral testing	Functional recovery after treatment
Tuszynski et al. (2003)	Dorsal hemisection at T7	Delayed delivery of NT-3-expressing fibroblasts placed in, and caudal to the injury site	Significant growth of CST axons up to 15 mm distal to the injury site	BBB locomotor scale	Modest recovery

In an effort to encourage axonal growth below the cell implants, Blits et al. (2003) used Schwann cell implants to bridge thoracic spinal cord transections and subsequently delivered adeno-associated viral vectors containing the gene sequence for BDNF and NT-3 caudal to the cellular bridge. This surgical treatment, however, failed to promote growth caudal to the level of the NT-3-secreting bridge. Using a similar strategy, Tuszynski and co-workers (2003) observed growth of CST axons up to 15 mm distal to the injury level after the implantation of NT-3-expressing fibroblasts in, as well as caudal to a thoracic dorsal hemisection. Despite the considerable difference in the extent of axonal growth reported in these two studies, both procedures led to equally modest recovery on the BBB locomotor scale (Blits et al. 2003; Tuszynski et al. 2003). Thus, the striking difference in the extents of NT-3-mediated regeneration observed between these studies is by no means reflected in the level of performance on the BBB locomotor scale. In summary, that axonal regeneration after neurotrophin treatment has a direct impact on the recovery of function, at least with regard to the motor modality, is yet to be established.

11 Conclusion

In its infancy only a few decades ago, spinal cord regeneration has become a vast and dynamic field of research in neuroscience. Over the last 30 years, many groups of scientists have concentrated their efforts to identify growth inhibitors that are present in the damaged spinal cord and to develop strategies to counteract their action. Others have sought to design ways to increase the levels of growth-promoting neurotrophin expression at the site of injury in an attempt to offset the effect of these inhibitory molecules. There is now compelling evidence that neurotrophic factors can promote the survival of neurons giving rise to the different ascending and descending fiber pathways running in the spinal cord and that are axotomized after an injury. Moreover, it is well substantiated that the action of inhibitory signalling molecules that act as a barrier to axonal regeneration can be reverted by the delivery of neurotrophins to the injured spinal cord. Collectively, these key findings represent significant advances towards the development of

therapeutic strategies to promote axonal regeneration through a spinal cord lesion. Further work such as subjecting promising anatomical outcomes to independent replication is required before these preclinical data can be translated to clinical trials.

Recovery of function after spinal cord injury remains an enormous challenge for neuroscientists working in the field of spinal cord regeneration. Indeed, although the literature examined in the present chapter has reported axonal growth in and even several millimeters below the level of injury, there is no compelling evidence to show that the regenerative benefits of neurotrophin treatment are directly associated with functional recovery. Several factors may contribute to this unfulfilled endeavor. First and foremost, functional recovery must be supported by anatomical evidence of reinnervation of former postsynaptic target cells. This includes the demonstration that appropriate synaptic contacts (i.e., axodendritic synapses) with the postsynaptic target cells have been reestablished (e.g., Taylor Alto et al. 2009). Second, behavioral evidence for functional recovery gains significant strength if supported by electrophysiological data. In this regard, the report by Ramer et al. (2000) that deafferented sensory neurons in the dorsal horn can be synaptically driven by stimulation of the peripheral nerve after neurotrophin treatment constitutes powerful evidence of functional recovery. The years to come will certainly bring us closer to resolving this difficult task.

References

Aglash, C., Gordon, T., & Posse de Chaves, E. I. (2008). cAMP promotes neurite outgrowth and extension through protein kinase A but independently of Erk activation in cultured rat motoneurons. *Neuropharmacology, 55*, 8–17.

Aharoni, R., Eilam, R., Domev, H., Labunskay, G., Sela, M., & Arnon, R. (2005). The immunomodulator glatiramer acetate augments the expression of neurotrophic factors in brains of experimental autoimmune encephalomyelitis mice. *Proceedings of National Academy of Science United States of America, 102*, 19045–19050.

Arthur, J. S., Fong, A. L., Dwyer, J. M., Davare, M., Reese, E., Obrietan, K., & Impey, S. (2004). Mitogen- and stress-activated protein kinase 1 mediates cAMP response element-binding protein phosphorylation and activation by neurotrophins. *Journal of Neuroscience, 24*, 4324–4332.

Atwal, J. K., Massie, B., Miller, F. D., & Kaplan, D. R. (2000). The TrkB-Shc signals neuronal survival and local axon growth via MEK and P13-kinase. *Neuron, 27*, 265–277.

Barde, Y. A., Edgar, D., & Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *EMBO Journal, 1*, 549–553.

Barnabé-Heider, F., & Miller, F. D. (2003). Endogenously produced neurotrophins regulate survival and differentiation of cortical progenitors via distinct signaling pathways. *Journal of Neuroscience, 15*, 5149–5160.

Basso, D. M., Beattie, M. S., & Bresnahan, J. C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. *Journal of Neurotrauma, 12*, 1–21.

Baumgartner, B. J., & Shine, H. D. (1997). Targeted transduction of CNS neurons with adenoviral vectors carrying neurotrophic factor genes confers neuroprotection that exceeds the transduced population. *Journal of Neuroscience, 17*, 6504–6511.

Baumgartner, B. J., & Shine, H. D. (1998a). Neuroprotection of spinal motoneurons following targeted transduction with an adenoviral vector carrying the gene for glial cell-line derived neurotrophic factor. *Experimental Neurology, 153*, 102–112.

Baumgartner, B. J., & Shine, H. D. (1998b). Permanent rescue of lesioned motoneurons and enhanced axonal regeneration by adenovirus mediated expression of glial cell·line derived neurotrophic factor. *Journal of Neuroscience Research, 54*, 766–777.

Benn, S. C., & Woolf, C. J. (2004). Adult neurons survival strategies- slamming on the brakes. *Nature Reviews Neuroscience, 5*, 686–700.

Berkemeier, L. R., Winslow, J. W., Kaplan, D. R., Nokolics, K., Goeddel, D. V., & Rosenthal, A. (1991). Neurotrophin5: A novel neurotrophic factor that activates trk and trkB. *Neuron, 7*, 857–866.

Bhattacharrya, A., Watson, F. L., Pomeroy, S. L., Zhang, Y. Z., Stiles, C. D., & Segal, R. A. (2002). High-resolution imaging demonstrates dynein-based vesicular transport of activated Trk receptors. *Journal of Neurobiology, 51*, 302–312.

Bibel, M., Hoppe, E., & Barde, Y. A. (1999). Biochemical and functional interaction between the neurotrophin receptors trk andp75NTR. *EMBO Journal, 18*, 616–622.

Blanquet, P. R., & Lamour, Y. (1997). Brain-derived neurotrophic factor increases Ca+/calmodulin-dependent protein kinase 2 activity in hippocampus. *Journal of Biological Chemistry, 272*, 24133–24136.

Blesch, A., & Tuszynski, M. H. (2003). Cellular GDNF delivery promotes growth of motor and dorsal column sensory axons after partial and complete spinal cord transections and induces remyelination. *The Journal of Comparative Neurology, 467*, 403–417.

Blesch, A., Yang, H., Weidner, N., Hoang, A., & Otero, D. (2004). Axonal responses to cellularly delivered NT-4/5 after spinal cord Injury. *Molecular and Cellular Neuroscience, 27*, 190–201.

Blits, B., Oudega, M., Boer, G. L., Bartlett Bunge, M., & Verhaagen, J. (2003). Adeno-associated viral vector-mediated neurotrophin gene transfer in the injured adult rat spinal cord improves hind-limb function. *Neuroscience, 118*, 271–281.

Bo, X., Wu, D., Yeh, J., & Zhang, Y. (2011). Gene therapy approaches for neuroprotection and axonal regeneration after spinal cord and spinal root injury. *Current Gene Therapy, 11*, 101–115.

Bonner, J. F., Blesch, A., Neuhuber, B., & Fisher, I. (2010). Promoting directional axon growth from neural progenitors grafted into the injured spinal cord. *Journal of Neuroscience Research, 88*, 1182–1192.

Bonnie, A., Brunet, A., West, A. E., Datta, S. R., Takasu, M. A., & Greenberg, M. E. (1999). Cell survival promoted by the Ras-Mapk signaling pathway by transcription-dependent and -independent mechanisms. *Science, 286*, 1358–1362.

Bradbury, E. J., & Carter, L. M. (2011). Manipulating the glial scar: Chondroitinase ABC as a therapy for spinal cord injury. *Brain Research Bulletin, 84*, 306–316.

Bregman, B. S., Broude, E., McAtee, M., & Kelley, M. S. (1998). Transplant and neurotrophic factors prevent atrophy of mature CNS neurons after spinal cord injury. *Experimental Neurology, 149*, 13–27.

Bretzner, F., Liu, J., Currie, E., Roskams, A. J., & tetzlaff, W. (2008). Undesired effects of a combinatorial treatment for spinal cord injury – Transplantation of olfactory ensheathing cells and BDNF infusion to the red nucleus. *European Journal of Neuroscience, 28*, 1797–1807.

Brückner, G., Bringmann, A., Härtig, W., Köppe, G., Delpech, B., & Brauer, K. (1999). Acute and long-lasting changes in extracellular matrix chondroitin-sulphate proteoglycans induced by injection of chondroitinase ABC in the adult rat brain. *Experimental Brain Research, 121*, 300–310.

Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Juo, P., Hu, L. S., Anderson, M. J., Arden, K. C., Blenis, J., & Greenberg, M. E. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell, 96*, 857–868.

Cai, D., Shen, Y., De Bellard, M., Tang, S., & Filbin, M. T. (1999). Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism. *Neuron, 22*, 89–101.

Canossa, M., Gärtner, A., Campana, G., Inagaki, N., & Thoenen, H. (2001). Regulated secretion of neurotrophins by metabotropic glutamate group 1(mGluR1) and Trk receptor

activation is mediated via phospholipase C signalling pathways. *The EMBO Journal, 20*, 1640–1650.

Chaudhry, N., & Filbin, M. T. (2007). Myelin-associated inhibitory signalling and strategies to overcome inhibition. *Journal of Cerebral Blood Flow and Metabolism, 27*, 1096–1107.

Chen, M. S., Huber, A. B., van der Haar, M. E., Frank, M., Schnell, L., Spillmann, A. A., Christ, F., & Schwab, M. E. (2000). Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature, 403*, 434–439.

Choi, D. Y., Toledo-Aral, J. J., Segal, R., & Halegoua, S. (2001). Sustained signaling by phospholipase C-gamma mediates nerve growth factor-triggered gene expression. *Molecular and Cellular Biology, 21*, 2695–2705.

Chowdary, P. D., Che, D. L., & Cui, B. (2012). Neurotrophin signaling via long-distance axonal transport. *Annual Review of Physical Chemistry, 63*, 25.1–25.24.

Cohen, S., Levi-Montalcini, R., & Hamberger, V. (1954). A nerve growth-stimulating factor isolated from snake venom. *Proceeding of National Academy of Sciences of the United States of America, 40*, 1014–1018.

Cohen, M. S., Orth, C. B., Kim, H. J., Jeon, N. L., & Jaffrey, S. R. (2010). Neurotrophin-mediated dendrite-to-nucleus signaling revealed by microfluidic compartmentalization of dendrites. *Proceedings of the National Academy of Sciences, 108*, 11246–11251.

Cunningham, M. E., & Greene, L. A. (1998). A function-structure model for NGF-activated TRK. *The EMBO Journal, 17*, 7282–7293.

Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y., & Greenberg, M. E. (1997). Akt phosphorylation of Bad couples survival signals to the cell-intrinsic death machinery. *Cell, 91*, 231–241.

Delcroix, J. D., Valletta, J. S., Wu, C., Kowal, A. S., & Mobley, W. C. (2003). NGF signaling in sensory neurons: Evidence that early endosomes carry NGF retrograde signals. *Neuron, 39*, 69–84.

Du, K., & Montminy, M. (1998). CREB is a regulatory target for the protein kinase Akt/PKB. *The Journal of Biological Chemistry, 273*, 32377–32379.

Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R., & Greenberg, M. E. (1997). Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science, 275*, 661–665.

Egan, D. A., Flumerfelt, B. A., & Gwyn, D. G. (1977). Axon reaction in the red nucleus of the rat. *Acta Neuropathologica, 37*, 13–19.

Enfors, P., Ibáññez, C. F., Ebendal, T., Olson, L., & Persson, H. (1990). Molecular cloning and neurotrophic activity of a protein with structural similarities to nerve growth factor. *Proceeding of the National Academy of Science of the United States of America, 87*, 5454–5458.

Fagan, A. M., Zhang, H., Landis, S., Smeyne, R. J., Solos-Santiago, I., & Barbacid, M. (1996). TrkA, but not TrkC, receptors are essential for survival of sympathetic neurons in vivo. *Journal of Neuroscience, 16*, 6208–6218.

Fausett, B. V., & Goldman, D. (2006). A role for α1 Tubulin-expressing Müller glia in regeneration of the injured zebrafish retina. *Journal of Neuroscience, 26*, 6303–6313.

Fernandes, K. J. L., Fan, D.-P., Tsui, B. J., Cassar, S. L., & Tetzlaff, W. (1999). Influence of the axotomy to cell body distance in rat rubrospinal and spinal motoneurons: Differential regulation of GAP-43, Tubulins, and neurofilament-M. *The Journal of Comparative Neurology, 414*, 495–510.

Finkbeiner, S., Tavazoie, S. F., Maloratsky, A., Jacobs, K. M., Harris, K. M., & Greenberg, M. E. (1997). CREB: A major mediator of neuronal neurotrophin responses. *Neuron, 19*, 1031–1047.

Frade, J. M., & Barde, Y.-A. (1998). Nerve growth factor: Two receptors, multiple functions. *Bioessays, 20*, 137–145.

Franke, T. F., Yang, S.-I., Chan, T. O., Datta, K., Kazlauskas, A., Morrison, D. K., Kaplan, D. R., & Tsichlis, P. N. (1995). The protein kinase encoded the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell, 81*, 1–20.

Frey, D., Laux, T., Xu, L., Schneider, C., & Caroni, P. (2000). Shared and unique roles of CAP23 and GAP43 in actin regulation, neurite outgrowth, and anatomical plasticity. *The Journal of Cell Biology, 149*, 1443–1454.

Fu, X., Zang, K., Zhou, Z., Reichardt, L. F., & Xu, B. (2010). Retrograde neurotrophic signalling requires a protein interacting with receptor tyrosine kinase viaC_2H_2zinc fingers. *Molecular Biology of the Cell, 21*, 36–49.

Gallo, V., & Bertolotto, A. (1990). Extracellular matrix of cultured glial cells: Selective expression of chondroitin 4-sulfate by type-2 astrocytes and their progenitors. *Experimental Cell Research, 187*, 211–223.

Gallo, V., Bertolotto, A., & Levi, G. (1987). The proteoglycan chondroitin sulfate is present in a subpopulation of cultured astrocytes and in their precursors. *Developments in Biologicals, 123*, 282–285.

Gerin, C. G., Madueke, I. C., Perkins, T., Hill, S., Smith, K., Haley, B., Allen, S. A., Garcia, R. P., Paunesku, T., & Woloschak, G. (2011). Combination strategies for repair: Plasticity, and regeneration using regulation of genes expression during the chronic phase after spinal cord injury. *Synapse, 65*, 1255–1281.

Ghage, G. D., Roos, R. P., Kang, U. J., Wollmann, R., Fishman, P. S., Kalynych, A. M., Barr, E., & Leiden, J. M. (1995). CNS gene delivery by retrograde transport of replication-defective adenoviruses. *Gene Therapy, 2*, 132–137.

Giehl, K. M., & Tetzlaff, W. (1996). BDNF and NT-3, but not NGF, prevent axotomy-induced death of rat corticospinal neurons in vivo. *European Journal of Neuroscience, 8*, 1167–1175.

Giger, R. J., Hollis, E. R., & Tuszynski, M. H. (2010). Guidance molecules in axon regeneration. *Cold Spring Harbor Perspectives in Biology, 2*, 1–21.

Ginty, D. D., & Segal, R. A. (2002). Retrograde neurotrophin signaling: Trk-ing along the axon. *Current Opinion in Neurobiology, 12*, 268–274.

Glass, D. J., Nye, S. H., Hantzopoulos, P., Macchi, M. J., Squinto, S. P., Goldfarb, M., & Yancopoulos, G. D. (1991). TrkB mediates BDNF/NT-3 dependent survival and proliferation in fibroblasts lacking the low affinity NGF receptor. *Cell, 66*, 405–413.

Gloster, A., Wu, W., Speelman, A., Weiss, S., Causing, C., Pozniak, C., Reynolds, B., Chang, E., Toma, J. G., & Miller, F. D. (1994). The Tα1 α-Tubulin promoter specifies gene expression as a function of neuronal growth and regeneration in transgenic mice. *Journal of Neuroscience, 14*, 7319–7330.

Goldshmit, Y., Galea, M. P., Wise, G., Bartlett, P. F., & Turnley, A. M. (2004). Axonal regeneration and lack of astrocytic gliosis in EphA4-deficient mice. *Journal of Neuroscience, 24*, 10064–10073.

Gonzalez, G. A., & Montminy, M. R. (1989). Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB ar serine 133. *Cell, 59*, 675–680.

Gosh, A., Carnahan, J., & Greenberg, M. E. (1994). Requirement for BDNF in activity-dependent survival of cortical neurons. *Science, 263*, 1618–1623.

Götz, R., Köster, R., Winkler, C., Raulf, F., Lottspeich, F., Schartl, M., & Thoenen, H. (1994). Neurotrophin-6 is a new member of the nerve growth factor family. *Nature, 372*, 266–269.

GrandPré, T., Li, S., & Strittmatter, S. M. (2002). Nogo-66 receptor antagonist peptide promotes axonal regeneration. *Nature, 417*, 547–551.

Grill, R. J., Blesch, A., & Tuszynski, M. H. (1997a). Robust growth of chronically injured spinal cord axons induced by grafts of genetically modified NGF-secreting cells. *Experimental Neurology, 148*, 444–452.

Grill, R., Murai, K., Blesch, A., Gage, F. H., & Tuszynski, M. H. (1997b). Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury. *Journal of Neuroscience, 17*, 5560–5572.

Ha, J., Lo, K. W.-H., Myers, K. R., Carr, T. M., Humsi, M. K., Rasoul, B. A., Segal, R. A., & Pfister, K. K. (2008). A neuron-specific cytoplasmic dynein isoform preferentially transports TrkB signaling endosomes. *Journal of Biological Chemistry, 181*, 1027–1039.

Hallböök, F., Ibánñez, C. F., & Persson, H. (1991). Evolutionary studies of the nerve growth factor family revealed a novel member abundantly expressed in *Xenopus* ovary. *Neuron, 6*, 845–858.

Hammond, E. N. L., Tetzlaff, W., Mestres, P., & Giehl, K. M. (1999). BDNF, but not NT-3, promotes long-term survival of axotomized adult rat corticospinal neurons in vivo. *NeuroReport, 10*, 2671–2675.

Harrington, A. W., St. Hilaire, C., Zweifel, L. S., Glebova, N. O., Philippisou, P., Halegoua, S., & Ginty, D. D. (2011). Recruitment of actin modifiers to TrkA endosomes governs retrograde NGF signaling and survival. *Cell, 146*, 421–434.

Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R., Parada, L. F., & Chao, M. V. (1991). High-affinity NGF binding requires co-expression of the trk proto-oncogene and the low-affinity NGF receptor. *Nature, 350*, 678–683.

Hiebert, G. W., Dyer, J. K., Tetzlaff, W., & Steeves, J. D. (2000). Immunological myelin disruption does not alter expression of regeneration-associated genes in intact or axotomized rubrospinal neurons. *Experimental Neurology, 163*, 149–156.

Hoffman, P. N. (1989). Expression of GAP-43, a rapidly transported growth-associated protein, and class II beta tubulin, a slowly transported cytoskeletal protein, are coordinated in regenerating neurons. *Journal of Neuroscience, 9*, 893–897.

Hoffman, P. N., & Cleveland, D. W. (1988). Neurofilament and tubulin expression recapitulates the developmental pattern during axonal regeneration: Induction of a specific β-tubulin isotype. *Proceeding of National Academy of Sciences of the United States of America, 84*, 4530–4533.

Hohn, A., Lerbrock, J., Bailey, K., & Barde, Y. A. (1990). Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature, 344*, 339–341.

Holgado-Madruga, M., Moscatello, D. K., Emlet, D. R., Dieterich, R., & Wong, A. J. (1997). Grb2-associated binder-1 mediates phosphatidylinositol 3-kinase activation and the promotion of cell survival by nerve growth factor. *Proceeding of the National Academy of Sciences of the United States of America, 94*, 12419–12424.

Hollis, E. R., & Tuszynski, M. H. (2011). Neurotrophins: Potential therapeutic tools for the treatment of spinal cord injury. *Neurotherapeutics, 8*(4), 694–703.

Houweling, D. A., Lankhorst, A. J., Gispen, W. H., Bär, P. R., & Jooten, E. A. J. (1998). Collagen containing neurotrophin-3 (NT-3) attracts regrowing injured corticospinal axons in the adult rat spinal cord and promotes partial functional recovery. *Experimental Neurology, 153*, 49–59.

Howe, C. L., & Mobley, W. C. (2005). Long-distance retrograde neurotrophic signaling. *Current Opinion in Neurobiology, 15*, 40–48.

Howe, C. L., Valetta, J. S., Rusnak, A. S., & Mobley, W. C. (2001). NGF signaling from clathrin-coated vesicles: Evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway. *Neuron, 32*, 801–814.

Huang, E. J., & Reichardt, L. F. (2003). Trk receptors: Roles in neuronal signal transduction. *Annual Review of Biochemistry, 72*, 609–642.

Ibáñez, C. F. (2007). Message in a bottle: Long-range retrograde signaling in the nervous system. *Trends in Cell Biology, 17*, 19–28.

Impey, S., Obrietan, K., Wong, S. T., Poser, S., Yano, S., Wayman, G., Deloulme, J. C., Chan, G., & Storm, D. R. (1998). Cross talk between ERK and PKA is required for Ca^{2+} stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron, 21*, 869–883.

Ip, N. Y., Stitt, T. N., Tapley, P., Klein, R., Glass, D. J., Fandl, J., Greene, L. A., Barbacid, M., & Yancopoulos, G. D. (1993). Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells. *Neuron, 10*, 137–149.

Jin, Y., Fisher, I., Tessler, A., & Houle, J. D. (2002). Transplant of fibroblasts genetically modified to express BDNF promote axonal regeneration from supraspinal neurons following chronic spinal cord injury. *Experimental Neurology, 177*, 265–275.

Jones, K. R., & Reichardt, L. F. (1990). Molecular cloning of a human gene that is a member of the nerve growth factor family. *Proceeding of the National Academy of Sciences of the United States of America, 87*, 8060–8064.

Jones, L. L., Sajed, D., & Tuszynski, M. H. (2003). Axonal regeneration through regions of chondroitin sulfate proteoglycan deposition after spinal cord injury: A balance of permissiveness and inhibition. *Journal of Neuroscience, 23*, 9276–9288.

Kaisho, Y., Yoshimura, K., & Nakahama, K. (1990). Cloning and expression of a cDNA encoding a novel human neurotrophic factor. *FEBS Letters, 266*, 187–191.

Kaplan, D. R., Hempstead, B. L., Martin-Zanca, D., Chao, M. V., & Parada, L. F. (1991). The trk proto-oncogene product: A signal transducting receptor for nerve growth factor. *Science, 252*, 554–558.

Kim, D., Schallert, T., Liu, Y., Browarak, T., Nayeri, N., Tessler, A., Fischer, I., & Murray, M. (2001). Transplantation of genetically modified fibroblasts expressing BDNF in adult rats with a subtotal hemisection improves specific motor and sensory functions. *Neurorehabilitation and Neural Repair, 15*, 141–150.

Klein, R., Nanduri, V., Jing, S., Lamballe, F., & Barbacid, M. (1991a). The trk proto-oncogene encodes a receptor for nerve growth factor. *Cell, 65*, 189–197.

Klein, R., Nanduri, V., Jing, S., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F., & Barbacid, M. (1991b). The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell, 66*, 395–403.

Kobayashi, N. R., Fan, D.-P., Giehl, K. M., Bedard, A. M., Wiegrand, S. J., & Tetzlaff, W. (1997). BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and Tα1-Tubulin mRNA expression, and promote axonal regeneration. *Journal of Neuroscience, 17*, 9583–9595.

Koda, M., Hashimoto, M., Murakami, M., Wa, H. S., Sakao, S., Ino, H., Yoshinaga, K., Ikeda, O., Yamakazi, M., Koshizuka, S., Kamada, T., & Moriya, H. (2004). Adenovirus vector-mediated in vivo gene transfer of brain-derived neurotrophic factor (BDNF) promotes rubrospinal axonal regeneration and functional recovery after complete transection of the adult spinal cord. *Journal of Neurotrauma, 21*, 329–337.

Koda, M., Kamada, T., Hashimoto, M., Murakami, M., Shirasawa, H., Sakao, S., Ino, H., Yoshinaga, K., Koshizuka, S., Moriya, H., & Yamazaki, M. (2007). Adenovirus vector-mediated ex vivo gene transfer of brain-derived neurotrophic factor to bone marrow stromal cells promotes axonal regeneration after transplantation in completely transected adult spinal cord. *European Spine Journal, 16*, 2206–2214.

Krishna, M., & Narang, H. (2008). The complexity of mitogen-activated protein kinases (MAPKs) made simple. *Cellular and Molecular Life Sciences, 65*, 3525–3544.

Kwon, B. K., Liu, J., Messerer, C., Kobayashi, N. R., McGraw, J., Oschipok, L., & Tetzlaff, W. (2002). Survival and regeneration of rubrospinal neurons 1 year after spinal cord injury. *Proceeding of the National Academy of Sciences, 99*, 3246–3251.

Kwon, B. K., Liu, J., Oschipok, L., Teh, J., Liu, Z. W., & Tetzlaff, W. (2004). Rubrospinal neurons fail to respond to brain-derived neurotrophic factor applied to the spinal cord injury site 2 months after cervical axotomy. *Experimental Neurology, 189*, 45–57.

Kwon, B. K., Liu, J., Lam, C., Plunet, W., Oschipok, L., Hauswirth, W., Di Polo, A., Blesch, A., & Tetzlaff, W. (2007). Brain-derived neurotrophic factor gene transfer with adeno-associated viral and lentiviral vectors prevents rubrospinal neuronal atrophy and stimulate regeneration-associated gene expression after cervical spinal cord injury. *Spine, 32*, 1164–1173.

Lai, K. O., Fu, W. Y., Ip, F. C., & Ip, N. Y. (1998). Cloning and expression of a novel neurotrophin, NT-7, from carp. *Molecular and Cellular Neuroscience, 11*, 64–76.

Lamballe, F., Klein, R., & Barbacid, M. (1991). TrkC, a new member of the trk family of tyrosine protein kinases, is a receptor for Neurotrophin-3. *Cell, 66*, 967–979.

Lemmon, M. A., & Schlessinger, J. (2010). Cell signaling by receptor tyrosine kinase. *Cell, 141*, 1117–1134.

Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. *Science, 237*, 1154–1162.

Li, S., & Strittmatter, S. M. (2003). Delayed systemic Nogo-66 receptor antagonist promotes recovery from spinal cord. *Journal of Neuroscience, 23*, 4219–4227.

Lin, C. H., Yeh, S. H., Lin, C. H., Lu, K. T., Leu, T. H., Chang, W. C., & Gean, P. W. (2001). A role for Pl-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala. *Neuron, 31*, 841–851.

Liu, Y., Kim, D., Himes, B. T., Chow, S. Y., Schallert, T., Murray, M., Tessler, A., & Fisher, I. (1999). Transplants of fibroblasts genetically modified to express BDNF promote regeneration of adult rat rubrospinal axons and recovery of forelimb function. *Journal of Neuroscience, 19*, 4370–4387.

Liu, Y., Himes, T., Murray, M., Tessler, A., & Fisher, I. (2002). Grafts of BDNF-producing fibroblasts rescues axotomized rubrospinal neurons and prevent their atrophy. *Experimental Neurology, 178*, 150–164.

Lonze, B. E., & Ginty, D. D. (2002). Function and regulation of CREB family transcription factors in the nervous system. *Neuron, 35*, 605–623.

Lu, P., Jones, L. L., & Tuszynski, M. H. (2005). BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury. *Experimental Neurology, 191*, 344–360.

Lu, P., Blesch, A., Graham, L., Wang, Y., Samara, R., Banos, K., Haringer, V., Havton, L., Weishaupt, N., Bennet, D., Fouad, K., & Tuszynski, M. H. (2012). Motor axonal regeneration after partial and complete spinal cord transection. *Journal of Neuroscience, 32*, 8208–8218.

Maisonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M., & Yancopoulos, G. D. (1990a). Neurotrophin-3: A neurotrophic factor related to NGF and BDNF. *Science, 247*, 1446–1451.

Maisonpierre, P. C., Belluscio, L., Friedman, B., Alderson, R. F., Wiegand, S. J., Furth, M. E., Lindsay, R. M., & Yancopoulos, G. D. (1990b). NT-3, BDNF, and NGF in the developing rat nervous system: Parallel as well as reciprocal patterns of expression. *Neuron, 5*, 501–509.

Markus, A., Zhong, J., & Snider, W. D. (2002). Raf and akt mediate distinct aspects of sensory axon growth. *Neuron, 35*, 65–76.

Mason, M. R. J., Lieberman, A. R., & Anderson, P. N. (2003). Corticospinal neurons up-regulate a range of growth-associated genes following intracortical, but not spinal, axotomy. *European Journal of Neuroscience, 18*, 789–802.

McKerrecher, L., David, S., Jackson, D. L., Kottis, V., Dunn, R. J., & Braun, P. E. (1994). Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite outgrowth. *Neuron, 13*, 805–811.

McTigue, D. M., Horner, P. J., Stokes, B. T., & Gage, F. H. (1998). Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. *Journal of Neuroscience, 18*, 5354–5365.

Mellitzer, G., Xu, Q., & Wilkinson, D. G. (1999). Eph receptors and ephrins restrict cell intermingling and communication. *Nature, 400*, 77–81.

Merkler, D., Metz, G. A. S., Taineteau, O., Dietz, V., Schwab, M. E., & Fouad, K. (2001). Locomotor recovery in spinal cord-injured rats treated with an antibody neutralizing the myelin-associated neurite growth inhibitor Nogo-A. *Journal of Neuroscience, 21*, 3665–3673.

Mikol, D. D., & Stefansson, K. (1988). A phosphatidylinositol-linked peanut agglutinin-binding glycoprotein in central nervous system myelin and on oligodendrocytes. *The Journal of Cell Biology, 106*, 1273–1279.

Mikol, D. D., Gulcher, J. R., & Stefansson, K. (1990). The oligodendrocyte-myelin glycoprotein belongs to a distinct family of proteins and contains the HNK-1 carbohydrate. *The Journal of Cell Biology, 110*, 471–479.

Miller, F. D., Tetzlaff, W., Bisby, M. A., Fawcett, J. W., & Milner, R. J. (1989). Rapid induction of the major embryonic α-tubulin mRNA, in adults following neuronal injury. *Journal of Neuroscience, 9*, 1452–1463.

Mizoguchi, Y., Monji, A., & Nabekuta, J. (2002). Brain-derived neurotrophic factor induces long-lasting Ca^{2+}−activated K^+ currents in rat visual cortex neurons. *The European Journal of Neuroscience, 16*, 1417–1424.

Morris, R., Morgan, B. S., Lewis, T. M., Pierce, K. D., Pisano, A., & Schofield, P. R. (2004). In vivo somatic gene delivery of plasmid DNA and retrograde transport to obtain cell-specific gene expression in the central nervous system. *Journal of Neurochemistry, 90*, 1445–1452.

Morris, R., Tosolini, A. P., Goldstein, J. D., & Whishaw, I. Q. (2011). Impaired arpeggio movement in skilled reaching by rubrospinal tract lesions in the rat: A behavioral/anatomical fractionation. *Journal of Neurotrauma, 28*, 2439–2451.

Mukhopadhyay, G., Doherty, P., Walsh, F. S., Crocker, P. R., & Filbin, M. T. (1994). A novel role for myelin associated glycoprotein as an inhibitor of axonal regeneration. *Neuron, 13*, 757–767.

Nakajima, H., Uchida, K., Kobayashi, S., Inukai, T., Horiuchi, Y., Yayama, T., Sato, R., & Baba, H. (2007). Rescue of rat anterior horn neurons after spinal cord injury by retrograde transfection of adenovirus vector carrying brain-derived neurotrophic factor gene. *Journal of Neurotrauma, 24*, 703–712.

Nakajima, H., Uchida, K., Yayama, T., Kobayashi, S., Guerrero, A. R., Furukawa, S., & Baba, H. (2010). Targeted retrograde gene delivery of brain-derived neurotrophic factor suppresses apoptosis of neurons and oligodendroglia after spinal cord injury in rats. *Spine, 35*, 497–504.

Namiki, J., Kojima, A., & Tator, C. H. (2000). Effect of brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 on functional recovery and regeneration after spinal cord injury in adult rats. *Journal of Neurotrauma, 17*, 1219–1231.

Nebrada, A. R., & Gavin, A.-C. (1999). Cell survival demands some Rsk. *Science, 286*, 1309–1310.

Odega, M., & Hagg, T. (1996). Nerve growth factor promotes regeneration of sensory axons into adult rat spinal cord. *Experimental Neurology, 140*, 218–229.

Onose, G., Anghelescu, A., Muresanu, D. F., Padure, L., Haras, M. A., Chendreanu, C. O., Onose, L. V., Mirea, A., Ciurea, A. V., El Masri, W. S., & von Wild, K. R. H. (2006). A review of published reports on neuroprotection in spinal cord injury. *Spinal Cord, 47*, 716–726.

Pastekamp, R. J. (2012). Getting neural circuits into shape with semaphorins. *Nature Reviews Neuroscience, 13*, 605–618.

Pasterkamp, R. J., & Verhaagen, J. (2006). Semaphorin in axon regeneration: Developmental guidance molecules gone wrong? *Philosophical Transactions of the Royal Society London Series B: Biological Sciences, 361*, 1499–1511.

Pasterkamp, R. J., Giger, R. J., Ruitenberg, M. J., Holtmaat, A. J., De Wit, J., De Winter, F., & Verhaagen, J. (1999). Expression of the gene encoding the chemorepellent semaphorin III is induced in the fibroblast component of neural scar tissue formed following injuries of adult but not neonatal CNS. *Molecular and Cellular Neurosciences, 13*, 143–166.

Perkinton, M., Ip, J., Wood, G. L., Crossthwaite, A. J., & Williams, R. J. (2002). Phosphatidylinositol 3-kinase is a central mediator of NMDA receptor signalling to MAP kinase (Erk1/2), Akt/PKB and CREB in striatal neurones. *Journal of Neurochemistry, 80*, 239–254.

Phillippidou, P., Valdez, G., Akmentin, W., Bowers, W. J., Federoff, H. J., & Halegoua, S. (2010). Trk retrograde signaling requires persistent, Pincher-directed endosomes. *Proceedings of the National Academy of Sciences, 108*, 852–857.

Ramer, M. S., Priestley, J. V., & McMohon, S. B. (2000). Functional regeneration of sensory axons into the adult spinal cord. *Nature, 403*, 312–316.

Ramer, M. S., Duraisingam, I., Priestley, J. V., & McMahon, S. B. (2001). Two-tiered inhibition of axon regeneration at the dorsal root entry zone. *The Journal of Neuroscience, 21*, 2651–2660.

Reichardt, L. F. (2006). Neurotrophin-regulated signalling pathways. *Philosophical Transaction of the Royal Society B: Biological Sciences, 36*, 1545.

Reiderer, B. M., Pellier, V., Antonsson, B., Di Paolo, G., Stimpson, S. A., Lutjens, R., Catsicas, S., & Grenningloh, G. (1997). Regulation of microtubules dynamics by the neuronal growth-associated protein SCG10. *Proceeding of the National Academy of Sciences of the United States of America, 94*, 741–745.

Riccio, A., Ahn, S., Davenport, C. M., Blendy, J. A., & Ginty, D. D. (1999). Mediation by a CREB family transcription factor of NGF-dependent survival of sympathetic neurons. *Science, 286*, 2358–2361.

Rodriguez-Tébar, A., Dechant, G., & Barde, Y.-A. (1990). Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron, 4*, 487–492.

Rodriguez-Viciana, P., Warne, P. H., Vanhaesebroeck, B., Gout, I., Fry, M. J., Waterfield, M. D., & Downward, J. (1994). Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature, 370*(6490), 527–532.

Rolls, A., Shechter, R., & Schwart, M. (2009). The bright side of the glial scar in CNS repair. *Nature Reviews Neuroscience, 10*, 235–241.

Romero, M. I., Rangappa, N., Garry, M. G., & Smith, G. M. (2001). Functional regeneration of chronically injured sensory afferents into adult spinal cord after neurotrophin gene therapy. *The Journal of Neuroscience, 21*, 8408–8416.

Rosenthal, A., Goeddel, D. V., Nguyen, T., Lewis, M., Shih, A., Laramee, G. R., Nicolics, K., & Winslow, J. W. (1990). Primary structure and biological activity of a novel human neurotrophic factor. *Neuron, 4*, 767–773.

Ruitenberg, M. J., Plant, G. W., Hamers, F. P., Wortel, J., Blits, B., Dijkhuizen, P. A., Gipsen, W. H., Boer, G. J., & Verhaagen, J. (2003). Ex vivo adenoviral vector-mediated neurotrophins gene transfer to olfactory ensheathing glia: Effects on rubrospinal tract regeneration, lesion sizes, and functional recovery after implantation in the injured rat spinal cord. *Journal of Neuroscience, 23*, 7045–7058.

Ruitenberg, M. J., Blits, B., Dijkhuizen, P. A., te Beek, E. T., Bakker, A., van Heerikhuize, J. J., Pool, C. W., Hermens, W. T. J., Boer, G. J., & Verhaagen, J. (2004). Adeno-associated viral vector-mediated gene transfer of brain-derived neurotrophic factor reverses atrophy of rubrospinal neurons following both acute and chronic spinal cord injury. *Neurobiology of Disease, 15*, 394–406.

Saini, H. S., Gorse, K. M., Boxer, L. M., & Sato-Bogbee, C. (2004). Neurotrophin-3 and CREB-mediated signalling pathway regulate Bcl-2 expression in oligodendrocyte progenitor cells. *Journal of Neurochemistry, 89*, 951–961.

Schnell, L., Schneider, R., Kolbeck, R., Barde, Y.-A., & Schwab, M. E. (1994). Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal cord lesion. *Nature, 367*, 170–173.

Segal, R. A., Pomeroy, S. L., & Stiles, C. D. (1995). Axonal growth and fasciculation linked to differential expression of BDNF and NT3 receptors in developing cerebellar granule cells. *Journal of Neuroscience, 15*, 4970–4981.

Sharma, K., Selzer, M. E., & Li, S. (2012). Scar-mediated inhibition and CSPG receptors in the CNS. *Experimental Neurology, 237*, 370–378.

Shumsky, J. S., Tobias, C. A., Tumolo, M., Long, W. D., Giszter, S. F., & Murray, M. (2003). Delayed transplantation of fibroblasts genetically modified to secrete BDNF and NT-3 into a spinal cord injury site is associated with limited recovery of function. *Experimental Neurology, 184*, 114–130.

Silver, J., & Miller, J. H. (2004). Regeneration beyond the scar. *Narure Reviews Neuroscience, 5*, 146–156.

Skaper, S. D. (2012a). The neurotrophin family of neurotrophic factors: An overview. *Methods in Molecular Biology, 846*, 1–12.

Skaper, S. D. (2012b). Neuronal growth-promoting and inhibitory cues in neuroprotection and neuroregeneration. *Methods in Molecular Biology, 846*, 13–22.

Smith, G. M., Falone, A. E., & Frank, E. (2012). Sensory axon regeneration: Rebuilding functional connections in the spinal cord. *Trends in Neurosciences, 35*, 156–163.

Soppet, D., Escandon, E., Maragos, J., Middlemas, D. S., Reid, S. W., Blair, J., Burton, L. E., Stanton, B. R., Kaplan, D. R., Hunter, T., Nikolics, K., & Parada, L. F. (1991). The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. *Cell, 65*, 895–903.

Spencer, T. K., Mellado, W., & Filbin, M. T. (2008). BDNF activates CaMKIV and PKA in parallel to block MAG-mediated inhibition of neurite outgrowth. *Molecular and Cellular Neuroscience, 38*, 110–116.

Squinto, S. P., Stitt, T. N., Aldrich, T. H., Davis, S., Bianco, S. M., Radziejewski, C., Glass, D. J., Masiakowski, P., Furth, M. E., Valenzuela, D. M., DiStefano, P. S., & Yancopoulos, C. D.

(1991). TrkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. *Cell, 65*, 885–893.

Starkey, M. L., Bartus, K., Barritt, A. W., & Bradbury, E. J. (2012). Chondroitinase ABC promotes compensatory sprouting of the intact corticospinal tract and recovery of forelimb function following unilateral pyramidotomy in adult mice. *European Journal of Neuroscience, 36*(12), 3665–3678. doi:10.1111/ejn.12017.

Stephens, R. M., Loeb, D. M., Copeland, T. D., Pawson, T., Greene, L. A., & Kaplan, D. R. (1994). Trk receptors use redundant signal transduction pathways involving SHC and PLC-γ1 to mediate NGF responses. *Neuron, 12*, 691–705.

Storer, P. D., & Houle, J. D. (2003). BetaII-tubulin and GAP-43 mRNA expression in chronically injured neurons of the red nucleus after a second spinal cord injury. *Experimental Neurology, 183*, 537–547.

Storer, P. D., Dolbeare, D., & Houle, J. D. (2003). Treatment of chronically injured spinal cord with neurotrophic factors stimulates betaII-tubulin and GAP-43 expression in rubrospinal tract neurons. *Journal of Neuroscience Research, 74*, 502–511.

Tang, X.-Q., Heron, P., Mashburn, C., & Smith, G. M. (2007). Targeting sensory axon regeneration in adult spinal cord. *Journal of Neuroscience, 27*, 6068–6078.

Taylor Alto, L., Havton, L. A., Conner, J. M., Hollis, E. R., Blesch, A., & Tuszynski, M. H. (2009). Chemotropic guidance facilitates axonal regeneration and synapse formation after spinal cord injury. *Nature Neuroscience, 9*, 1106–1113.

Taylor, L., Jones, L., Tuszynski, M. H., & Blesch, A. (2006). Neurotrophin-3 gradients established by lentiviral gene delivery promote short-distance axonal bridging beyond cellular grafts in the injured spinal cord. *Journal of Neuroscience, 26*, 9713–9721.

Tedeschi, A. (2011). Tuning the orchestra: Transcriptional pathways controlling axon regeneration. *Frontiers in Molecular Neuroscience, 4*, 1–12.

Tetzlaff, W., Alexander, S. W., Miller, F. D., & Bisby, M. A. (1991). Response of facial and rubrospinal neurons to axotomy: Changes in mRNA expression for cytoskeletal protein and GAP-43. *Journal of Neuroscience, 11*, 2528–2544.

Tobias, C. A., Shumsky, J. S., Shibata, M., Tuszynski, M. H., Fisher, I., Tessler, A., & Murray, M. (2003). Delaying grafting of BDNF and NT-3 producing fibroblasts into the injured spinal cord stimulates sprouting, partially rescues axotomized neurons from loss and atrophy, and provides limited regeneration. *Experimental Neurology, 184*, 97–113.

Tobias, C. A., Han, S. S. W., Shumsky, J. S., Kim, D., Tumolo, M., Dhoot, N. O., Wheatley, M. A., Fisher, I., Tessler, A., & Murray, M. (2005). Alginate encapsulated BDNF-producing fibroblast grafts permit recovery of function after spinal cord injury in the absence of immune suppression. *Journal of Neurotrauma, 22*, 138–156.

Tosolini, A. P., & Morris, R. (2012). Spatial characterization of the motor neuron columns supplying the rat forelimb. *Neuroscience, 200*, 19–30.

Tuszynski, M. H., Grill, R., Jones, L. L., Brant, A., Blesch, A., Löw, K., Lacroix, S., & Lu, P. (2003). NT-3 delivery elicits growth of chronically injured corticospinal axons and modestly improves functional deficits after chronic scar resection. *Experimental Neurology, 181*, 47–56.

Uchida, K., Nakajima, H., Inukai, T., Takamura, T., Kobayashi, s., Furukawa, S., & Baba, H. (2008). Adenovirus-mediated retrograde transfer of neurotrophin-3 gene enhances survival of anterior horn neurons of *Twy*/*Twy* mice with chronic mechanical compression of the spinal cord. *Journal of Neuroscience Research, 86*, 1789–1800.

Uchida, K., Nakajima, H., Hirai, T., Yayama, T., Chen, K., Rodriguez Guerrero, A., Johnson, W. E., & Baba, H. (2012). The retrograde delivery of adenovirus vector carrying the gene for brain-derived neurotrophic factor protects neurons and oligodendrocytes from apoptosis in the chronically compressed spinal cord of *twy*/*twy* mice. *Spine*, *37*, 2125–2135.

Ullrich, A., & Schlessinger, J. (1990). Signal transduction by receptors with tyrosine kinase activity. *Cell, 61*, 203–212.

Vaillant, A. R., Mazzoni, I., Tudan, C., Boudreau, M., Kaplan, D. R., & Miller, F. D. (1999). Depolarization and neurotrophins converge on the phosphatidylinositol 3-kinase-Akt pathway to synergistically regulate neuronal survival. *The Journal of Cell Biology, 146*, 955–966.

Vyas, A. A., Blixt, O., Paulson, J. C., & Schnaar, R. L. (2005). Potent glycan inhibitors of myelin-associated glycoprotein enhance axon outgrowth in vitro. *Journal of Biological Chemistry, 280*, 16305–16310.

Watson, F. L., Heerssen, H. M., Bhattacharyya, A., Klesse, L., Lin, M. Z., & Segal, R. A. (2001). Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. *Nature Neuroscience, 4*, 981–988.

Wehrman, T., He, X., Raab, B., Dukipatti, A., Blau, H., & Garcia, K. C. (2007). Structural and mechanistic insight into nerve growth factor interaction with the TrkA and p75 receptors. *Neuron, 53*, 25–38.

Weinmann, O., Schnell, L., Ghosh, A., Montani, L., Wiessner, C., Wannier, T., Rouiller, E., Mir, A., & Schwab, M. E. (2006). Intrathecally infused antibodies against Nogo-A penetrate the CNS and downregulate the endogenous neurite growth inhibitor Nogo-A. *Molecular and Cellular Neuroscience, 32*, 161–173.

Wennerberg, K. (2005). The Ras superfamily at a glance. *Journal of Cell Science, 118*(5), 843–846.

Whishaw, I. Q., Gorny, B., & Sarna, J. (1998). Paw and limb use in skilled and spontaneous reaching after pyramidal tract, red nucleus and combined lesions in the rat: Behavioral and anatomical dissociation. *Behavioural Brain Research, 93*, 167–183.

Xu, X. M., Guenard, V., Kleitman, N., Aebischer, P., & Bunge, M. B. (1995). A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. *Experimental Neurology, 134*, 261–272.

Xu, Q., Mellitzer, G., Robinson, V., & Wilkinson, D. G. (1999). In vivo cell sorting in complementary segmental domains mediated by Eph receptors and ephrins. *Nature, 399*, 267–271.

Yamada, M., Numakawa, T., Koshimizu, H., Tanabe, K., Wada, K., Koizumi, S., & Hatanaka, H. (2002). Distinct usages of phospholipase C gamma and Shc in intracellular signaling stimulated by neurotrophins. *Brain Research, 955*, 183–190.

Yuan, J., & Yankner, B. A. (2000). Apoptosis in the nervous system. *Nature, 407*, 802–809.

Zhang, Y., Dijkhuizen, P. A., Anderson, P. N., & Lieberman, A. R. (1998). NT-3 delivery by an adenoviral vector induces injured dorsal root axons to regenerate into the spinal cord of adult rats. *Journal of Neuroscience Research, 54*, 554–562.

Zhang, H., Qiao, C., Wang, C.-H., Li, J., Yuan, Z., Zhang, C., & Xiao, X. (2010). Efficient retrograde transport of adeno-associated virus type 8 to spinal cord and dorsal root ganglion after vector delivery in muscle. *Human Gene Therapy, 21*, 87–97.

Zheng, J., Shen, W.-H., Lu, T.-J., Zhou, Y., Chen, Q., Wang, Z., Xiang, T., Zhu, Y.-C., Zhang, C., Duan, S., & Xiong, Z.-Q. (2008). Clathrin-dependent endocytosis is required for TrkB-dependent Akt-mediated neuronal protection. *Journal of Biological Chemistry, 283*, 13280–13288.

Zhou, L., Baumgartner, B. J., Hill-Felberg, S. J., McGowen, L. R., & Shine, H. D. (2003). Neurotrophin-3 expressed in situ induces axonal plasticity in the adult injured spinal cord. *Journal of Neuroscience, 23*, 1424–1431.

Neurotoxicity and Stroke

Victoria O'Collins, David Howells, and Romesh Markus

Contents

Abstract

Stroke is the clinical manifestation of the occlusion (ischemic stroke) or rupture of a blood vessel to the brain (hemorrhagic stroke). Neurotoxicity is triggered in stroke by the failure of oxygen delivery and the buildup of metabolites resulting in a cascade of harmful physiological events. Here, we review and contrast neurotoxicity and its corollary – neuroprotection – in the context of preclinical

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DOI 10.1007/978-1-4614-5836-4_132,

(animal) and clinical (patient) drug trials. Preclinical tests are distinguished by greater homogeneity, earlier time windows for treatment, and greater probability of success. Many of the same processes have been targeted in preclinical and clinical trials – such as excitotoxicity, oxidation, inflammation, and trophic factors – however, it is not a seamless flow from bench to bedside and back. Current trends in stroke neuroprotection include combination neuroprotection and improved trial designs. Areas where drug development in stroke could be improved include better target identification, development of lead drug candidates prior to preclinical testing and use of computational tool for prediction of toxicity based on biochemical pathway and drug characteristics.

Keywords

Neurotoxicity • Stroke • Neuroprotection • Brain ischemia • Animal models • Clinical trials

List of Abbreviations

AMPA	3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)
AO	Antioxidant
FRS	Free radical scavenger
ICA	Internal carotid artery
MCA	Middle cerebral artery
MCAo	Middle cerebral artery occlusion
NMDA	*N*-methyl-D-aspartate
tPA	Tissue plasminogen activator
WHO	World Health Organization

1 Introduction to Stroke

1.1 Definition of Stroke

Stroke is the clinical manifestation of the occlusion (ischemic stroke) or rupture of a blood vessel to the brain (hemorrhagic stroke). It has been formally defined by the World Health Organization as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting more than 24 hours (unless interrupted by surgery or death) with no apparent cause other than a vascular origin" (WHO 1988). This review is limited to ischemic stroke which accounts for approximately 85 % of strokes in humans.

1.2 Pathophysiology of Ischemic Stroke

Knowledge of the mechanisms by which ischemic stroke causes brain damage has been derived from autopsy, from animal experiments, and more recently from

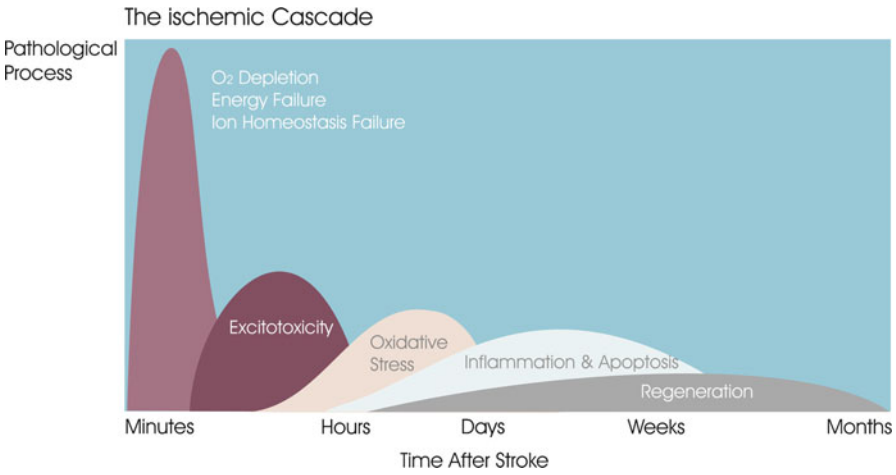

Fig. 1 The ischemic cascade (Adapted from Heiss et al. 1999)

imaging of the human brain. These data suggest that the extent to which blood flow is reduced in the brain is an important determinant of the response of cells (Astrup et al. 1981; Bandera et al. 2006).

Occlusion of a cerebral artery results in a reduction in oxygen, glucose, and nutrients in the territory of the brain supplied by the artery. The brain attempts to compensate for small reductions in blood flow (hypoperfusion) by recruiting blood flow through collateral vessels and by autoregulatory vasodilation (Derdeyn et al. 1999). Further reductions in blood flow are compensated for by increasing the amount of oxygen extracted from blood (Derdeyn et al. 1999). A failure to normalize perfusion pressure and blood flow will result in cellular swelling, disturbances in the redox state, lactic acidosis, loss of membrane dynamics, and ultimately cell death (Derdeyn et al. 1999).

On the basis of the degree to which blood flow and metabolism are compromised, brain tissue is often divided into normal healthy tissue, oligemic tissue, penumbral tissue, and infarcted tissue, in order of increasing severity of damage. Infarction initially occurs in the most severely hypoperfused region ("core"). The surrounding nonfunctional but potentially salvageable brain tissue ("penumbra") progresses to infarction with time as a result of a cascade of ischemia-induced biochemical changes resulting in cellular death (Markus et al. 2003).

Time is a critical determinant of the viability of brain tissue following a stroke. Evidence from animal models of stroke has demonstrated that the occlusion of a blood vessel sets in train a series of physiological events which have been termed the "ischemic cascade" (Dirnagl et al. 1999; Heiss et al. 1999). Primary damage following the loss of blood flow includes oxygen depletion, energy failure, and the failure of ion homeostasis (Fig. 1) (Heiss et al. 1999).

1.3 Neurotoxicity and Stroke

Ischemic stroke is a complex, multifaceted, and heterogeneous disease; thus, neurotoxicity is not easy to define in the context of stroke. Whilst the lack of oxygen and nutrients is the ultimate "neurotoxin," secondary damage may result from the following mechanisms: (1) "excitotoxicity," a surge in extracellular excitatory amino acids such as glutamate which become toxic because they are not buffered by normal cellular reuptake mechanisms (mainly astrocytes) (Benveniste et al. 1984; Benveniste 1991; Rothman and Olney 1986); (2) "oxidative stress," the massive release of free radicals that follow when endogenous production mechanisms become overactive (e.g., NOX enzymes) or when protective mechanisms (e.g., glutathione, superoxide dismutase) are swamped (Uenohara et al. 1988); and (3) inflammation and associated cell death, the inflammatory and apoptotic response marked by the migration and activation of microglia and macrophages at the lesion site and apoptotic signaling mechanisms (e.g., misplaced mitochondrial proteins which lead to further cell death) (Abraham and Lazar 2000; Mabuchi et al. 2000). Neurotoxicity may also result from disturbances in physiological processes such as acidosis, hyperthermia, blood sugar imbalance, and excessively high or low blood pressure.

Neurotoxicity may also be used to describe side effects of medications administered to manage stroke or a patient's comorbidities. For instance, tissue plasminogen activator (tPA) – expressed endogenously and administered in recombinant form therapeutically to break down clots – is a lifesaving therapy, but in animal models it has also been associated with neuronal damage, especially if the blood–brain barrier is not intact or if administered outside the vascular compartment (Wang et al. 1998, 1999; Kaur et al. 2004). Similarly, early agents targeting excitotoxicity (e.g., NMDA antagonists like MK-801) had poor profiles in terms of their effect on cognition (Muir and Lees 1995).

Neurotoxicity, however, is not the most common term designating damage in stroke research; when it does, it is often when implicating substances related to damage in other areas of medicine such as iron-induced neurotoxicity (Selim and Ratan 2004). Blood is neurotoxic if it gets outside of the vessels; the iron in hemoglobin is toxic to the brain and blood-signaling proteins like cytokines and chemokines. This causes inappropriate stimulation of the neuraxis and plays a role in directing invasion of inflammatory cells which themselves cause further damage but also help in regeneration. These regenerative responses are believed to be activated in the days and weeks following stroke onset (Wiltrout et al. 2007), but by this time most of the damage has been done.

One reason why endogenous substances upregulated during the ischemic cascade are not automatically designated as neurotoxic is that they might also take on neuroprotective roles, depending upon the time after iachemic onset and cell type. For instance, microglia has both neurotrophic and proinflammatory roles (Kim and de Vellis 2005) and may play a complex role after ischemia. Neuroprotection, rather than neurotoxicity, has therefore been the focus of intense interest in stroke research over the past 25 years.

2 Neuroprotection and Stroke

2.1 Clinical Management of Acute Stroke

Current evidence supports the use of a limited number of acute stroke interventions (Donnan et al. 2008): (1) management of patients in hospital stroke units, (2) reestablishment of blood flow using recombinant tissue activator (tPA) (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group 1995), (3) aspirin (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group 1997; International Stroke Trial Collaborative Group 1997), and (4) hemicraniectomy (Vahedi et al. 2007). Of these treatments, the most significant and widely available gains during the acute phase of stroke have been secured through caring for patients in a dedicated hospital stroke unit (Donnan et al. 2008). The benefits of these interventions are not mutually exclusive; however, for patients in hospital stroke units are probably more likely to have access to the other three interventions.

Therapies that target the cerebral vasculature by restoration of cerebral blood flow (thrombolytics, mechanical embolectomy) and preventing clot formation (aspirin) have shown unequivocal benefit in clinical trials and are approved for use in clinical practice. Neuroprotective strategies targeting the injurious biochemical and molecular cascade are designed to restrict injury to the ischemic brain by preventing neuronal cell death, especially in the salvageable penumbral region. While the concept of neuroprotection has shown promise in experimental studies, thus far there has been failure of translation into clinical success.

2.2 Neuroprotection and Animal Models of Stroke

Over the past 50 years, animal models have been used to test the efficacy of potential neuroprotective stroke therapies both before and after they have gone to clinical trial. Most commonly, these animal models entail occlusion of the middle cerebral artery to induce a "focal" area of brain damage. The standard method of evaluating treatments has been by applying a dye or stain to fine slices of brain tissue after the animal has been sacrificed. Various functional tests for animals have been derived from their clinical counterparts and purport to measure sensory and motor deficits. Such tests have been the mainstay of preclinical testing of potential therapies to reduce the impact of stroke.

To obtain global estimates of efficacy in animal models, data were pooled from two systematic reviews (O'Collins et al. 2006, 2012), which identified studies reporting the efficacy of interventions in animal experiments published 1978–2010. Testing the effect of therapies on infarct size was undertaken in 3,288 acute ischemic stroke experiments (47,899 animals); 94 % of these experiments were undertaken in rats or mice. Data were combined using meta-analysis and meta-regression, partitioned on the basis of stroke model

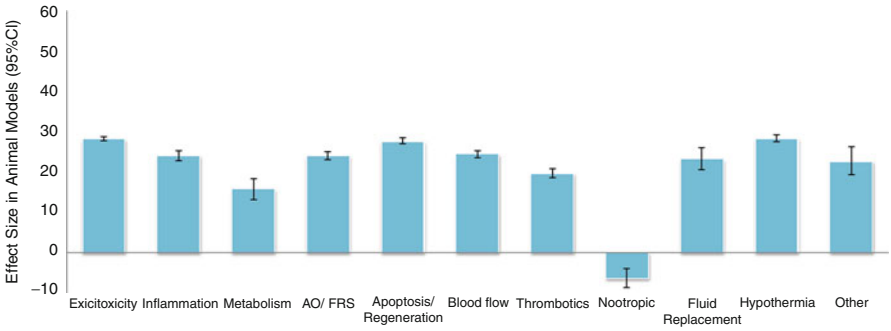

Fig. 2 Testing of acute stroke treatments in animal models. *Bar chart* of data from 3,288 controlled experimental tests of therapies in acute stroke in animal models (47,899 animals), subject to meta-analysis. A higher effect size indicates greater efficacy of the therapeutic class, compared to the control condition. Abbreviations: *AO* antioxidant, *FRS* free radical scavenger

and therapeutic target (e.g., excitotoxicity, thrombolysis, oxidative stress). Because of the heterogeneity, we used random effects meta-analyses (DerSimonian and Laird 1986).

Across all 502 potential therapies included in the dataset, the studies indicated on average a substantial reduction in brain damage (infarct) through use of a therapy (effect size = 25.1 ± .15, 95%CI 24–25.4). Of the 3,288 experiments, 24 % involved testing of excitotoxicity agents, 17 % involved agents targeting apoptosis or regeneration, 13 % involved free radical scavengers/antioxidants, and 12 % deployed hypothermia. The most effective of the therapeutic classes was hypothermia (effect size = 28.4 ± 0.4, 95%CI 27.6–29.3) and anti-excitotoxic drugs (28.3 ± 0.3, 95%CI 27.7–28.8) (see Fig. 2). However, classification of treatment mechanisms is notoriously difficult with treatments with multiple mechanisms of action and no objective measure of the strength of action in a domain.

2.3 Clinical Trials of Neuroprotection in Stroke Versus Animal Data

The Internet Stroke Center website provides a comprehensive database of neuroprotective agents that have been studied in humans (Internet Stroke Center 2013). Table 1 summarizes the key Phase III neuroprotection trials in humans, none of which have shown unequivocal clinical benefit (Table 1). Details of the drugs tested are given below. No neuroprotective strategy – in a narrow sense of the term – is currently approved for clinical use. By contrast, individual therapies tested in animal models and taken to Phase III clinical trial, estimates of efficacy from meta-analysis (Table 2) tend to suggest a positive effect. Note that these estimates are representative and not exhaustive of all animal model studies for each of the drugs listed below.

Table 1 Neuroprotection in acute ischemic stroke. Phase III trials in humans

Category	Drug name	Mechanism	Time window	Patients (drug/placebo)	Clinical Phase	Year	Trial	Reference
Antioxidant	NXY-059	Free radical scavenger	6	1,646/1,660	III	2007	SAINT II	(Shuaib et al. 2007)
	NXY-059	Free radical scavenger	6	853/852	III	2006	SAINT I	(Lees et al. 2006)
	Tirilazad	Free radical scavenger	6	276/280	III	1996	RANTTAS	(The RANTTAS Investigators 1996)
Calcium modulators	Nimodipine	Calcium antagonist	6	225/229	III	2001	VENUS	(Horn et al. 2001)
	Nimodipine	Calcium antagonist	48	800/264	III	1992		(The American Nimodipine Study Group 1992)
	Nimodipine	Calcium antagonist	48	607/608	III	1990	TRUST	(Trust Study Group 1990)
	Ginsenoside	Calcium antagonist	72	290/96	III	2012		(Liu et al. 2012)
Excitotoxicity	Aptiganel	NMDA ion channel blocker	12	414/214	II/III	2002		(Albers et al. 2001)
	Cervene (Nalmefene)	Opioid antagonist	6	163/167	III	2000		(Clark et al. 2000)
	Clomethiazole	GABA agonist	12	599/599	III	2002	CLASS-1	(Lyden et al. 2002)
	Clomethiazole	GABA agonist	12	680/680	III	1999	CLASS	(Wahlgren et al. 1999)
	Diazepam	GABA agonist	12	426/417	III	2006	EGASIS	(Lodder et al. 2006)
	Gavestinel (GV150526A)	NMDA glycine antagonist	6	701/666	III	2001	GAIN AMERICAS	(Sacco et al. 2001)
	Gavestinel (GV150526A)	NMDA glycine antagonist	6	891/897	III	2000	GAIN INTERNATIONAL	(Lees et al. 2000)
	Lubeluzole	Sodium/calcium channel blocker/NOS inhibitor	6	365/360	III	1998		(Diener 1998)

(*continued*)

Table 1 (continued)

Category	Drug name	Mechanism	Time window	Patients (drug/placebo)	Clinical Phase	Year	Trial	Reference
	Magnesium	NMDA antagonist/Ca channel blocker	12	1,292/1,297	III	2004	IMAGES	(Muir et al. 2004)
	Selfotel (CGS-19755)	NMDA antagonist	6	281/286	III	2000		(Davis et al. 2000)
Membrane stabilizers	Piracetam	AMPA (Na) modulator	12	464/463	III	1997	PASS	(De Deyn et al. 1997)
	Citicoline	Phospholipid synthesis	24	1,148/1,150	III	2012	ICTUS	(Dávalos et al. 2012)
	Repinotan	Serotonin antagonist	4.5	342/337	III	2009		(Teal et al. 2009)
Trophic factor	Cerebrolysin	Neurotrophic	12	529/541	III	2012	CASTA	(Heiss et al. 2012)
	Erythropoietin	Anti-apoptosis, oxygen delivery	6	266/256	III	2009		(Ehrenreich et al. 2009)
	Trafermin	Growth factor	6	286	II/III	2002		(Bogousslavsky et al. 2002)

Table 2 Neuroprotection in acute ischemic stroke: preclinical animal studies

Category	Drug name	Mechanism	Time window	Number of animals (drug/control)	No. Experiments	Effect size ± SE	95%CI	Year
Antioxidant	NXY-059	FRS	5 min	217/117	25	38 ± 2	[33,42]	1999
	Tirilazad	FRS	10 min	205/135	23	26 ± 2	[23,35]	1991
Calcium modulators	Ginsenoside	Calcium antagonist	0 min	217/79	29	41 ± 3	[36,47]	2004
	Nimodipine	Calcium antagonist	−5 min	306/217	36	24 ± 2	[21,27]	1986
Excitotoxicity	Aptiganel (Cerestat)	NMDA ion channel blocker	15 min	100/52	10	68 ± 3	[63,73]	1993
	Cervene (Nalmefene)	Opioid antagonist	–	0/0	0			None
	Clomethiazole	GABA agonist	15 min	60/39	8	47 ± 3	[41,54]	1995
	Diazepam (Valium)	GABA agonist	30 min	5/5	1	−13 ± 25	[−61,35]	2004
	Gavestinel (GV150526A)	NMDA glycine antagonist	7 min	161/70	21	65 ± 8	[49,80]	1997
	Lubeluzole	Sodium/calcium channel blocker/ NOS inhibitor	1 min	231/115	19	40 ± 2	[37,43]	1996
	Magnesium sulfate	NMDA antagonist/Ca channel blocker	−12.5 min	125/71	14	25 ± 3	[19,307]	1996
	Selfotel (CGS-19755)	NMDA antagonist	5 min	31/21	5	33 ± 2	[28,37]	1990
	YM872	AMPA receptor antagonist	10 min	180/112	18	24 ± 3	[30,98]	1999
Membrane stabilizers	Citicoline	Nootropic	1 h	481/185	48	27 ± 1	[24,29]	1996
	Piracetam	AMPA (Na) modulator	6 h	25/22	4	10 ± 5	[0,20]	2002
Nootropic/ stimulant	Cerebrolysin	Nootropic	24 h	120/40	12	8 ± 4	[0,17]	2007
Trophic factors	Erythropoietin	Anti-apoptosis oxygen delivery	6 h	157/65	20	19 ± 2	[15, 23]	1999
	Trafermin[a]	Growth factor	30 min	248/174	29	32 ± 2	[28,36]	1994
Others	Repinotan	Serotonin antagonist	1 min	192/108	19	53 ± 1	[50,54]	1998

[a]rhbFGF basic fibroblast growth factor. Time window is median time of drug delivery post occlusion. Control groups are adjusted for the number of comparisons

2.3.1 Glutamate Antagonists

Glutamate excitotoxicity is an important mediator of ischemia-induced neural injury via the *N*-methyl-D-aspartate (NMDA) and the 3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors.

NMDA Receptor Antagonists

Aptiganel (CNS-1102), a noncompetitive NMDA receptor antagonist (Albers et al. 2001), and Selfotel (CGS 19755) (Davis et al. 2000), a competitive NMDA receptor antagonist, have been studied in Phase III clinical studies. Although preclinical studies indicated that neuroprotection with these agents occurred only after very early administration, a time window of up to 6 h after stroke onset was utilized in human studies. Both agents exhibited significant neurobehavioral toxicity, resulting in a lower dosage being used in human studies than that predicted to provide neuroprotection from comparative preclinical animal studies. A higher mortality was observed in the Aptiganel group with a trend towards higher mortality in the Selfotel group, raising the possibility that NMDA receptor-mediated neuronal survival mechanisms were adversely affected by the delayed administration (Hoyte et al. 2004).

NMDA receptor antagonists have been relatively well studied in animal models of stroke compared with other classes, although even amongst these drugs, relatively few tests were published prior to clinical trial. Aptiganel has been studied in rat models of stroke and has been found to reduce infarct size when administered minutes after stroke onset with effects studied within the few days (Minematsu et al. 1993; Pitsikas et al. 2001; Zhao et al. 2010). When administered subcutaneously in Fisher 344 rats – as opposed to intravenously in more commonly used rat strains – beneficial effects were only seen in a narrow dose range (Pitsikas et al. 2001).

Selfotel has been investigated in focal ischemia models, global ischemia, and spinal cord ischemia, both in cat focal ischemia models (Okada et al. 1997) and in rat models (Jolkkonen et al. 1999; Simon and Shiraishi 1990; Minger et al. 1998), although testing in focal ischemia models is limited. Following adverse effects of NMDA antagonists in clinical trials, psychomimetic effects (e.g., hyperlocomotion, head weaving, and stereotyped sniffing) were studied in a small cohort of stroke rats and similar behavioral effects noted after administration (Loscher et al. 1998).

Gavestinel (GV150526), a NMDA receptor glycine site antagonist (Lees et al. 2000; Sacco et al. 2001) within 6 h of onset, showed no benefit in clinical outcome, mortality, or, in an imaging substudy, infarct attenuation in comparison with placebo (Warach et al. 2006). Gavestinel was tested in the Glaxo-Wellcome Research Laboratories, predominantly in permanent models of ischemia, and benefits on infarct measures of damage were found (Bordi et al. 1997; Di Fabio et al. 1999; Reggiani et al. 2001). Subsequent authors have hypothesized that NMDA receptor antagonists may hinder survival and regeneration (Hoyte et al. 2004); these outcomes were not typically measured in the studies.

AMPA Receptor Antagonists

Two clinical studies using YM872 (an AMPA receptor antagonist) within 6 h of stroke onset with and without concomitant rtPA, were prematurely terminated in 2003 after an interim futility analysis (Internet Stroke Center 2013). The study results have not been published. Animal studies have been undertaken in permanent and temporary occlusion models in rats (Kawasaki-Yatsugi et al. 1998; Shimizu-Sasamata et al. 1998; Haberg et al. 1998; Ni et al. 1998; Suzuki et al. 2003) and cats (Takahashi et al. 1998). While the overall result was positive, a number of individual trials showed minimal or no effect on infarct outcome in animal models.

2.3.2 GABA Agonists

Studies using clomethiazole, a GABA(A) agonist (Wahlgren et al. 1999; Lyden et al. 2002), and diazepam, a GABA-ergic agent (Lodder et al. 2006) within 12 h of stroke onset showed no clinical benefit in comparison with placebo. In contrast, preclinical studies of clomethiazole in rats and marmoset models of stroke conducted by AstraZeneca and others tended to show a reduction in infarct volume and edema compared to control groups (Baldwin et al. 1994; Sydserff et al. 1995, 1996, 2000; Green and Cross 1994; Snape et al. 1993; Marshall et al. 1999, 2000). Studies of diazepam in animal stroke models are few: Diazepam did not reduce infarct volume in a filament occlusion model of stroke (Kuhmonen et al. 2002) but did so in a photothrombotic model (Aerden et al. 2004).

2.3.3 Magnesium

The Intravenous Magnesium Efficacy in Stroke Trial (IMAGES) that randomized 2,589 patients within 12 h of stroke onset showed no clinical benefit for IV magnesium in comparison with placebo with a trend to higher mortality in the IV magnesium-treated group (Muir et al. 2004). The median time to treatment was 7 h, and only 3 % were treated within 3 h. The Field Administration of Stroke Therapy – Magnesium (FAST-MAG) Trial is currently recruiting to evaluate the safety and effectiveness of paramedic initiation of IV magnesium within 2 h of stroke onset (Internet Stroke Center 2013).

Magnesium has been relatively well characterized in investigator-sponsored trials in animal models of ischemia (Marinov et al. 1996; Lin et al. 2002; Westermaier et al. 2003; Meloni et al. 2006; O'Collins et al. 2011). It has been suggested that the effect of magnesium is via its effect on temperature, for where spontaneous hypothermia was prevented, no protective effect was seen (Zhu et al. 2004; Song et al. 2012; Campbell et al. 2008). A number of studies have found that magnesium is more effective when combined with other neuroprotective strategies (Schmid-Elsaesser et al. 1999; Zhao et al. 2010; Chung et al. 2004; Meloni et al. 2009; Campbell et al. 2008; Song et al. 2012; Wang et al. 2012), but not always (Lee et al. 1999). It has been argued that the sulfate salt is possibly more effective than the chloride salt (Izumi et al. 1991).

2.3.4 Antioxidants

NXY-059

NXY-059, a nitrone with free radical scavenging properties, was subjected to more extensive preclinical testing than most other agents prior to entering clinical trials in humans although subsequently several deficiencies were identified (Savitz 2007). The SAINT I (Lees et al. 2006) and SAINT II (Shuaib et al. 2007) studies randomized 1,722 and 3,306 patients, respectively, to receive a 72-h infusion of NXY-059 commencing within 6 h of stroke onset. The SAINT I trial reported a small but statistically significant shift of the modified Rankin score (mRS) at 3 months, but there was no change in the Barthel index or NIHSS scale. The SAINT II trial failed to confirm the data reported in SAINT 1. When taken together, the investigators concluded that NXY-059 was ineffective for the treatment of acute ischemic stroke.

NXY-059 is interesting from a preclinical perspective in that it was one of the first drugs said to have been conducted according to rigorous experimental standards (the STAIR guidelines) (Kuroda et al. 1999; Marshall et al. 2001; Zhao et al. 2001; Lapchak et al. 2002a, b; Sydserff et al. 2002; Yoshimoto et al. 2002a, b; Lapchak and Araujo 2003; Marshall et al. 2003a, b; Lapchak et al. 2004). There was much hope that it would succeed at clinical trial, and after this failed, it received criticism for falling short in terms of methodological quality (Macleod et al. 2008; Bath et al. 2009; Savitz 2007). Nevertheless, the preclinical literature is still stronger than many other drugs which have been taken to clinical trial.

Tirilazad

Tirilazad, an aminosteroid that inhibits free radical-induced lipid peroxidation, showed no clinical benefit in acute stroke when initiated within 6 h of stroke onset (The RANTTAS Investigators 1996). A subsequent meta-analysis revealed that tirilazad increased death and disability by about one-fifth when given to patients with acute ischemic stroke (Tirilazad International Steering Committee 2000). In animal models, tirilazad (U74006F) has reduced infarct size in a rat and rabbit model of stroke but not cat (Alessandri et al. 2000; Beck and Bielenberg 1991; Hellstrom et al. 1994; Orozco et al. 1995; Oktem et al. 2000; Park and Hall 1994; Schmid-Elsaesser et al. 1998, 1999; Scholler et al. 2004; Takeshima et al. 1994; Umemura et al. 1994; Wilson et al. 1992; Xue et al. 1992; Zausinger et al. 2003a, b; He et al. 1993).

2.3.5 Membrane-Stabilizing Agents

Citicoline

Citicoline is an exogenous form of cytidine 5′-diphosphate choline, which is essential for the biosynthesis of membrane phospholipids. The ICTUS study (Dávalos et al. 2012) that randomized 2,298 patients to receive citicoline or placebo within 24 h of stroke onset did not confirm the clinical benefits that had been suggested by a meta-analysis of four smaller trials using this agent (Dávalos et al. 2012). In animal models of stroke, citicoline is one of the best agents characterized,

second only to tissue plasminogen activator (Schabitz et al. 1996; Onal et al. 1997; Andersen et al. 1999; Shuaib et al. 2000; Katsumata et al. 2001; Ataus et al. 2004; Hurtado et al. 2005, 2007; Adibhatla et al. 2006; Ramos-Cabrer et al. 2011; Xu et al. 2011; Diederich et al. 2012).

Piracetam, a GABA derivative, is also used clinically to improve cognitive function. Although taken to clinical trial, only a few studies have reported its function in animal models (Tortiglione et al. 2002; Muley et al. 2012). Repinotan (BAY x 3702) is a selective 5-HT1A receptor agonist developed and tested in animal models by Bayer Health Care (Berends et al. 2005) and tested in focal models (Semkova et al. 1998; Mauler and Horvath 2005).

2.3.6 Calcium Modulators

Voltage-Gated Calcium Antagonists

A meta-analysis published in 2001 of 29 studies that included 7,665 patients with acute ischemic stroke treated with calcium antagonists reported no benefit with respect to clinical outcome or mortality (Horn and Limburg 2001). The calcium antagonists used were nimodipine in 23 studies, flunarizine in 3 studies, and isradipine and nicardipine in 1 study each. In 25 studies, including the Phase III TRUST (1,215 patients) and the American Nimodipine study (1,064 patients), treatment was started up to 48 h after stroke onset. There was no beneficial effect observed in the subgroup (660 nimodipine, 619 placebo) that received oral nimodipine within 12 h. Subsequent to this meta-analysis, the Very Early Nimodipine Use in Stroke (VENUS) trial reported the use of oral nimodipine within 6 h of stroke onset. This study was prematurely terminated by the steering committee because of sample size concerns. At the time 454 patients had been randomized and nimodipine was not associated with reduction of death or dependency at 3 months.

In animal studies, nimodipine was one of the earliest drugs trialed. More recently, it has been used a positive control against newer treatments, especially in studies of traditional Chinese medicines (Tian et al. 2005; Lu et al. 2011; He et al. 2012). It has been tested in mice, rats, and cats (Barnett et al. 1986; Gotoh et al. 1986; Hakim 1986; Sauter and Rudin 1986; Germano et al. 1987; Berger and Hakim 1988; Sauter et al. 1989; Jacewicz et al. 1990; Sakaki et al. 1991; Prehn et al. 1993; Herz et al. 1996; Cramer and Toorop 1998; Kawaguchi et al. 1999; Sobrado et al. 2003; Zausinger et al. 2003). In the 36 experimental comparisons included here, the median time for treatment was 5 minutes prior to ischemia.

Ginsenoside

Ginsenoside is a receptor-mediated calcium channel antagonist that does not lower blood pressure like voltage-gated calcium antagonists such as nimodipine discussed above. Liu et al. (2012) randomized 390 patients with acute ischemic stroke in a 3:1 ratio to receive a 14-day intravenous infusion of ginsenoside or placebo within 72 h after the onset of stroke. The primary outcome was positive with improved distribution of disability scores on the modified Rankin

scale (mRS) at 90 days in the group receiving ginsenoside. This is a promising result that needs verification in a larger RCT, bearing in mind that the initial NXY-059 trial reported a similar positive result based on shift of the mRS scores that was not confirmed in a second larger trial.

Ginsenosides (ginseng saponins) are the active component isolated from the traditional Chinese medicine ginseng, from the root of Panax ginseng. Over 30 different ginsenosides have been isolated, a number of these have been recently studied in animal models: ginsenoside Rb(1), ginsenoside Rd, ginsenoside Rh2, ginsenoside Rg(2), ginsenoside RG3, and fermented ginseng (Bae et al. 2004). Animal models suggest that the mode of action is on mitochondrial energy metabolism and altered activity of the mitochondrial permeability transition pore (Tian et al. 2009) and potentially via its effect on neural plasticity (Cheng et al. 2005) and angiogenesis (Yang et al. 2012). Rodent models have shown dose-dependent reductions in infarct size; however, sample sizes tend to have been small, and in many studies animals were treated prior to ischemia (Park et al. 2004; Tian et al. 2005; Zhang et al. 2006; Lu et al. 2011; Ye et al. 2011a, b, c; He et al. 2012; Yang et al. 2012; Zhu et al. 2012).

2.3.7 Anti-apoptotic and Trophic Factors

Cerebrolysin

First developed in Russia in the 1970s, cerebrolysin is a porcine brain-derived preparation of low-molecular-weight neuropeptides (10 kDa) and free amino acids that shows pharmacodynamic properties similar to those of naturally occurring neurotrophic factors. The CASTA study investigators randomized 1,070 patients to receive cerebrolysin or placebo within 12 h of stroke onset (Heiss et al. 2012). There was no difference in the primary outcome of a global functional scale, but there was a trend towards lower disability and reduced mortality in the cerebrolysin group for patients with more severe stroke (NIHSS >12). Cerebrolysin has demonstrated efficacy in a limited set of animal focal ischemia experiments (Schwab et al. 1998; Ren et al. 2007; Hanson et al. 2009; Zhang et al. 2010).

Erythropoietin, either alone or in combination with another agent after ischemic stroke, has attracted much interest in the last decade. Where animal tests have been undertaken, the time window for delivery tends to have been longer than other potential neuroprotectives and more emphasis has been placed on behavioral tests (Bernaudin et al. 1999; Brines et al. 2000; Wang et al. 2004, 2007; Esneault et al. 2008; Belayev et al. 2009; Fletcher et al. 2009; Jia et al. 2010; Zechariah et al. 2010).

2.4 Possible Reasons for Failure of Neuroprotection

With such positive data from animal experiments, how is it then that the promise of neuroprotection has thus far failed to translate into clinical benefit in human stroke studies (Ginsberg 2008; Sutherland et al. 2012)? There are numerous

preclinical and clinical reasons that could account for this failure. Failure of the initial clinical trials led to the development of the STAIR guidelines in 1999 for preclinical animal testing (STAIR Group 1999). In the wake of negative human clinical trials of compounds such as NXY-059 that had appeared promising in animal trials that fulfilled these guidelines, the criteria were revised in 2009 (Fisher et al. 2009).

A key issue in the human clinical trials is whether the agent actually reached the target tissue, the ischemic penumbra. The time window for effective thrombolysis in humans is 4.5 h after sstroke onset with greatest efficacy at earlier time points when the penumbra is maximal. By contrast, the completed Phase II/III studies of neuroprotective agents in humans had time windows of up to 48 h although efficacy was demonstrated at a shorter time window in the corresponding animal studies where there is no evidence of effective neuroprotection beyond 6 h. In the animal data surveyed above, the median time of treatment administration was 10 min after stroke onset.

Ischemic stroke in humans is heterogeneous with a multitude of subtypes and mechanisms. In contrast, animal studies have used relatively homogenous MCA occlusion models, but this particular stroke subtype accounts for only about 25 % of ischemic strokes in humans. Potential therapies have been tested almost exclusively in male animals (unless a female hormone is implicated in the mode of action) and in young animals (our data suggests that less than 1 % of testing occurs in middle-aged or old animals).

Neurotoxicity or neuroprotection may also occur as a covariate in animal models, e.g., anesthetic agents such as ketamine have effects on regional cerebral blood flow, EEG, sensation, and memory. Finally, there are important anatomical differences in gray and white matter distribution animal and human brains. The ischemic cascade has been studied primarily in gray matter that accounts for 90 % of rodent brain, but white matter accounts for approximately 50 % of human brain tissue involved by ischemic stroke (Ho et al. 2005).

3 The Future of Neuroprotection in Ischemic Stroke

3.1 Current Trends

The large numbers of negative clinical trials of neuroprotection in humans have led to calls for abandoning future trials, citing ineffectiveness of this strategy (The Lancet 2006). Others have reasonably argued for improved design of future trials of neuroprotectants in humans, primarily with shorter time windows and use in conjunction with thrombolytic therapy (Ginsberg 2008; Sutherland et al. 2012). Current ongoing trials of neuroprotection strategies in ischemic stroke incorporating these changes include intravenous magnesium within 2 h of onset (Internet Stroke Center 2013) and uric acid, an endogenous antioxidant in conjunction with rtPA (Amaro et al. 2010). In preclinical development, current trends

in neuroprotection include a focus on the microvasculature, inflammation, and the neurovascular unit and, secondly, a focus on endogenous neuroprotection and regeneration (stem cells, hormones, oxygen metabolism).

3.2 Improved Drug Development Procedures

Few of the agents tested in animal models of stroke have been developed specifically for treatment of ischemic stroke. This will reflect a bias against publication of potentially patentable drugs. However, it also suggests a need for better funding of stroke target identification and validation, together with better development of lead drug candidates before taking candidates to preclinical studies.

3.3 Toxicity in Animal Models of Stroke

Although toxicity is a frequent cause of drug failure at clinical trial, preclinical investigation of toxicity is generally looked at further down the preclinical pathway. Simultaneous optimization of drug efficacy and ADMET properties (absorption, distribution, metabolism, elimination, toxicology) is a strategy which could be looked at in greater detail in stroke drug development. To this end, improved computational tools for better prediction of toxicity based on biochemical pathways and drug characteristics may assist.

Expanding preclinical outcome measures beyond the standard measures of brain damage and neurological score may help identify potential neurotoxicity of drugs earlier. Cognitive deficit which has been noted following administration of NMDA antagonists could have been detected in animal models with tests typically deployed in other areas of neuroscience, for instance, locomotion (hyperlocomotion), prepulse inhibition disturbances, and disruptions to social interactions. Side effects of stroke drugs can include neurotoxicity but may also extend to genotoxicity, carcinogenicity, reproductive and developmental toxicity, and skin sensitization or irritation.

4 Conclusion

Ischemic stroke is a complex disease that straddles the vascular and nervous systems and is frequently confounded by comorbidities such as hypertension, diabetes and age-related degeneration. Thus, identifying and targeting individual causes of neurotoxicity in stroke is a great challenge for the clinical testing and laboratory development of novel therapeutics. The clinical and animal trials reviewed here have tended to focus on protecting the brain against damage caused by free radical scavengers and excitoxicity, or have used pharmacological means to target calcium modulation, membrane stabilization and trophic factors.

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References

Abraham, H., & Lazar, G. (2000). Early microglial reaction following mild forebrain ischemia induced by common carotid artery occlusion in rats. *Brain Research, 862*(1–2), 63–73.

Adibhatla, R. M., Hatcher, J. F., et al. (2006). CDP-choline significantly restores phosphatidylcholine levels by differentially affecting phospholipase A2 and CTP: Phosphocholine cytidylyltransferase after stroke. *The Journal of Biological Chemistry, 281*(10), 6718–6725.

Aerden, L. A., Kessels, F. A., et al. (2004). Diazepam reduces brain lesion size in a photothrombotic model of focal ischemia in rats. *Neuroscience Letters, 367*(1), 76–78.

Albers, G. W., Goldstein, L. B., et al. (2001). Aptiganel hydrochloride in acute ischemic stroke: A randomized controlled trial. *JAMA: The Journal of the American Medical Association, 286*(21), 2673–2682.

Alessandri, B., Basciani, R., et al. (2000). Chronic effects of an aminosteroid on microdialytically measured parameters after experimental middle cerebral artery occlusion in the rat. *Journal of Clinical Neuroscience, 7*(1), 47–51.

Amaro, S., Cánovas, D., et al. (2010). The URICO-ICTUS study, a phase 3 study of combined treatment with uric acid and rtPA administered intravenously in acute ischaemic stroke patients within the first 4.5 h of onset of symptoms. *International Journal of Stroke, 5*(4), 325–328.

Andersen, M., Overgaard, K., et al. (1999). Effects of citicoline combined with thrombolytic therapy in a rat embolic stroke model. *Stroke, 30*(7), 1464–1471.

Astrup, J., Siesjö, B. K., et al. (1981). Thresholds in cerebral ischemia – The ischemic penumbra. *Stroke, 12*(6), 723–725.

Ataus, S. A., Onal, M. Z., et al. (2004). The effects of citicoline and lamotrigine alone and in combination following permanent middle cerebral artery occlusion in rats. *The International Journal of Neuroscience, 114*(2), 183–196.

Bae, E. A., Hyun, Y. J., et al. (2004). Protective effect of fermented red ginseng on a transient focal ischemic rats. *Archives of Pharmacal Research, 27*(11), 1136–1140.

Baldwin, H. A., Williams, J. L., et al. (1994). Attenuation by chlormethiazole administration of the rise in extracellular amino acids following focal ischaemia in the cerebral cortex of the rat. *British Journal of Pharmacology, 112*(1), 188–194.

Bandera, E., Botteri, M., et al. (2006). Cerebral blood flow threshold of ischemic penumbra and infarct core in acute ischemic stroke: A systematic review. *Stroke, 37*(5), 1334–1339.

Barnett, G. H., Bose, B., et al. (1986). Effects of nimodipine on acute focal cerebral ischemia. *Stroke, 17*(5), 884–890.

Bath, P. M., Gray, L. J., et al. (2009). Effects of NXY-059 in experimental stroke: An individual animal meta-analysis. *British Journal of Pharmacology, 157*(7), 1157–1171.

Beck, T., & Bielenberg, G. W. (1991). The effects of two 21-aminosteroids on overt infarct size 48 hours after middle cerebral artery occlusion in the rat. *Brain Research, 560* (1–2), 159–162.

Belayev, L., Khoutorova, L., et al. (2009). A novel neurotrophic therapeutic strategy for experimental stroke. *Brain Research, 1280*, 117–123.

Benveniste, H. (1991). The excitotoxin hypothesis in relation to cerebral ischemia. *Cerebrovascular and Brain Metabolism Reviews, 3*(3), 213–245.

Benveniste, H., Drejer, J., et al. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *Journal of Neurochemistry, 43*(5), 1369–1374.

Berends, A. C., Luiten, P. G., et al. (2005). A review of the neuroprotective properties of the 5-HT1A receptor agonist repinotan HCl (BAYx3702) in ischemic stroke. *CNS Drug Reviews, 11*(4), 379–402.

Berger, L., & Hakim, A. M. (1988). Calcium channel blockers correct acidosis in ischemic rat brain without altering cerebral blood flow. *Stroke, 19*(10), 1257–1261.

Bernaudin, M., Marti, H. H., et al. (1999). A potential role for erythropoietin in focal permanent cerebral ischemia in mice. *Journal of Cerebral Blood Flow and Metabolism, 19*(6), 643–651.

Bogousslavsky, J., Victor, S. J., et al. (2002). Fiblast (trafermin) in acute stroke: Results of the European-Australian phase II/III safety and efficacy trial. *Cerebrovascular Diseases, 14* (3–4), 239–251.

Bordi, F., Pietra, C., et al. (1997). The glycine antagonist GV150526 protects somatosensory evoked potentials and reduces the infarct area in the MCAo model of focal ischemia in the rat. *Experimental Neurology, 145*(2 Pt 1), 425–433.

Brines, M. L., Ghezzi, P., et al. (2000). Erythropoietin crosses the blood–brain barrier to protect against experimental brain injury. *Proceedings of the National Academy of Sciences of the United States of America, 97*(19), 10526–10531.

Campbell, K., Meloni, B. P., et al. (2008a). Combined magnesium and mild hypothermia (35 degrees C) treatment reduces infarct volumes after permanent middle cerebral artery occlusion in the rat at 2 and 4, but not 6 h. *Brain Research, 1230*, 258–264.

Campbell, K., Meloni, B. P., et al. (2008b). Magnesium treatment and spontaneous mild hypothermia after transient focal cerebral ischemia in the rat. *Brain Research Bulletin, 77*(5), 320–322.

Cheng, Y., Shen, L. H., et al. (2005). Anti-amnestic and anti-aging effects of ginsenoside Rg1 and Rb1 and its mechanism of action. *Acta Pharmacologica Sinica, 26*(2), 143–149.

Chung, S. Y., Lin, J. Y., et al. (2004). Synergistic efficacy of magnesium sulfate and FK506 on cerebral ischemia-induced infarct volume in gerbil. *Medical Science Monitor, 10*(4), BR105–BR108.

Clark, W. M., Raps, E. C., et al. (2000). Cervene (Nalmefene) in acute ischemic stroke: Final results of a phase III efficacy study. The Cervene Stroke Study Investigators. *Stroke, 31*(6), 1234–1239.

Cramer, W. C., & Toorop, G. P. (1998). Focal cerebral ischemia in the mouse: Hypothermia and rapid screening of drugs. *General Pharmacology, 30*(2), 195–200.

Dávalos, A., Castillo, J., et al. (2002). Oral citicoline in acute ischemic stroke: An individual patient data pooling analysis of clinical trials. *Stroke, 33*(12), 2850–2857.

Dávalos, A., Alvarez-Sabín, J., et al. (2012). Citicoline in the treatment of acute ischaemic stroke: An international, randomised, multicentre, placebo-controlled study (ICTUS trial). *Lancet, 380*(9839), 349–357.

Davis, S. M., Lees, K. R., et al. (2000). Selfotel in acute ischemic stroke: Possible neurotoxic effects of an NMDA antagonist. *Stroke, 31*(2), 347–354.

De Deyn, P. P., Reuck, J. D., et al. (1997). Treatment of acute ischemic stroke with piracetam. Members of the Piracetam in Acute Stroke Study (PASS) Group. *Stroke, 28*(12), 2347–2352.

Derdeyn, C. P., Grubb, R. L., Jr., et al. (1999). Cerebral hemodynamic impairment: Methods of measurement and association with stroke risk. *Neurology, 53*(2), 251–259.

DerSimonian, R., & Laird, N. (1986). Meta-analysis in clinical trials. *Controlled Clinical Trials, 7*(3), 177–188.

Di Fabio, R., Conti, N., et al. (1999). Substituted analogues of GV150526 as potent glycine binding site antagonists in animal models of cerebral ischemia. *Journal of Medicinal Chemistry, 42*(18), 3486–3493.

Diederich, K., Frauenknecht, K., et al. (2012). Citicoline enhances neuroregenerative processes after experimental stroke in rats. *Stroke, 43*(7), 1931–1940.

Diener, H. C. (1998). Multinational randomised controlled trial of lubeluzole in acute ischaemic stroke. European and Australian Lubeluzole Ischaemic Stroke Study Group. *Cerebrovascular Diseases, 8*(3), 172–181.

Dirnagl, U., Iadecola, C., et al. (1999). Pathobiology of ischaemic stroke: An integrated view. *Trends in Neurosciences, 22*(9), 391–397.

Donnan, G. A., Fisher, M., et al. (2008). Stroke. *Lancet, 371*(9624), 1612–1623.

Ehrenreich, H., Weissenborn, K., et al. (2009). Recombinant human erythropoietin in the treatment of acute ischemic stroke. *Stroke, 40*(12), e647–e656.

Esneault, E., Pacary, E., et al. (2008). Combined therapeutic strategy using erythropoietin and mesenchymal stem cells potentiates neurogenesis after transient focal cerebral ischemia in rats. *Journal of Cerebral Blood Flow and Metabolism, 28*(9), 1552–1563.

Fisher, M., Feuerstein, G., et al. (2009). Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke, 40*(6), 2244–2250.

Fletcher, L., Kohli, S., et al. (2009). Intranasal delivery of erythropoietin plus insulin-like growth factor-I for acute neuroprotection in stroke. Laboratory investigation. *Journal of Neurosurgery, 111*(1), 164–170.

Germano, I. M., Bartkowski, H. M., et al. (1987). The therapeutic value of nimodipine in experimental focal cerebral ischemia. Neurological outcome and histopathological findings. *Journal of Neurosurgery, 67*(1), 81–87.

Ginsberg, M. D. (2008). Neuroprotection for ischemic stroke: Past, present and future. *Neuropharmacology, 55*(3), 363–389.

Gotoh, O., Mohamed, A. A., et al. (1986). Nimodipine and the haemodynamic and histopathological consequences of middle cerebral artery occlusion in the rat. *Journal of Cerebral Blood Flow and Metabolism, 6*(3), 321–331.

Green, A. R., & Cross, A. J. (1994). Attenuation by chlormethiazole of oedema following focal ischaemia in the cerebral cortex of the rat. *Neuroscience Letters, 173*(1–2), 27–30.

Haberg, A., Takahashi, M., et al. (1998). Neuroprotective effect of the novel glutamate AMPA receptor antagonist YM872 assessed with in vivo MR imaging of rat MCA occlusion. *Brain Research, 811*(1–2), 63–70.

Hakim, A. M. (1986). Cerebral acidosis in focal ischemia: II. Nimodipine and verapamil normalize cerebral pH following middle cerebral artery occlusion in the rat. *Journal of Cerebral Blood Flow and Metabolism, 6*(6), 676–683.

Hanson, L., Liu, X., et al. (2009). Cerebrolysin reduces infarct volume in a rat model of focal cerebral ischemic damage. *American Journal of Neuroprotection and Neuroregeneration, 1*, 62–69.

He, Y. Y., Hsu, C. Y., et al. (1993). Polyethylene glycol-conjugated superoxide dismutase in focal cerebral ischemia-reperfusion. *The American Journal of Physiology, 265*(1 Pt 2), H252–H256.

He, B., Chen, P., et al. (2012). Neuroprotective effect of 20(R)-ginsenoside Rg(3) against transient focal cerebral ischemia in rats. *Neuroscience Letters, 526*(2), 106–111.

Heiss, W. D., Thiel, A., et al. (1999). Which targets are relevant for therapy of acute ischemic stroke? *Stroke, 30*(7), 1486–1489.

Heiss, W.-D., Brainin, M., et al. (2012). Cerebrolysin in patients with acute ischemic stroke in Asia: Results of a double-blind, placebo-controlled randomized trial. *Stroke, 43*(3), 630–636.

Hellstrom, H. O., Wanhainen, A., et al. (1994). Effect of tirilazad mesylate given after permanent middle cerebral artery occlusion in rat. *Acta Neurochirurgica, 129*(3–4), 188–192.

Herz, R. C., De Wildt, D. J., et al. (1996). The effects of gamma 2-melanocyte-stimulating hormone and nimodipine on cortical blood flow and infarction volume in two rat models of middle cerebral artery occlusion. *European Journal of Pharmacology, 306*(1–3), 113–121.

Ho, P. W., Reutens, D. C., et al. (2005). Is white matter involved in patients entered into typical trials of neuroprotection? *Stroke, 36*(12), 2742–2744.
Horn, J., & Limburg, M. (2001). Calcium antagonists for ischemic stroke: A systematic review. *Stroke, 32*(2), 570–576.
Horn, J., De Haan, R. J., et al. (2001). Very early nimodipine use in stroke (VENUS): A randomized, double-blind, placebo-controlled trial. *Stroke, 32*(2), 461–465.
Hoyte, L., Barber, P. A., et al. (2004). The rise and fall of NMDA antagonists for ischemic stroke. *Current Molecular Medicine, 4*(2), 131–136.
Hurtado, O., Moro, M. A., et al. (2005). Neuroprotection afforded by prior citicoline administration in experimental brain ischemia: Effects on glutamate transport. *Neurobiology of Disease, 18*(2), 336–345.
Hurtado, O., Cardenas, A., et al. (2007). A chronic treatment with CDP-choline improves functional recovery and increases neuronal plasticity after experimental stroke. *Neurobiology of Disease, 26*(1), 105–111.
International Stroke Trial Collaborative Group. (1997). The International Stroke Trial (IST): A randomised trial of aspirin, subcutaneous heparin, both, or neither among 19435 patients with acute ischaemic stroke. International Stroke Trial Collaborative Group. *Lancet, 349*(9065), 1569–1581.
Internet Stroke Center. (2013). Stroke trials registry. http://www.strokecenter.org/trials/
Izumi, Y., Roussel, S., et al. (1991). Reduction of infarct volume by magnesium after middle cerebral artery occlusion in rats. *Journal of Cerebral Blood Flow and Metabolism, 11*(6), 1025–1030.
Jacewicz, M., Brint, S., et al. (1990). Continuous nimodipine treatment attenuates cortical infarction in rats subjected to 24 hours of focal cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism, 10*(1), 89–96.
Jia, L., Chopp, M., et al. (2010). Erythropoietin in combination of tissue plasminogen activator exacerbates brain hemorrhage when treatment is initiated 6 hours after stroke. *Stroke, 41*(9), 2071–2076.
Jolkkonen, J., Puurunen, K., et al. (1999). Neuroprotection by the alpha2-adrenoceptor agonist, dexmedetomidine, in rat focal cerebral ischemia. *European Journal of Pharmacology, 372*(1), 31–36.
Katsumata, T., Katayama, Y., et al. (2001). Delayed administration of JTP-2942, a novel thyrotropin-releasing hormone analogue, improves cerebral blood flow and metabolism in rat postischaemic brain. *Clinical and Experimental Pharmacology & Physiology, 28*(1–2), 48–54.
Kaur, J., Zhao, Z., et al. (2004). The neurotoxicity of tissue plasminogen activator? *Journal of Cerebral Blood Flow and Metabolism, 24*(9), 945–963.
Kawaguchi, K., Henshall, D. C., et al. (1999). Parallel dose–response studies of the voltage-dependent Na^+ channel antagonist BW619C89, and the voltage-dependent Ca^{2+} channel antagonist nimodipine, in rat transient focal cerebral ischaemia. *European Journal of Pharmacology, 364*(2–3), 99–105.
Kawasaki-Yatsugi, S., Yatsugi, S., et al. (1998). A novel AMPA receptor antagonist, YM872, reduces infarct size after middle cerebral artery occlusion in rats. *Brain Research, 793*(1–2), 39–46.
Kim, S. U., & de Vellis, J. (2005). Microglia in health and disease. *Journal of Neuroscience Research, 81*(3), 302–313.
Kuhmonen, J., Lukkarinen, J., et al. (2002). Diazepam does not reduce infarct size in rats subjected to transient occlusion of the middle cerebral artery when normothermia is maintained. *The Journal of Pharmacy and Pharmacology, 54*(11), 1565–1569.
Kuroda, S., Tsuchidate, R., et al. (1999). Neuroprotective effects of a novel nitrone, NXY-059, after transient focal cerebral ischemia in the rat. *Journal of Cerebral Blood Flow and Metabolism, 19*(7), 778–787.
Langhorne, P., Williams, B. O., et al. (1993). Do stroke units save lives? *Lancet, 342*(8868), 395–398.

Langhorne, P., Williams, B. O., et al. (1995). A formal overview of stroke unit trials. *Revista de Neurologia, 23*(120), 394–398.

Lapchak, P. A., & Araujo, D. M. (2003). Development of the nitrone-based spin trap agent NXY-059 to treat acute ischemic stroke. *CNS Drug Reviews, 9*(3), 253–262.

Lapchak, P. A., Araujo, D. M., et al. (2002a). Effects of the spin trap agent disodium- [tert-butylimino)methyl]benzene-1,3-disulfonate N-oxide (generic NXY-059) on intracerebral hemorrhage in a rabbit Large clot embolic stroke model: Combination studies with tissue plasminogen activator. *Stroke, 33*(6), 1665–1670.

Lapchak, P. A., Araujo, D. M., et al. (2002b). Neuroprotective effects of the spin trap agent disodium-[(tert-butylimino)methyl]benzene-1,3-disulfonate N-oxide (generic NXY-059) in a rabbit small clot embolic stroke model: Combination studies with the thrombolytic tissue plasminogen activator. *Stroke, 33*(5), 1411–1415.

Lapchak, P. A., Song, D., et al. (2004). Coadministration of NXY-059 and tenecteplase six hours following embolic strokes in rabbits improves clinical rating scores. *Experimental Neurology, 188*(2), 279–285.

Lee, E. J., Ayoub, I. A., et al. (1999). Mexiletine and magnesium independently, but not combined, protect against permanent focal cerebral ischemia in Wistar rats. *Journal of Neuroscience Research, 58*(3), 442–448.

Lees, K. R., Asplund, K., et al. (2000). Glycine antagonist (gavestinel) in neuroprotection (GAIN International) in patients with acute stroke: A randomised controlled trial. GAIN International Investigators. *Lancet, 355*(9219), 1949–1954.

Lees, K. R., Zivin, J. A., et al. (2006). NXY-059 for acute ischemic stroke. *The New England Journal of Medicine, 354*(6), 588–600.

Lin, J. Y., Chung, S. Y., et al. (2002). Effects of magnesium sulfate on energy metabolites and glutamate in the cortex during focal cerebral ischemia and reperfusion in the gerbil monitored by a dual-probe microdialysis technique. *Life Sciences, 71*(7), 803–811.

Liu, X., Wang, L., et al. (2012). Ginsenoside-Rd improves outcome of acute ischaemic stroke – A randomized, double-blind, placebo-controlled, multicenter trial. *European Journal of Neurology, 19*(6), 855–863.

Lodder, J., van Raak, L., et al. (2006). Diazepam to improve acute stroke outcome: Results of the early GABA-Ergic activation study in stroke trial. A randomized double-blind placebo-controlled trial. *Cerebrovascular Diseases, 21*(1–2), 120–127.

Loscher, W., Wlaz, P., et al. (1998). Focal ischemia enhances the adverse effect potential of *N*-methyl-D-aspartate receptor antagonists in rats. *Neuroscience Letters, 240*(1), 33–36.

Lu, T., Jiang, Y., et al. (2011). Intranasal ginsenoside Rb1 targets the brain and ameliorates cerebral ischemia/reperfusion injury in rats. *Biological & Pharmaceutical Bulletin, 34*(8), 1319–1324.

Lyden, P., Shuaib, A., et al. (2002). Clomethiazole Acute Stroke Study in ischemic stroke (CLASS-I): Final results. *Stroke, 33*(1), 122–128.

Mabuchi, T., Kitagawa, K., et al. (2000). Contribution of microglia/macrophages to expansion of infarction and response of oligodendrocytes after focal cerebral ischemia in rats. *Stroke, 31*(7), 1735–1743.

Macleod, M. R., van der Worp, H. B., et al. (2008). Evidence for the efficacy of NXY-059 in experimental focal cerebral ischaemia is confounded by study quality. *Stroke, 39*(10), 2824–2829.

Marinov, M. B., Harbaugh, K. S., et al. (1996). Neuroprotective effects of preischemia intraarterial magnesium sulfate in reversible focal cerebral ischemia. *Journal of Neurosurgery, 85*(1), 117–124.

Markus, R., Reutens, D. C., et al. (2003). Topography and temporal evolution of hypoxic viable tissue identified by 18F-fluoromisonidazole positron emission tomography in humans after ischemic stroke. *Stroke, 34*(11), 2646–2652.

Marshall, J. W., Cross, A. J., et al. (1999). Functional benefit from clomethiazole treatment after focal cerebral ischemia in a nonhuman primate species. *Experimental Neurology, 156*(1), 121–129.

Marshall, J. W., Cross, A. J., et al. (2000). Clomethiazole protects against hemineglect in a primate model of stroke. *Brain Research Bulletin, 52*(1), 21–29.

Marshall, J. W., Duffin, K. J., et al. (2001). NXY-059, a free radical–trapping agent, substantially lessens the functional disability resulting from cerebral ischemia in a primate species. *Stroke, 32*(1), 190–198.

Marshall, J. W., Cummings, R. M., et al. (2003a). Functional and histological evidence for the protective effect of NXY-059 in a primate model of stroke when given 4 hours after occlusion. *Stroke, 34*(9), 2228–2233.

Marshall, J. W., Green, A. R., et al. (2003b). Comparison of the neuroprotective effect of clomethiazole, AR-R15896AR and NXY-059 in a primate model of stroke using histological and behavioural measures. *Brain Research, 972*(1–2), 119–126.

Mauler, F., & Horvath, E. (2005). Neuroprotective efficacy of repinotan HCl, a 5-HT1A receptor agonist, in animal models of stroke and traumatic brain injury. *Journal of Cerebral Blood Flow and Metabolism, 25*(4), 451–459.

Meloni, B. P., Zhu, H., et al. (2006). Is magnesium neuroprotective following global and focal cerebral ischaemia? A review of published studies. *Magnesium Research, 19*(2), 123–137.

Meloni, B. P., Campbell, K., et al. (2009). In search of clinical neuroprotection after brain ischemia: The case for mild hypothermia (35 degrees C) and magnesium. *Stroke, 40*(6), 2236–2240.

Minematsu, K., Fisher, M., et al. (1993). Diffusion and perfusion magnetic resonance imaging studies to evaluate a noncompetitive *N*-methyl-D-aspartate antagonist and reperfusion in experimental stroke in rats. *Stroke, 24*(12), 2074–2081.

Minger, S. L., Geddes, J. W., et al. (1998). Glutamate receptor antagonists inhibit calpain-mediated cytoskeletal proteolysis in focal cerebral ischemia. *Brain Research, 810*(1–2), 181–199.

Muir, K. W., & Lees, K. R. (1995). Clinical experience with excitatory amino acid antagonist drugs. *Stroke, 26*(3), 503–513.

Muir, K. W., Lees, K. R., et al. (2004). Magnesium for acute stroke (Intravenous Magnesium Efficacy in Stroke trial): Randomised controlled trial. *Lancet, 363*(9407), 439–445.

Muley, M. M., Thakare, V. N., et al. (2012). Silymarin improves the behavioural, biochemical and histoarchitecture alterations in focal ischemic rats: A comparative evaluation with piracetam and protocatechuic acid. *Pharmacology, Biochemistry, and Behavior, 102*(2), 286–293.

Ni, J. W., Takahashi, M., et al. (1998). Effects of YM872 on atrophy of substantia nigra reticulata after focal ischemia in rats. *Neuroreport, 9*(16), 3719–3724.

O'Collins, V. E., Macleod, M. R., et al. (2012). Evaluation of combination therapy in animal models of cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism, 32*, 585–597.

O'Collins, V. E., Macleod, M. R., et al. (2011). Preclinical drug evaluation for combination therapy in acute stroke using systematic review, meta-analysis, and subsequent experimental testing. *Journal of Cerebral Blood Flow and Metabolism, 31*(3), 962–975.

O'Collins, V. E., Macleod, M. R., et al. (2006). 1,026 experimental treatments in acute stroke. *Annals of Neurology, 59*(3), 467–477.

Okada, M., Ueda, H., et al. (1997). Effect of D-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid on focal cerebral ischemia in cat. *Arzneimittel-Forschung, 47*(6), 703–705.

Oktem, I. S., Menku, A., et al. (2000). Therapeutic effect of tirilazad mesylate (U-74006F), mannitol, and their combination on experimental ischemia. *Research in Experimental Medicine. Zeitschrift für die Gesamte Experimentelle Medizin Einschliesslich Experimenteller Chirurgie, 199*(4), 231–242.

Onal, M. Z., Li, F., et al. (1997). Synergistic effects of citicoline and MK-801 in temporary experimental focal ischemia in rats. *Stroke, 28*(5), 1060–1065.

Orozco, J., Mendel, R. C., et al. (1995). Influence of a 'brain protector' drug 21-amino steroid on the effects of experimental embolic stroke treated by thrombolysis. *Neurological Research, 17*(6), 423–425.

Park, C. K., & Hall, E. D. (1994). Dose–response analysis of the effect of 21-aminosteroid tirilazad mesylate (U-74006F) upon neurological outcome and ischemic brain damage in permanent focal cerebral ischemia. *Brain Research, 645*(1–2), 157–163.

Park, E. K., Choo, M. K., et al. (2004). Ginsenoside Rh2 reduces ischemic brain injury in rats. *Biological & Pharmaceutical Bulletin, 27*(3), 433–436.

Pitsikas, N., Brambilla, A., et al. (2001). Effects of cerestat and NBQX on functional and morphological outcomes in rat focal cerebral ischemia. *Pharmacology, Biochemistry, and Behavior, 68*(3), 443–447.

Prehn, J. H., Welsch, M., et al. (1993). Effects of serotonergic drugs in experimental brain ischemia: Evidence for a protective role of serotonin in cerebral ischemia. *Brain Research, 630*(1–2), 10–20.

Ramos-Cabrer, P., Agulla, J., et al. (2011). Serial MRI study of the enhanced therapeutic effects of liposome-encapsulated citicoline in cerebral ischemia. *International Journal of Pharmaceutics, 405*(1–2), 228–233.

Reggiani, A., Pietra, C., et al. (2001). The neuroprotective activity of the glycine receptor antagonist GV150526: An in vivo study by magnetic resonance imaging. *European Journal of Pharmacology, 419*(2–3), 147–153.

Ren, J., Sietsma, D., et al. (2007). Cerebrolysin enhances functional recovery following focal cerebral infarction in rats. *Restorative Neurology and Neuroscience, 25*(1), 25–31.

Rothman, S. M., & Olney, J. W. (1986). Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Annals of Neurology, 19*, 105–111.

Sacco, R. L., DeRosa, J. T., et al. (2001). Glycine antagonist in neuroprotection for patients with acute stroke: GAIN Americas: A randomized controlled trial. *JAMA: The Journal of the American Medical Association, 285*(13), 1719–1728.

Sakaki, T., Tsunoda, S., et al. (1991). The influence of the calcium antagonist nimodipine and induced hypertension on the behavior of the cerebral pial arteries, the blood–brain barrier, cerebral edema, and cerebral infarction in cats with one-hour occlusion of the middle cerebral artery. *Neurosurgery, 28*(2), 267–272.

Sauter, A., & Rudin, M. (1986). Calcium antagonists reduce the extent of infarction in rat middle cerebral artery occlusion model as determined by quantitative magnetic resonance imaging. *Stroke, 17*(6), 1228–1234.

Sauter, A., Rudin, M., et al. (1989). Cerebrovascular, biochemical, and cytoprotective effects of isradipine in laboratory animals. *The American Journal of Medicine, 86*(4A), 134–146.

Savitz, S. I. (2007). A critical appraisal of the NXY-059 neuroprotection studies for acute stroke: A need for more rigorous testing of neuroprotective agents in animal models of stroke. *Experimental Neurology, 205*(1), 20–25.

Schabitz, W. R., Weber, J., et al. (1996). The effects of prolonged treatment with citicoline in temporary experimental focal ischemia. *Journal of the Neurological Sciences, 138*(1–2), 21–25.

Schmid-Elsaesser, R., Zausinger, S., et al. (1998). Monotherapy with dextromethorphan or tirilazad–but not a combination of both–improves outcome after transient focal cerebral ischemia in rats. *Experimental Brain Research, 122*(1), 121–127.

Schmid-Elsaesser, R., Hungerhuber, E., et al. (1999a). Neuroprotective efficacy of combination therapy with two different antioxidants in rats subjected to transient focal ischemia. *Brain Research, 816*(2), 471–479.

Schmid-Elsaesser, R., Zausinger, S., et al. (1999b). Neuroprotective effects of combination therapy with tirilazad and magnesium in rats subjected to reversible focal cerebral ischemia. *Neurosurgery, 44*(1), 163–171; discussion 171–162.

Scholler, K., Zausinger, S., et al. (2004). Neuroprotection in ischemic stroke–combination drug therapy and mild hypothermia in a rat model of permanent focal cerebral ischemia. *Brain Research, 1023*(2), 272–278.

Schwab, M., Antonow-Schlorke, I., et al. (1998). Brain-derived peptides reduce the size of cerebral infarction and loss of MAP2 immunoreactivity after focal ischemia in rats. *Journal of Neural Transmission. Supplementum, 53*, 299–311.

Seenan, P., Long, M., et al. (2007). Stroke units in their natural habitat: Systematic review of observational studies. *Stroke, 38*(6), 1886–1892.

Selim, M. H., & Ratan, R. R. (2004). The role of iron neurotoxicity in ischemic stroke. *Ageing Research Reviews, 3*(3), 345–353.

Semkova, I., Wolz, P., et al. (1998). Neuroprotective effect of 5-HT1A receptor agonist, Bay X 3702, demonstrated in vitro and in vivo. *European Journal of Pharmacology, 359*(2–3), 251–260.

Shimizu-Sasamata, M., Kano, T., et al. (1998). YM872, a highly water-soluble AMPA receptor antagonist, preserves the hemodynamic penumbra and reduces brain injury after permanent focal ischemia in rats. *Stroke, 29*(10), 2141–2148.

Shuaib, A., Yang, Y., et al. (2000). Evaluating the efficacy of citicoline in embolic ischemic stroke in rats: Neuroprotective effects when used alone or in combination with urokinase. *Experimental Neurology, 161*(2), 733–739.

Shuaib, A., Lees, K. R., et al. (2007). NXY-059 for the treatment of acute ischemic stroke. *The New England Journal of Medicine, 357*(6), 562–571.

Simon, R., & Shiraishi, K. (1990). *N*-methyl-D-aspartate antagonist reduces stroke size and regional glucose metabolism. *Annals of Neurology, 27*(6), 606–611.

Snape, M. F., Baldwin, H. A., et al. (1993). The effects of chlormethiazole and nimodipine on cortical infarct area after focal cerebral ischaemia in the rat. *Neuroscience, 53*(3), 837–844.

Sobrado, M., Lopez, M. G., et al. (2003). Combined nimodipine and citicoline reduce infarct size, attenuate apoptosis and increase bcl-2 expression after focal cerebral ischemia. *Neuroscience, 118*(1), 107–113.

Song, W., Wu, Y. M., et al. (2012). Intra-carotid cold magnesium sulfate infusion induces selective cerebral hypothermia and neuroprotection in rats with transient middle cerebral artery occlusion. *Neurological Sciences, 34*(4), 479.

STAIR Group. (1999). Recommendations for standards regarding preclinical neuroprotective and restorative drug development. *Stroke, 30*(12), 2752–2758.

Sutherland, B. A., Minnerup, J., et al. (2012). Neuroprotection for ischaemic stroke: Translation from the bench to the bedside. *International Journal of Stroke, 7*(5), 407–418.

Suzuki, M., Sasamata, M., et al. (2003). Neuroprotective effects of YM872 coadministered with t-PA in a rat embolic stroke model. *Brain Research, 959*(1), 169–172.

Sydserff, S. G., Cross, A. J., et al. (1995a). The neuroprotective effect of chlormethiazole on ischaemic neuronal damage following permanent middle cerebral artery ischaemia in the rat. *Neurodegeneration, 4*(3), 323–328.

Sydserff, S. G., Cross, A. J., et al. (1995b). The effect of chlormethiazole on neuronal damage in a model of transient focal ischaemia. *British Journal of Pharmacology, 114*(8), 1631–1635.

Sydserff, S. G., Green, A. R., et al. (1996). The effect of oedema and tissue swelling on the measurement of neuroprotection; a study using chlormethiazole and permanent middle cerebral artery occlusion in rats. *Neurodegeneration, 5*(1), 81–85.

Sydserff, S. G., Cross, A. J., et al. (2000). Clomethiazole is neuroprotective in models of global and focal cerebral ischemia when infused at doses producing clinically relevant plasma concentrations. *Brain Research, 862*(1–2), 59–62.

Sydserff, S. G., Borelli, A. R., et al. (2002). Effect of NXY-059 on infarct volume after transient or permanent middle cerebral artery occlusion in the rat; studies on dose, plasma concentration and therapeutic time window. *British Journal of Pharmacology, 135*(1), 103–112.

Takahashi, M., Ni, J. W., et al. (1998). YM872, a novel selective alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor antagonist, reduces brain damage after permanent

focal cerebral ischemia in cats. *The Journal of Pharmacology and Experimental Therapeutics, 284*(2), 467–473.

Takeshima, R., Kirsch, J. R., et al. (1994). Tirilazad treatment does not decrease early brain injury after transient focal ischemia in cats. *Stroke, 25*(3), 670–676.

Teal, P., Davis, S., et al. (2009). A randomized, double-blind, placebo-controlled trial to evaluate the efficacy, safety, tolerability, and pharmacokinetic/pharmacodynamic effects of a targeted exposure of intravenous repinotan in patients with acute ischemic stroke: modified Randomized Exposure Controlled Trial (mRECT). *Stroke, 40*(11), 3518–3525.

The American Nimodipine Study Group. (1992). Clinical trial of nimodipine in acute ischemic stroke. *Stroke, 23*(1), 3–8.

The Lancet. (2006). Neuroprotection: the end of an era? *Lancet*, *368*(9547), 1548.

The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. (1995). Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. *The New England Journal of Medicine*, *333*(24), 1581–1587.

The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. (1997). CAST: Randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke. CAST (Chinese Acute Stroke Trial) Collaborative Group. *Lancet*, *349*(9066), 1641–1649.

The RANTTAS Investigators. (1996). A randomized trial of tirilazad mesylate in patients with acute stroke (RANTTAS). *Stroke, 27*(9), 1453–1458.

Tian, J., Fu, F., et al. (2005). Neuroprotective effect of 20(S)-ginsenoside Rg3 on cerebral ischemia in rats. *Neuroscience Letters, 374*(2), 92–97.

Tian, J., Zhang, S., et al. (2009). 20(S)-ginsenoside Rg3, a neuroprotective agent, inhibits mitochondrial permeability transition pores in rat brain. *Phytotherapy Research, 23*(4), 486–491.

Tirilazad International Steering Committee. (2000). Tirilazad mesylate in acute ischemic stroke: A systematic review. *Stroke, 31*(9), 2257–2265.

Tortiglione, A., Minale, M., et al. (2002). The 2-oxopyrrolidinacetamide piracetam reduces infarct brain volume induced by permanent middle cerebral artery occlusion in male rats. *Neuropharmacology, 43*(3), 427–433.

Trust Study Group. (1990). Randomised, double-blind, placebo-controlled trial of nimodipine in acute stroke. *Lancet, 336*(8725), 1205–1209.

Uenohara, H., Imaizumi, S., et al. (1988). The protective effect of mannitol, vitamin E and glucocorticoid on ischaemic brain injury: Evaluation by chemiluminescence, energy metabolism and water content. *Neurological Research, 10*(2), 73–80.

Umemura, K., Wada, K., et al. (1994). Effect of 21-aminosteroid lipid peroxidation inhibitor, U74006F, in the rat middle cerebral artery occlusion model. *European Journal of Pharmacology, 251*(1), 69–74.

Vahedi, K., Hofmeijer, J., et al. (2007). Early decompressive surgery in malignant infarction of the middle cerebral artery: A pooled analysis of three randomised controlled trials. *Lancet Neurology, 6*(3), 215–222.

Wahlgren, N. G., Ranasinha, K. W., et al. (1999). Clomethiazole Acute Stroke Study (CLASS): Results of a randomized, controlled trial of clomethiazole versus placebo in 1360 acute stroke patients. *Stroke, 30*(1), 21–28.

Wang, Y. F., Tsirka, S. E., et al. (1998). Tissue plasminogen activator (tPA) increases neuronal damage after focal cerebral ischemia in wild-type and tPA-deficient mice. *Nature Medicine, 4*(2), 228–231.

Wang, X., Asahi, M., et al. (1999). Tissue type plasminogen activator amplifies hemoglobin-induced neurotoxicity in rat neuronal cultures. *Neuroscience Letters, 274*(2), 79–82.

Wang, L., Zhang, Z., et al. (2004). Treatment of stroke with erythropoietin enhances neurogenesis and angiogenesis and improves neurological function in rats. *Stroke, 35*(7), 1732–1737.

Wang, Y., Zhang, Z. G., et al. (2007). Post-ischemic treatment with erythropoietin or carbamylated erythropoietin reduces infarction and improves neurological outcome in a rat model of focal cerebral ischemia. *British Journal of Pharmacology, 151*(8), 1377–1384.

Wang, L. C., Huang, C. Y., et al. (2012). Magnesium sulfate and nimesulide have synergistic effects on rescuing brain damage after transient focal ischemia. *Journal of Neurotrauma, 29*(7), 1518–1529.

Warach, S., Kaufman, D., et al. (2006). Effect of the Glycine Antagonist Gavestinel on cerebral infarcts in acute stroke patients, a randomized placebo-controlled trial: The GAIN MRI Substudy. *Cerebrovascular Diseases, 21*(1–2), 106–111.

Westermaier, T., Hungerhuber, E., et al. (2003). Neuroprotective efficacy of intra-arterial and intravenous magnesium sulfate in a rat model of transient focal cerebral ischemia. *Acta Neurochirurgica, 145*(5), 393–399; discussion 399.

WHO. (1988). The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): A major international collaboration. WHO MONICA Project Principal Investigators. *Journal of Clinical Epidemiology, 41*, 105–114.

Wilson, J. T., Bednar, M. M., et al. (1992). The effect of the 21-aminosteroid U74006F in a rabbit model of thromboembolic stroke. *Neurosurgery, 31*(5), 929–933; discussion 933–924.

Wiltrout, C., Lang, B., et al. (2007). Repairing brain after stroke: A review on post-ischemic neurogenesis. *Neurochemistry International, 50*(7–8), 1028–1041.

Xu, F., Hongbin, H., et al. (2011). Greatly improved neuroprotective efficiency of citicoline by stereotactic delivery in treatment of ischemic injury. *Drug Delivery, 18*(7), 461–467.

Xue, D., Slivka, A., et al. (1992). Tirilazad reduces cortical infarction after transient but not permanent focal cerebral ischemia in rats. *Stroke, 23*(6), 894–899.

Yang, N., Chen, P., et al. (2012). Beneficial effects of ginsenoside-Rg1 on ischemia-induced angiogenesis in diabetic mice. *Acta Biochimica et Biophysica Sinica, 44*(12), 999–1005.

Ye, R., Kong, X., et al. (2011a). Ginsenoside Rd attenuates redox imbalance and improves stroke outcome after focal cerebral ischemia in aged mice. *Neuropharmacology, 61*(4), 815–824.

Ye, R., Yang, Q., et al. (2011b). Ginsenoside Rd attenuates early oxidative damage and sequential inflammatory response after transient focal ischemia in rats. *Neurochemistry International, 58*(3), 391–398.

Ye, R., Zhang, X., et al. (2011c). Ginsenoside Rd attenuates mitochondrial dysfunction and sequential apoptosis after transient focal ischemia. *Neuroscience, 178*, 169–180.

Yoshimoto, T., Kanakaraj, P., et al. (2002a). NXY-059 maintains Akt activation and inhibits release of cytochrome C after focal cerebral ischemia. *Brain Research, 947*(2), 191–198.

Yoshimoto, T., Kristian, T., et al. (2002b). Effect of NXY-059 on secondary mitochondrial dysfunction after transient focal ischemia; comparison with cyclosporin A. *Brain Research, 932*(1–2), 99–109.

Zausinger, S., Scholler, K., et al. (2003a). Combination drug therapy and mild hypothermia after transient focal cerebral ischemia in rats. *Stroke, 34*(9), 2246–2251.

Zausinger, S., Westermaier, T., et al. (2003b). Neuroprotection in transient focal cerebral ischemia by combination drug therapy and mild hypothermia: Comparison with customary therapeutic regimen. *Stroke, 34*(6), 1526–1532.

Zechariah, A., ElAli, A., et al. (2010). Combination of tissue-plasminogen activator with erythropoietin induces blood–brain barrier permeability, extracellular matrix disaggregation, and DNA fragmentation after focal cerebral ischemia in mice. *Stroke, 41*(5), 1008–1012.

Zhang, B., Hata, R., et al. (2006). Prevention of ischemic neuronal death by intravenous infusion of a ginseng saponin, ginsenoside Rb(1), that upregulates Bcl-x(L) expression. *Journal of Cerebral Blood Flow and Metabolism, 26*(5), 708–721.

Zhang, C., Chopp, M., et al. (2010). Cerebrolysin enhances neurogenesis in the ischemic brain and improves functional outcome after stroke. *Journal of Neuroscience Research, 88*(15), 3275–3281.

Zhao, Z., Cheng, M., et al. (2001). NXY-059, a novel free radical trapping compound, reduces cortical infarction after permanent focal cerebral ischemia in the rat. *Brain Research, 909*(1–2), 46–50.

Zhao, X., Strong, R., et al. (2010). Caffeinol at the receptor level: Anti-ischemic effect of *N*-methyl-D-aspartate receptor blockade is potentiated by caffeine. *Stroke, 41*(2), 363–367.

Zhu, H. D., Martin, R., et al. (2004). Magnesium sulfate fails to reduce infarct volume following transient focal cerebral ischemia in rats. *Neuroscience Research, 49*(3), 347–353.

Zhu, J., Jiang, Y., et al. (2012). Suppression of local inflammation contributes to the neuroprotective effect of ginsenoside Rb1 in rats with cerebral ischemia. *Neuroscience, 202*, 342–351.

Neurotoxicity in Huntington Disease

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Abstract

Huntington Disease (HD) is an autosomal dominant, progressive, neurodegenerative disorder, due to abnormal CAG expansion in the Chromosome 4 *Huntingtin* (*HTT*) gene. Typical age of onset is in the 30s-40s, and symptoms include involuntary movements, cognitive/behavioural symptoms and psychiatric disorders. As a monogenic disorder, the aetiology of HD is well established - however our understanding of the pathogenesis of HD is still evolving. In particular, mutation in the *HTT* gene confers neurotoxicity on multiple levels, ranging from the genetic/protein level, intracellular level, intercellular level, to the end-organ level. On a genetic/protein level, a *HTT* mutation is associated with toxic gain of function, loss of normal function and altered transcription of other genes. On an intracellular level, it is associated with mitochondrial dysfunction and impaired cytoskeleton/intra-cellular trafficking. On an intercellular level, it is associated with impaired synaptic transmission, excitoxicity and inflammation. On an organ level, both the brain and peripheral tissues are affected. We will review each of these areas in this article.

Keywords

Huntington disease • Molecular genetics • Neurodegeneration • Neurotoxicity • Polyglutamine • Tandem repeat disorder

1 Introduction

Huntington Disease (HD) is an autosomal-dominant, progressive, neurodegenerative disorder, due to abnormal CAG expansion in the chromosome 4 *Huntingtin (HTT)* gene. It is one of the most common neurogenetic disorders (MacMillan and Harper 1991), and has devastating consequences for patients and their families (Vamos et al. 2007). Typical age of onset is in the 30s–40s, and symptoms include involuntary movements, cognitive/behavioral symptoms, and psychiatric disorders (Walker 2007). While symptomatic treatments exist, these do not prolong survival, and median survival is about 20 years (Walker 2007). HD strikes at a productive period in work and family life, and requires a high level of complex care. Consequently, the direct and indirect cost of HD has been informally estimated to be two billion dollars per year, in the United States alone (Society for Neuroscience 2005).

2 Overview

As a monogenic disorder, the etiology of HD is well established (The Huntington's Disease Collaborative Research Group 1993), however our understanding of the pathogenesis of HD is still evolving. In particular, mutation in the *HTT* gene appears to confer neurotoxicity on multiple levels, ranging from the genetic/protein level, intracellular level, intercellular level, to the end-organ level (Table 1). We will review each of these areas in turn.

Table 1 Mutation in the Huntingtin gene leads to neurotoxicity on multiple levels

	Consequences of mutation in the *Huntingtin* gene
Neurotoxicity on a genetic/protein level	Toxic gain of function:
	1. Altered conformation
	2. Aberrant posttranslational modifications
	3. Impaired clearance
	4. Toxic proteolytic cleavage products
	5. Increased propensity to aggregate
	Loss of normal function
	Altered transcription of other genes
Neurotoxicity on an intracellular level	Role of the intranuclear inclusion body
	Mitochondrial dysfunction
	Impaired cytoskeleton and intracellular trafficking
Neurotoxicity on an intercellular level	Impaired synaptic transmission
	Excitotoxicity and the kynurenine pathway
	Inflammation (?)
Neurotoxicity on the end-organ level	Brain
	Peripheral tissues

3 Neurotoxicity on a Genetic/Protein Level

The *HTT* gene is located on the short arm of chromosome 4 (4p16.3) (Meyer et al. 2012). It has 67 exons and encodes a 348-kDa nuclear protein which is widely expressed in the human brain as well as other tissues (Strong et al. 1993). Exon 1 of the *HTT* gene contains a polymorphic CAG repeat, which is expanded among people with HD. The general population carries up to 26 repeats at this locus, and people carrying 40 or more repeats almost invariably develop clinical HD (ACMG/ASHG 1998). People with 36–39 CAG repeats have reduced penetrance. People with 27–35 CAG repeats do not develop clinical HD, but their offspring may do so, due to meiotic instability of the CAG repeat and further expansion across generations (Leeflang et al. 1995).

3.1 Toxic Gain of Function

From a genetic point of view, the prevailing hypothesis is that the expanded CAG repeats lead to a toxic gain of function. This is supported by: (1) the dominant mode of inheritance, (2) association of longer CAG repeats with earlier age of onset (Lee et al. 2012), and (3) no worsening of clinical HD phenotype among people

without a normal copy of *HTT* gene (i.e., people with homozygous HD mutations) (Wexler et al. 1987). In addition, a transgene expressing the human *HTT* Exon 1 with expanded CAG repeats was sufficient to cause a HD-like phenotype in the R6 lines of transgenic mice (Mangiarini et al. 1996).

Accordingly, the mutant huntingtin protein (mHTT) has been studied to elucidate a potential pathogenic mechanism for HD. Compared to wildtype huntingtin protein (wHTT), mHTT has (1) altered conformation, (2) aberrant posttranslational modifications, (3) impaired clearance, (4) toxic proteolytic cleavage products, and (5) increased propensity to aggregate (Ross and Tabrizi 2011).

3.1.1 Altered Conformation

In wHTT, the CAG expansion translates to an N-terminal polyglutamine tail which is flexible and can adopt a variety of conformations (Kim et al. 2009). However, when the CAG region is expanded into the pathogenic range, mHTT develops a compact two-strand hairpin conformation (Peters-Libeu et al. 2012). This hairpin epitope was identified using the 3B5H10 antibody, which also predicts the severity of neurodegeneration upon live cell imaging (Miller et al. 2011). Exactly how this hairpin structure leads to neurodegeneration is currently unclear, although early pathogenic processes are likely to include changes in protein solubility, aggregation, and protein–protein interactions.

3.1.2 Aberrant Posttranslational Modifications

Posttranslational modification (PTM) of mHTT is altered compared to wHTT. Examples of these PTM include phosphorylation, palmitoylation, ubiquitination, and acetylation. Huntingtin is constitutively phosphorylated at multiple sites (Schilling et al. 2006), and in general, mHTT is less phosphorylated than wHHT (Ehrnhoefer et al. 2011). For instance, in cell models, phosphorylation of Threonine-3 in HTT declines in a dose–response manner, with increasing CAG repeat length (Aiken et al. 2009). When phosphorylation was mimicked using site-specific mutagenesis for Serines-13 and -16, mHTT aggregation was reduced in vitro, and motor/psychiatric symptoms improved in a mouse model (Gu et al. 2009). Palmitoylation at Cysteine-214 of the HTT protein is also reduced, in the presence of a pathogenic CAG repeat (Yanai et al. 2006). Cells expressing mHTT resistant to palmitoylation are associated with faster inclusion body formation (Yanai et al. 2006). Ubiquitination is associated with protein targeting for proteasome degradation. Mutant HTT with expanded glutamine repeats have a slower rate of degradation in the proteasome (Jana et al. 2001). In addition, a protein specific to the striatum, the Ras Homologue Enriched in the Striatum (Rhes) protein, selectively increases SUMO-ylation (Small Ubiquitin-like modifier) of mHTT. This results in disaggregation of mHTT and cytotoxicity, and is consistent with the anatomical distribution of HTT (Subramaniam et al. 2009). Finally, acetylation at Lysine-444 of mHTT is associated with tagging for autophagy in vitro. This reverses toxic effects of mHTT in *C. elegans* and cell models, and is an example where alteration in posttranslational modification of mHTT is beneficial rather than deleterious (Jeong et al. 2009).

3.1.3 Impaired Clearance

Clearance of mHTT is impaired. In addition to the slower rate of degrading mHTT in the proteasome, N-terminal fragments of mHTT were found to directly impair functioning of the Uniquitin/Proteosome System (UPS) in a cellular model (Bence et al. 2001). More recently, this has been confirmed in vivo, using a UPS reporter/inducible HD mouse model (Ortega et al. 2010). UPS dysfunction was found transiently after expression of N-terminal mutant HTT, and recovered with appearance of inclusion bodies.

3.1.4 Toxic Proteolytic Cleavage Products

A number of experiments support the notion that neurotoxicity in HD arises from toxic proteolytic cleavage products of mHTT rather than the full mHTT molecule. These include: (1) sufficiency of *HTT* Exon 1 to cause an HD-like phenotype in a transgenic mouse model (Mangiarini et al. 1996), and (2) normal phenotype in a mouse model expressing caspase-6 resistant mHTT (Graham et al. 2006). In addition to caspases, calpains also play a role in proteolytic cleavage (Southwell et al. 2011). More recently, CAG repeat length-dependent aberrant splicing of the *HTT* gene has been found to be an alternative mechanism in generating N-terminal mHTT fragments (Sathasivam et al. 2013).

3.1.5 Increased Propensity to Aggregate

Early neuropathologic studies of the brains of people with HD found intranuclear inclusion bodies that contained protein aggregates immunoreactive to antibodies raised to the N-terminal 17 amino acids, but not an internal epitope for HTT (Becher et al. 1998). This raised the question as to whether these N-terminal HTT fragments, aggregated in inclusion bodies, were pathogenic. As evidence was generated showing that the inclusion bodies could be protective rather than pathogenic (Arrasate et al. 2004) (see Sect. 4.1 for further details), attention was shifted to polyglutamine monomers and oligomers, which may be more neurotoxic (Miller et al. 2011). Oligomer formation is faster and more extensive for HTT fragments with longer polyglutamine tracts (Legleiter et al. 2010). This increased propensity for mHTT to aggregate also provides an explanation for the inverse relationship between CAG repeat length and age of onset in HD. Interestingly, an aggregation kinetic study has found a static population of oligomers despite ongoing conversion of mHTT monomers to inclusion bodies in a cell models (Olshina et al. 2010). This suggests that neurotoxicity due to oligomers may be a constant process, and correlates poorly with inclusion body formation.

3.2 Loss of Normal Function

While the prevailing hypothesis for neurotoxicity of CAG expansion is a toxic gain of function, there is also an argument that loss of normal function may play a role (Cattaneo et al. 2005). Mouse models without a normal copy of the murine

HTT analogue gene are embryonically lethal (Duyao et al. 1995; Nasir et al. 1995). Inactivation of the murine *HTT* analogue genes postnatally also leads to a progressive neurodegenerative phenotype (Dragatsis et al. 2000). These underline the role of wHTT in normal neuronal functioning, which include: (1) upregulation of brain-derived neurotrophic factor (BDNF) (Zuccato et al. 2001), (2) interaction with the motor proteins and microtubules in BDNF transport (Gauthier et al. 2004), and (3) interaction with literally hundreds more proteins (Kaltenbach et al. 2007), including ones important for synaptic function (Milnerwood and Raymond 2010).

3.3 Altered Transcription of Other Genes

Wildtype HTT upregulates BDNF by sequestering repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF) in the cytoplasm, reducing its availability to bind neuron restrictive silencer element (NRSE) in the nucleus, thus allowing increased transcription (Zuccato et al. 2003). This mechanism also applies to a number of other NRSE-containing genes, and is impaired in mHTT. NRSE is only one of many pathways through which gene transcription is altered in HD. Other examples include nuclear receptor compressor (N-CoR) (Boutell et al. 1999), transcription factor Sp1 (Dunah et al. 2002), the Histone Acetyltransferase cAMP response element-binding protein (CREB) (Nucifora et al. 2001), and RNA-mediated gene silencing through association with the Argonaute (Savas et al. 2008). As a consequence, mHTT is associated with widespread alteration in gene transcription both in development (Feyeux et al. 2012) and diseased brains (Hodges et al. 2006). Drugs targeting some of these transcriptional changes, e.g., Histone Deacetylase Inhibitors, appear to be promising therapeutic agents (Steffan et al. 2001).

4 Neurotoxicity on an Intracellular Level

4.1 Role of the Intranuclear Inclusion Body

A characteristic feature of the neuropathology HD is the intranuclear inclusion body, which stains positive for ubiquitin and HTT. They also stain positive for antibodies raised to the N-terminal 17 amino acids, but not those raised to an internal epitope of HTT. This was the case in both the R6/2 mouse model (Mangiarini et al. 1996) and human archival tissue (Becher et al. 1998). The role of inclusion bodies in pathogenesis was clarified with automated microscopy and live cell imaging, where inclusion body formation was associated with improved neuron survival rather than death (Arrasate et al. 2004). Since then, the focus on mHTT has shifted to mHTT monomers and oligomers.

4.2 Mitochondrial Dysfunction

Mutant HTT can impair cellular mitochondrial function directly and indirectly. Direct effects of mHTT on the mitochondria include altered calcium handling and mitochondrial dynamics. Mitochondrial capacity to retain calcium is impaired in HD (Panov et al. 2002), where mHTT is thought to interrupt membrane integrity (Choo et al. 2004; Rockabrand et al. 2007). This, in turn, adds to the effect of glutamate/calcium-mediated excitotoxicity. Mutant HTT contributes toward increased intracellular calcium by sensitizing activation of the type 1 inositol-(1,4,5)-triphosphate receptor, thus increasing calcium release from the endoplasmic reticulum (Tang et al. 2003).

Mitochondria undergo continual fissure and fusion, and alteration of its dynamics can lead to neurodegeneration (Knott et al. 2008). Mutant HTT triggers mitochondrial fragmentation and arrests mitochondrial transport, through abnormal interaction with the GTPase dynamin-related protein 1 (DRP1) (Song et al. 2011). Interestingly these cellular changes can be rescued by reducing DRP1 activity.

Finally, mHTT also impairs mitochondrial function indirectly by inhibiting expression of peroxisome proliferator-activated receptor [PPAR]-gamma coactivator-1 alpha (PGC-1 alpha), a transcriptional coactivator that regulates mitochondrial function (Cui et al. 2006). The mechanism for this is likely to be through interference with the CREB transcription pathway.

4.3 Impaired Cytoskeleton and Intracellular Trafficking

Wildtype HTT binds to monomeric tubulin and microtubules, while mHTT disrupts integrity of microtubule filaments (Trushina et al. 2003). Mutant HTT is associated with neurite retraction in a cell culture model, indicating cytoskeletal collapse. Toxicity of mHTT can also be modulated by microtubule stabilization using low-dose taxol (Trushina et al. 2003).

Mutant HTT is associated with impaired axonal transport, with possible mechanisms including: titration of motor proteins such as kinesin from other cargoes (Gunawardena et al. 2003), direct blockage of axonal transport by aggregates (Lee et al. 2004), and by reducing kinesin-1 binding to microtubules through mHTT associated cJun N-terminal kinase (JNK) activation (Morfini et al. 2009).

Loss of normal function of wHTT, as an integrator of molecular motors, may also contribute toward the disrupted intracellular trafficking in HD (Caviston and Holzbaur 2009). An example of one such molecular binding partner for HTT is Huntingtin-Associated Protein 1 (HAP1) (Li et al. 1995). HAP1 is concentrated in axon terminals and promotes neurite extension (Li et al. 2000). Mutant HTT reduces HAP1 binding to molecular motors, resulting in decreased level of the

nerve growth factor receptor Tropomyosin-receptor kinase A (TrkA), and neurite outgrowth (Rong et al. 2006). Translating to human studies, an amino-acid substituting polymorphism in *HAP1* was found to be associated with a later age of onset in HD (Metzger et al. 2008). Finally, mHTT also impairs transport of mitochondria (Song et al. 2011) and vesicles (Gauthier et al. 2004).

5 Neurotoxicity on an Intercellular Level

5.1 Impaired Synaptic Transmission

In addition to vesicular transport, synaptic transmission is also affected in HD (Smith et al. 2005). In exocytosis, mHTT reduces the level of Complexin II, a protein involved in the transport vesicle/presynaptic protein-binding process (Edwardson et al. 2003). In that cell model, there was a corresponding reduction in calcium-triggered exocytosis of neurotransmitters.

Mutant HTT impairs endocytosis through interaction with caveolin-1, which has a role in forming invaginations of the plasma membrane (Trushina et al. 2006). There is some suggestion that mHTT could impair clathrin-mediated endocytosis; however, the data is conflicting (Trushina et al. 2006; Borgonovo et al. 2013).

These, together with other factors, have resulted in complex changes of glutamate and dopamine levels in the brain (Andre et al. 2010). Another neurotransmitter affected by mHTT is Gamma-AminoButyric Acid (GABA) (Pearson and Reynolds 1994).

5.2 Excitotoxicity and the Kynurenine Pathway

The association between excitotoxicity and HD was made prior to molecular characterization of HTT. This was based on the shared, extrinsic axon sparing phenotype, between the human HD brain and lesion models using excitatory amino acids (Schwarcz et al. 2010). In particular, the Quinolinic acid (QUIN) lesioned rats were found to have similar neurochemical profiles to human HD brains. These neurochemical profiles were characterized by depletion of GABA and Substance P, and relative sparing of Somatostatin/Neuropeptide Y neurons (Beal et al. 1986). QUIN level was also raised in early stage HD brains (Guidetti et al. 2004). QUIN is a downstream product of the kynurenine pathway (KP), which catabolizes the amino-acid tryptophan. It forms part of the neuroinflammatory response and acts as an excitotoxin through the *N*-methyl-D-aspartate (NMDA) receptor (Guillemin 2012). Recent interest in the KP for HD has grown, following reports that modulation of the KP led to improved life span in a mouse model (Zwilling et al. 2011), and a *Drosophila* model of HD (Campesan et al. 2011).

5.3 Inflammatory Response

There are a few lines of evidence, in addition to QUIN, that suggest a role of inflammation in HD (Soulet and Cicchetti 2011). In a mouse model where mHTT is selectively expressed in astrocytes only, the phenotype remained similar to the corresponding fully expressed HD mouse, albeit with a late onset (Bradford et al. 2009). Microglial activation measured on Positron Emission Tomography scans also correlated with clinical severity of people with HD (Pavese et al. 2006). Nonetheless, the exact role of inflammation in the pathogenesis of HD remains unclear.

6 Neurotoxicity on an Organ Level

6.1 Brain

Since Graveland et al.'s Golgi impregnation study in 1985, medium-sized spiny neurons in the striatum have been considered the primary cell type affected in HD (Graveland et al. 1985). Medium-sized spiny neurons require a high level of energy to maintain their unusually high transmembrane potentials. This makes them more vulnerable than other cell-types to the disrupted cellular respiration in HD (Mitchell et al. 1999).

However, the striatum is not the only brain region affected in HD. Recently, a large HD brain autopsy series ($N > 500$) (Hadzi et al. 2012) highlighted the role of the cerebral cortex. Forty-one neuropathologic ratings from various regions of the brain were categorised into two clusters: the striatal and cortical clusters. Neurodegeneration in the striatal cluster had a stronger correlation with HD CAG repeat size, but the cortical cluster had a stronger correlation with brain weight. These suggested that neurodegeneration in the striatum and the cortex do not necessarily proceed in a coupled manner.

6.2 Peripheral Tissues

Finally, the effect of mHTT reaches beyond the brain (Sassone et al. 2009; van der Burg et al. 2009). For instance, derangement in the hypothalamic–pituitary–adrenal axes is not the only potential mechanism for weight loss in HD. Other contributory mechanisms include the effect of mHTT on the gastrointestinal tract (impaired motility and malabsorption) (van der Burg et al. 2011), pancreas (altered glucose homeostasis) (Lalic et al. 2008), adipose tissue (Phan et al. 2009), and a hypermetabolic state (Mochel et al. 2007).

7 Conclusion

Given the complex and varied mechanisms leading to neurotoxicity in HD, it is unlikely that a therapeutic agent based on any single mechanism will be able to reverse all disease manifestations of HD. This makes oligonucleotides (RNA interference and antisense oligonucleotides) (Sah and Aronin 2011) a particularly attractive therapeutic approach, as they will be acting upstream of most other neurotoxic mechanisms. Animal studies using oligonucleotides have been promising (Kordasiewicz et al. 2012; Yu et al. 2012), and the first round of human safety trials is keenly awaited. However, complementary therapeutic approaches, addressing a range of pathogenic targets, need to be addressed in parallel, to combat this devastating disease.

References

ACMG/ASHG. (1998). ACMG/ASHG statement. Laboratory guidelines for Huntington disease genetic testing. The American College of Medical Genetics/American Society of Human Genetics Huntington Disease Genetic Testing Working Group. *American Journal of Human Genetics, 62*(5), 1243–1247.

Aiken, C. T., Steffan, J. S., et al. (2009). Phosphorylation of threonine 3: Implications for Huntingtin aggregation and neurotoxicity. *The Journal of Biological Chemistry, 284*(43), 29427–29436.

Andre, V. M., Cepeda, C., et al. (2010). Dopamine and glutamate in Huntington's disease: A balancing act. *CNS Neuroscience & Therapeutics, 16*(3), 163–178.

Arrasate, M., Mitra, S., et al. (2004). Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature, 431*(7010), 805–810.

Beal, M. F., Kowall, N. W., et al. (1986). Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature, 321*(6066), 168–171.

Becher, M. W., Kotzuk, J. A., et al. (1998). Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: Correlation between the density of inclusions and IT15 CAG triplet repeat length. *Neurobiology of Disease, 4*(6), 387–397.

Bence, N. F., Sampat, R. M., et al. (2001). Impairment of the ubiquitin-proteasome system by protein aggregation. *Science, 292*(5521), 1552–1555.

Borgonovo, J. E., Troncoso, M., et al. (2013). Mutant huntingtin affects endocytosis in striatal cells by altering the binding of AP-2 to membranes. *Experimental Neurology, 241*, 75–83.

Boutell, J. M., Thomas, P., et al. (1999). Aberrant interactions of transcriptional repressor proteins with the Huntington's disease gene product, huntingtin. *Human Molecular Genetics, 8*(9), 1647–1655.

Bradford, J., Shin, J. Y., et al. (2009). Expression of mutant huntingtin in mouse brain astrocytes causes age-dependent neurological symptoms. *Proceedings of the National Academy of Sciences of the United States of America, 106*(52), 22480–22485.

Campesan, S., Green, E. W., et al. (2011). The kynurenine pathway modulates neurodegeneration in a Drosophila model of Huntington's disease. *Current Biology, 21*(11), 961–966.

Cattaneo, E., Zuccato, C., et al. (2005). Normal huntingtin function: An alternative approach to Huntington's disease. *Nature Reviews. Neuroscience, 6*(12), 919–930.

Caviston, J. P., & Holzbaur, E. L. (2009). Huntingtin as an essential integrator of intracellular vesicular trafficking. *Trends in Cell Biology, 19*(4), 147–155.

Choo, Y. S., Johnson, G. V., et al. (2004). Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. *Human Molecular Genetics, 13*(14), 1407–1420.

Cui, L., Jeong, H., et al. (2006). Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell, 127*(1), 59–69.

Dragatsis, I., Levine, M. S., et al. (2000). Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. *Nature Genetics, 26*(3), 300–306.

Dunah, A. W., Jeong, H., et al. (2002). Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science, 296*(5576), 2238–2243.

Duyao, M. P., Auerbach, A. B., et al. (1995). Inactivation of the mouse Huntington's disease gene homolog Hdh. *Science, 269*(5222), 407–410.

Edwardson, J. M., Wang, C. T., et al. (2003). Expression of mutant huntingtin blocks exocytosis in PC12 cells by depletion of complexin II. *The Journal of Biological Chemistry, 278*(33), 30849–30853.

Ehrnhoefer, D. E., Sutton, L., et al. (2011). Small changes, big impact: Posttranslational modifications and function of huntingtin in Huntington disease. *The Neuroscientist, 17*(5), 475–492.

Feyeux, M., Bourgois-Rocha, F., et al. (2012). Early transcriptional changes linked to naturally occurring Huntington's disease mutations in neural derivatives of human embryonic stem cells. *Human Molecular Genetics, 21*(17), 3883–3895.

Gauthier, L. R., Charrin, B. C., et al. (2004). Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell, 118*(1), 127–138.

Graham, R. K., Deng, Y., et al. (2006). Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. *Cell, 125*(6), 1179–1191.

Graveland, G. A., Williams, R. S., et al. (1985). Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science, 227*(4688), 770–773.

Gu, X., Greiner, E. R., et al. (2009). Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. *Neuron, 64*(6), 828–840.

Guidetti, P., Luthi-Carter, R. E., et al. (2004). Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. *Neurobiology of Disease, 17*(3), 455–461.

Guillemin, G. J. (2012). Quinolinic acid, the inescapable neurotoxin. *The FEBS Journal, 279*(8), 1356–1365.

Gunawardena, S., Her, L. S., et al. (2003). Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. *Neuron, 40*(1), 25–40.

Hadzi, T. C., Hendricks, A. E., et al. (2012). Assessment of cortical and striatal involvement in 523 Huntington disease brains. *Neurology, 79*(16), 1708–1715.

Hodges, A., Strand, A. D., et al. (2006). Regional and cellular gene expression changes in human Huntington's disease brain. *Human Molecular Genetics, 15*(6), 965–977.

Jana, N. R., Zemskov, E. A., et al. (2001). Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release. *Human Molecular Genetics, 10*(10), 1049–1059.

Jeong, H., Then, F., et al. (2009). Acetylation targets mutant huntingtin to autophagosomes for degradation. *Cell, 137*(1), 60–72.

Kaltenbach, L. S., Romero, E., et al. (2007). Huntingtin interacting proteins are genetic modifiers of neurodegeneration. *PLoS Genetics, 3*(5), e82.

Kim, M. W., Chelliah, Y., et al. (2009). Secondary structure of Huntingtin amino-terminal region. *Structure, 17*(9), 1205–1212.

Knott, A. B., Perkins, G., et al. (2008). Mitochondrial fragmentation in neurodegeneration. *Nature Reviews. Neuroscience, 9*(7), 505–518.

Kordasiewicz, H. B., Stanek, L. M., et al. (2012). Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. *Neuron, 74*(6), 1031–1044.

Lalic, N. M., Maric, J., et al. (2008). Glucose homeostasis in Huntington disease: Abnormalities in insulin sensitivity and early-phase insulin secretion. *Archives of Neurology, 65*(4), 476–480.

Lee, W. C., Yoshihara, M., et al. (2004). Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America, 101*(9), 3224–3229.

Lee, J. M., Ramos, E. M., et al. (2012). CAG repeat expansion in Huntington disease determines age at onset in a fully dominant fashion. *Neurology, 78*(10), 690–695.

Leeflang, E. P., Zhang, L., et al. (1995). Single sperm analysis of the trinucleotide repeats in the Huntington's disease gene: Quantification of the mutation frequency spectrum. *Human Molecular Genetics, 4*(9), 1519–1526.

Legleiter, J., Mitchell, E., et al. (2010). Mutant huntingtin fragments form oligomers in a polyglutamine length-dependent manner in vitro and in vivo. *The Journal of Biological Chemistry, 285*(19), 14777–14790.

Li, X. J., Li, S. H., et al. (1995). A huntingtin-associated protein enriched in brain with implications for pathology. *Nature, 378*(6555), 398–402.

Li, S. H., Li, H., et al. (2000). Expression of huntingtin-associated protein-1 in neuronal cells implicates a role in neuritic growth. *Molecular and Cellular Neurosciences, 16*(2), 168–183.

MacMillan, J. C., & Harper, P. S. (1991). Single-gene neurological disorders in South Wales: An epidemiological study. *Annals of Neurology, 30*(3), 411–414.

Mangiarini, L., Sathasivam, K., et al. (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell, 87*(3), 493–506.

Metzger, S., Rong, J., et al. (2008). Huntingtin-associated protein-1 is a modifier of the age-at-onset of Huntington's disease. *Human Molecular Genetics, 17*(8), 1137–1146.

Meyer, L. R., Zweig, A. S., Hinrichs, A. S., Karolchik, D., Kuhn, R. M., Wong, M., Sloan, C. A., Rosenbloom, K. R., Roe, G., Rhead, B., Raney, B. J., Pohl, A., Malladi, V. S., Li, C. H., Lee, B. T., Learned, K., Kirkup, V., Hsu, F., Heitner, S., Harte, R. A., Haeussler, M., Guruvadoo, L., Goldman, M., Giardine, B. M., Fujita, P. A., Dreszer, T. R., Diekhans, M., Cline, M. S., Clawson, H., Barber, G. P., Haussler, D., & Kent, W. J. (2012). The UCSC Genome Browser database: Extensions and updates 2013. *Nucleic Acids Research*, D918–D923. doi:10.1093/nar/gkr1055.

Miller, J., Arrasate, M., et al. (2011). Identifying polyglutamine protein species in situ that best predict neurodegeneration. *Nature Chemical Biology, 7*(12), 925–934.

Milnerwood, A. J., & Raymond, L. A. (2010). Early synaptic pathophysiology in neurodegeneration: Insights from Huntington's disease. *Trends in Neurosciences, 33*(11), 513–523.

Mitchell, I. J., Cooper, A. J., et al. (1999). The selective vulnerability of striatopallidal neurons. *Progress in Neurobiology, 59*(6), 691–719.

Mochel, F., Charles, P., et al. (2007). Early energy deficit in Huntington disease: Identification of a plasma biomarker traceable during disease progression. *PLoS One, 2*(7), e647.

Morfini, G. A., You, Y. M., et al. (2009). Pathogenic huntingtin inhibits fast axonal transport by activating JNK3 and phosphorylating kinesin. *Nature Neuroscience, 12*(7), 864–871.

Nasir, J., Floresco, S. B., et al. (1995). Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell, 81*(5), 811–823.

Nucifora, F. C., Jr., Sasaki, M., et al. (2001). Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science, 291*(5512), 2423–2428.

Olshina, M. A., Angley, L. M., et al. (2010). Tracking mutant huntingtin aggregation kinetics in cells reveals three major populations that include an invariant oligomer pool. *The Journal of Biological Chemistry, 285*(28), 21807–21816.

Ortega, Z., Diaz-Hernandez, M., et al. (2010). Acute polyglutamine expression in inducible mouse model unravels ubiquitin/proteasome system impairment and permanent recovery attributable to aggregate formation. *The Journal of Neuroscience, 30*(10), 3675–3688.

Panov, A. V., Gutekunst, C. A., et al. (2002). Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nature Neuroscience, 5*(8), 731–736.

Pavese, N., Gerhard, A., et al. (2006). Microglial activation correlates with severity in Huntington disease: A clinical and PET study. *Neurology, 66*(11), 1638–1643.

Pearson, S. J., & Reynolds, G. P. (1994). Neocortical neurotransmitter markers in Huntington's disease. *Journal of Neural Transmission. General Section, 98*(3), 197–207.

Peters-Libeu, C., Miller, J., et al. (2012). Disease-associated polyglutamine stretches in monomeric huntingtin adopt a compact structure. *Journal of Molecular Biology, 421*(4–5), 587–600.

Phan, J., Hickey, M. A., et al. (2009). Adipose tissue dysfunction tracks disease progression in two Huntington's disease mouse models. *Human Molecular Genetics, 18*(6), 1006–1016.

Rockabrand, E., Slepko, N., et al. (2007). The first 17 amino acids of Huntingtin modulate its subcellular localization, aggregation and effects on calcium homeostasis. *Human Molecular Genetics, 16*(1), 61–77.

Rong, J., McGuire, J. R., et al. (2006). Regulation of intracellular trafficking of huntingtin-associated protein-1 is critical for TrkA protein levels and neurite outgrowth. *The Journal of Neuroscience, 26*(22), 6019–6030.

Ross, C. A., & Tabrizi, S. J. (2011). Huntington's disease: From molecular pathogenesis to clinical treatment. *Lancet Neurology, 10*(1), 83–98.

Sah, D. W., & Aronin, N. (2011). Oligonucleotide therapeutic approaches for Huntington disease. *The Journal of Clinical Investigation, 121*(2), 500–507.

Sassone, J., Colciago, C., et al. (2009). Huntington's disease: The current state of research with peripheral tissues. *Experimental Neurology, 219*(2), 385–397.

Sathasivam, K., Neueder, A., et al. (2013). Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington disease. *Proceedings of the National Academy of Sciences of the United States of America, 110*, 2366–2370.

Savas, J. N., Makusky, A., et al. (2008). Huntington's disease protein contributes to RNA-mediated gene silencing through association with Argonaute and P bodies. *Proceedings of the National Academy of Sciences of the United States of America, 105*(31), 10820–10825.

Schilling, B., Gafni, J., et al. (2006). Huntingtin phosphorylation sites mapped by mass spectrometry. Modulation of cleavage and toxicity. *The Journal of Biological Chemistry, 281*(33), 23686–23697.

Schwarcz, R., Guidetti, P., et al. (2010). Of mice, rats and men: Revisiting the quinolinic acid hypothesis of Huntington's disease. *Progress in Neurobiology, 90*(2), 230–245.

Smith, R., Brundin, P., et al. (2005). Synaptic dysfunction in Huntington's disease: A new perspective. *Cellular and Molecular Life Sciences, 62*(17), 1901–1912.

Society for Neuroscience. (2005). Huntington's disease: Making a difference today. *Society for Neuroscience*.

Song, W., Chen, J., et al. (2011). Mutant huntingtin binds the mitochondrial fission GTPase dynamin-related protein-1 and increases its enzymatic activity. *Nature Medicine, 17*(3), 377–382.

Soulet, D., & Cicchetti, F. (2011). The role of immunity in Huntington's disease. *Molecular Psychiatry, 16*(9), 889–902.

Southwell, A. L., Bugg, C. W., et al. (2011). Perturbation with intrabodies reveals that calpain cleavage is required for degradation of huntingtin exon 1. *PLoS One, 6*(1), e16676.

Steffan, J. S., Bodai, L., et al. (2001). Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. *Nature, 413*(6857), 739–743.

Strong, T. V., Tagle, D. A., et al. (1993). Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. *Nature Genetics, 5*(3), 259–265.

Subramaniam, S., Sixt, K. M., et al. (2009). Rhes, a striatal specific protein, mediates mutant-huntingtin cytotoxicity. *Science, 324*(5932), 1327–1330.

Tang, T. S., Tu, H., et al. (2003). Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. *Neuron, 39*(2), 227–239.

The Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell, 72*(6), 971–983.

Trushina, E., Heldebrant, M. P., et al. (2003). Microtubule destabilization and nuclear entry are sequential steps leading to toxicity in Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America, 100*(21), 12171–12176.

Trushina, E., Singh, R. D., et al. (2006). Mutant huntingtin inhibits clathrin-independent endocytosis and causes accumulation of cholesterol in vitro and in vivo. *Human Molecular Genetics, 15*(24), 3578–3591.

Vamos, M., Hambridge, J., et al. (2007). The impact of Huntington's disease on family life. *Psychosomatics, 48*(5), 400–404.

van der Burg, J. M., Bjorkqvist, M., et al. (2009). Beyond the brain: Widespread pathology in Huntington's disease. *Lancet Neurology, 8*(8), 765–774.

van der Burg, J. M., Winqvist, A., et al. (2011). Gastrointestinal dysfunction contributes to weight loss in Huntington's disease mice. *Neurobiology of Disease, 44*(1), 1–8.

Walker, F. O. (2007). Huntington's disease. *Lancet, 369*(9557), 218–228.

Wexler, N. S., Young, A. B., et al. (1987). Homozygotes for Huntington's disease. *Nature, 326*(6109), 194–197.

Yanai, A., Huang, K., et al. (2006). Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. *Nature Neuroscience, 9*(6), 824–831.

Yu, D., Pendergraff, H., et al. (2012). Single-stranded RNAs use RNAi to potently and allele-selectively inhibit mutant huntingtin expression. *Cell, 150*(5), 895–908.

Zuccato, C., Ciammola, A., et al. (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science, 293*(5529), 493–498.

Zuccato, C., Tartari, M., et al. (2003). Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nature Genetics, 35*(1), 76–83.

Zwilling, D., Huang, S.-Y., et al. (2011). Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. *Cell, 145*(6), 863–874.

Neurotoxicity: A Complex Multistage Process Involving Different Mechanisms

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Abstract

Neurotoxicity is defined as alterations causing physical damage or disruption in functions of the cells (neurons, glial cells) of the nervous system. There are several mechanisms by which neurotoxic processes take place. These include high concentrations of glutamate and/or calcium ions, increased oxidative damage, and reactive species (RS) such as free radicals, mechanisms linked to execution of

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apoptosis, glial dysfunction injury, and/or genetic-epigenetic defects in protein clearance. Each of these mechanisms reciprocally modulates their own actions and is capable of neuronal cell death followed by nervous system dysfunction.

Keywords

Apoptosis • Calcium ions • DNA damage • Epigenetic • Glia activation • Glutamate • Neurotoxicity mechanism • Neurotoxicity pathway • Oxidative stress

List of Abbreviations

5-HT	Serotonin
AIF	Proapoptotic inducting factor
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Apaf-1	Apoptotic protease activating factor-1
ATP	Adenosine triphosphate
CAT	Catalase
CNS	Central nervous system
CpG	Cytosine-guanine dinucleotide
CREB	cAMP response element binding
CYP	Cytochrome P450
DISC	Death-inducing signaling complex
DNMT	DNA methyltransferase
EAAT	Excitatory amino-acid transporter
endo-G	Endonuclease G
esiRNA	Endogenous small interfering RNA (endo-siRNA)
FADD	Fas-associated death domain
FasL	Fas ligand
GABA	ɣ-aminobutyric acid
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HDM	Histone demethylase
HMT	Histone methyltransferase
IAP	Inhibitor of apoptosis protein
IL	Interleukin
JNK	c-Jun NH_2-terminal kinase
lncRNA	Long noncoding RNA
MAO	Monoamine oxidase
MDA	Malondialdehyde
miRNA	microRNA
MMP	Metalloproteinase
ncRNA	Noncoding RNA
NMDA	*N*-methyl-D-aspartic acid

NOS	Nitric oxide synthase
NSL	Nuclear localization signal
PARP	Poly-ADP ribose polymerase
piRNA	Piwi-interacting RNA
RBS	Reactive bromine species
RCS	Reactive chloride species
RIP	Receptor-interacting protein
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RS	Reactive species
SMAC	Small mitochondria-derived activator of caspase
SOD	Superoxide dismutase
SODD	Silencer of death domain
TNF	Tumor necrosis factor
TRADD	TNF receptor-associated death domain
TRAF2	TNF-R-associated factor 2
TRPM	Transient receptor potential melastin
VGLUT	Vesicular glutamate transporter
xCT	Cystine-glutamate antiporter

1 Introduction

Neurotoxicity is defined as alterations in the normal activity of the nervous system to cause its physical damage (e.g., cell death) or disruption in functions (e.g., neurocognitive deficits, poor judgments, and diminished decision-making capacity). Within the central nervous system (CNS), neuronal cells are key factors for neurotoxicity and degeneration in many diseases. However, in the mid-1990s research has shown that glial cells (i.e., astroglia, microglia, and oligodendrocytes) also play a number of active roles in the brain and are sensitive indicators for pathology of the CNS (see also Clark et al. 2013). Thus, astrocytes – apart from provision of nutrients to the nervous tissue, maintenance of both extracellular ion balance and the blood–brain barrier, and a role in the repair and scarring process of the brain and spinal cord following traumatic injuries – are engaged in the release or absorption of neural transmitters (called gliotransmission) (Ben Achour and Pascual 2012; Santello et al. 2012). For example, astrocytes propagate intercellular calcium waves over long distances in response to stimulation and, similar to neurons, release transmitters in a Ca^{2+}-dependent manner (Zorec et al. 2012), while a new report indicates also that astrocytes from adult human brain are ɣ-aminobutyric (GABA)-ergic cells possessing enzymes and receptors and modulating microglial activity representing GABAceptive cells (Lee et al. 2011). The concept of a "tripartite synapse" referring to the relationship occurring at synapses among a presynaptic and a postsynaptic element as well as a glial element has been

introduced (Santello et al. 2012). On the other hand, microglial cells are activated due to neuronal damage leading to reactive gliosis and to cytoarchitectonic changes accompanied by alterations in surface receptor and channel expression as well as to release of neuroactive soluble factors (see below) that in turn exert actions on neuronal function and cell death. Recently, it was found that oligodendroglia in the CNS is not only linked to myelin formation and maintenance, but they actively participate in neuronal survival and development as well as in neurotransmission and synaptic function including neurotoxic processes (Deng and Poretz 2003).

Neurotoxicity can result from exposure to several factors (intrinsic and exogenous xenobiotics) that disturb the normal physiology and function of the nervous system. The exogenous and intrinsic factors influence each other's actions.

2 Intrinsic Factors Engaged in Neurotoxicity

There are several endogenous brain toxins which in high amount may lead to neurotoxicity. When present in high concentrations, glutamate, calcium ions, and reactive species (RS) such as free radicals, proinflammatory cytokines, tumor necrosis factor alpha (TNF-α), or neurotrophins released by microglia are potentially neurotoxic (Aloisi 2001; Aloisi et al. 2001; Hanisch 2002). All of them reciprocally modulate their own actions and are responsible for neuronal cell death followed by nervous system dysfunction (e.g., loss of motor control; cognitive deterioration; loss of memory, vision, and/or intellect; uncontrollable obsessive and/or compulsive behaviors; delusions; headache; or neurodegenerative diseases). The most common to damage cells within the CNS are glutamate, increased cell calcium concentrations, RS and increased oxidative damage, mitochondrial dysfunction linked to apoptotic pathways, glial dysfunction injury, and/or genetic-epigenetic defects in protein clearance (cf. Halliwell 2006).

2.1 Neurotoxicity Induced by Glutamate

Glutamate serves a principal brain neurotransmitter, while its neurotransmission impairment such as high concentrations (>μM in extracellular fluids) evokes excitotoxicity to neurons. The glutamate concentration equilibrium is extremely delicate and is under control of glutamate transporters that pump the glutamate back from the neuronal synapse into the glia and neurons and allow glutamate to be recycled for repeated release via glutamate-glutamine neuronal glial cycle.

There are two general classes of glutamate transporters, those that are dependent on a sodium ion gradient (so-called the excitatory amino-acid transporters; EAATs) and those that are not dependent (vesicular glutamate transporters (VGLUTs) and cystine-glutamate antiporter (xCT)). EAATs are also dependent on transmembrane K^+ and H^+ concentration gradients and are known also as "sodium- and potassium-coupled glutamate transporters" or "high-affinity glutamate transporters." In humans and rodents, five subtypes of EAATs have been identified: subtypes EAAT1–2 are

found in membranes of glial cells (astrocytes, microglia, and oligodendrocytes) and in endothelial cells, EAAT3–4 are located on neurons, while EAAT5 is found in the retina. Four VGLUTs (VGLUTs 1–3 and sialin) are found in the membrane of glutamate-containing synaptic vesicles. The xCT located to the plasma membrane of cells prefers cystine and glutamate as its substrates in the way that the anionic form of cystine is transported in exchange for glutamate to glial cells. The xCT protein is upregulated in glial cells upon oxidative stress and plays an essential role to protect neurons. The glial transporters – in particular the various splice variants of EAAT2 and xCT – play the largest role (>90 %) in regulating extracellular glutamate levels. Data suggest that astrocytes also signal to neurons through Ca^{2+}-dependent release of glutamate (Hua et al. 2004).

In the event of any disruption in the function of glutamate transporters, a high concentration of glutamate accumulates at the glutamate receptors. Exposing neurons to high glutamate concentrations results in the activation of ionotropic glutamate receptors such as the *N*-methyl-D-aspartic acid (NMDA) receptors or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which induce faster mitochondrial Ca^{2+} uptake (Rego and Oliveira 2003). NMDA receptor overstimulation allows high levels of calcium ions to enter to cells, causing formation of mitochondrial RS and lipid peroxidation (Maciel et al. 2001). Moreover, extrasynaptic NMDA receptor activation promotes a dominant CREB (cAMP response element binding) dephosphorylation pathway that leads to protective CREB function shutoff causing loss of mitochondrial membrane potential and finally apoptosis (Hardingham and Bading 2010).

It should be added that NMDA glutamatergic receptors are stimulated also by quinine, being a product of the autoxidative pathway of dopamine metabolism (Lieb et al. 1995) which was shown to be neurotoxic in striatal neurons (Ben-Shachar et al. 1995).

2.2 Neurotoxicity Induced by Calcium Ions

High Ca^{2+} cell concentration in the cytosol initiates apoptosis (a cell death that leads to the elimination of cells without releasing harmful substances into the surrounding area) through cleaved caspase processing and activation of a number of several intracellular enzymes (e.g., phospholipase A_2, nitric oxide synthase, calcineurin, endonucleases, and xanthine dehydrogenase) and proteases (e.g., calpain) which by themselves can induce the formation of RS or damage the cytoskeleton, membrane, or DNA (Rego and Oliveira 2003). Another damaging result of excess Ca^{2+} in the cytosol is the opening of the mitochondrial permeability transition pore. Opening this mitochondrial pore may cause release of reactive oxygen species (ROS), more Ca^{2+}, more proteins that can lead to apoptosis and finally to cessation of adenosine triphosphate (ATP) production (Court and Coleman 2012).

A rise in Ca^{2+} intracellular concentration is generated – among others – by glutamate high synaptic concentrations (see above) or products of lipid peroxidation (e.g., 4-hydroxynoneral; see below).

2.3 Neurotoxicity Induced by RS

RS include free radicals and non-radical molecules. A free radical means any species capable of independent existence ("free") that contains one or more unpaired valence shell electrons (Halliwell and Gutteridge 1988). These chemically reactive molecules include ROS, reactive bromine species (RBS), reactive chloride species (RCS), and reactive nitrogen species (RNS). It should be stated that the term ROS is usually used as a collective term for both oxygen radicals that include free radicals (e.g., superoxide $O_2^{\bullet -}$, hydroxyl $OH^{\bullet}$, hydroperoxyl $HO_2^{\bullet}$) and non-radicals (e.g., hydrogen peroxide H_2O_2, peroxynitrite $ONOO^-$). The collective definition exists also for RBS (free radical: atomic bromide Br and non-radicals: bromine gas Br_2, hypobromous acid, HOBr), for RCS (free radical: atomic chlorine Cl and non-radicals: hypochlorous acid HOCl, chloramines), and for RNS (free radicals: nitric oxide NO, nitrogen dioxide NO_2, and nitrate NO_3 and non-radicals: nitrous acid HNO_2, nitrosyl anion NO^-, peroxynitrite $ONOO^-$, and peroxynitrate O_2NOO^-). Among RS, the most neurotoxic are $O_2^{\bullet -}$, $OH^{\bullet}$, and H_2O_2 (Bartosz 2009; Matés et al. 2010), the first radical leaking from the respiratory chain in mitochondria, the second radical resulting from the dismutation of $O_2^{\bullet -}$ or directly from the action of oxidase enzymes, and the non-radical species causing modification of purine and pyrimidine bases that lead to DNA damage.

The most important source of RS for aerobic cells is O_2 due to its high consumption, especially by the mammalian brain (Halliwell 2006). When formed during the normal metabolism of oxygen, RS have important roles in cell signaling and homeostasis; however, strong environmental stressors (e.g., UV, heat exposure, ionizing radiation, chemical substances including addictive drugs) increase their brain levels. In the brain, formation of oxygen radicals is linked to an increase in the Ca^{2+} concentration inside a cell (via membrane Ca^{2+} exporters, transient receptor potential melastatin-related (TRPM)2 cation channels located on some neurons and glia or via Ca^{2+} pumps in the endoplasmic reticulum) due to damage of mitochondrial function by neurotoxins following disruption of ATP production. High intracellular Ca^{2+} levels can activate neuronal nitric oxide synthase (NOS), phospholipase A_2, and calpains and/or interfere with mitochondrial function. The interaction between the Ca^{2+} and Ca^{2+}-dependent NOS pathway results in the production of $NO^{\bullet}$ which reacts with O_2 forming $ONOO^-$. $ONOO^-$ and other RS may decrease glutamate transporter activity on glial cells and inactivate glutamine synthethase (Aksenov et al. 1997; Trotti et al. 1996; Noack et al. 1998; Koppal et al. 1999), while 4-hydroxynonenal (a major aldehydic product formed by peroxidation of ω-6-unsaturated fatty acids regarded as a specific marker of lipid peroxidation) can impair the function of glutamate transporters (Mattson and Chan 2003). Disturbances in glutamate clearance may promote the high Ca^{2+} intracellular levels and lead to mitochondrial and protein dysfunctions.

Other potential sources of RS are cytochromes P450 (CYPs) and activated microglia. CYPs are enzymes localized to almost every cell, including some brain regions (Miksys and Tyndale 2004). Among CYPs, the CYP2E1 leaks electrons during the catalytic cycle and is especially linked to RS production (Gonzalez 2005);

however, the CYP2E1-dependent RS generation is important following exposure to, e.g., solvents, tobacco, or ethanol, as these substances significantly increase the enzyme levels.

It was also found that neurotoxin-activated microglia can produce $O_2^{\bullet -}$, H_2O_2, and cytokines (interleukin (IL)-1, interleukin-6, and TNF α). In turn, cytokines lead to activation of microglia and then to production of more RS, inducible NOS and $NO^{\bullet}$ (Duncan and Heales 2005). Of note, cytokines may be also produce by activated astroglia (Duncan and Heales 2005).

RS are also generated by endogenous sources such as neurotransmitters. In fact, catecholamines such as dopamine, its precursor L-DOPA and norepinephrine, as well as indolamine serotonin (5-HT) react with O_2 to produce $O_2^{\bullet -}$ (Spencer et al. 1998). The best recognized is dopamine-induced generation of RS. Thus, dopamine metabolism includes either a nonenzymatic pathway (via molecular oxygen through autoxidation) to generate hydrogen peroxide (H_2O_2) and a superoxide anion ($O_2^{\bullet -}$) or an enzymatic pathway by monoamine oxidase (MAO) to form H_2O_2. Both of these radicals may react via the Haber-Weiss/Fenton reaction with transition metal ions to produce the highly toxic $OH^{\bullet}$ (Hermida-Ameijeiras et al. 2004).

As the brain is the most vulnerable to increased levels of RS due to its low oxidative capacity and because neurons are characterized as postmitotic cells, it means that neurons live with accumulated damage over the years. Harmful effects of RS on the cell include induction of apoptotic pathways, damage of DNA, oxidations of polyunsaturated fatty acids in lipids (lipid peroxidation and malondialdehyde (MDA) are widely studied; MDA is a product of polyunsaturated fatty acid peroxidation), oxidations of amino acids in proteins, and oxidatively inactivated specific enzymes by oxidation of their cofactors. Of note, there is positive feedback response between the mitochondrial production of RS and glutamate concentration at the synapse which further gives rise to postsynaptic Ca^{2+} levels via RS-mediated inhibition of glial EAAT2 function.

Whether RS will act as damaging or signaling molecules and whether "oxidative stress" occurs depends on the delicate equilibrium between RS production and scavenging/detoxification. There are several cellular or extracellular defensive mechanisms comprising of the nonenzymic as well as enzymic antioxidants to break down RS. The enzymic antioxidants include proteins such as superoxide dismutases (SOD), catalases (CAT), peroxiredoxins, glutathione peroxidase (GPx), glutathione reductases (GR), thioredoxin reductases, heme oxygenase-1, eosinophil peroxidase, and metallothionein (Matés et al. 2010). An essential defense role against oxidative stress in all aerobic organisms is played by SOD which converts O_2^- to H_2O_2 and molecular oxygen. Four classes of SOD have been identified: cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, extracellular-SOD, and mitochondrial Fe-SODs; the first three SODs exist in humans. CAT, GRx, or peroxiredoxins convert molecules of H_2O_2 into water and oxygen, while GR catalyzes the reduction of glutathione disulfide (GSSG) to glutathione (GSH) and, thus, maintains a cellular redox state (Matés et al. 2008).

A potent nonenzymatic antioxidant defense system is comprised of vitamin C (ascorbic acid), vitamin E (α-tocopherol), vitamin A, β-carotene, polyamines,

melatonin, NADPH, adenosine, urate, coenzyme Q-10 (ubiquinol), polyphenols, flavonoids, phytoestrogens, GSH, cysteine, homocysteine, taurine, methionine, *S*-adenosyl-L-methionine, nitroxides, and selenium (Matés et al. 2008). Among these small antioxidant molecules ascorbate, GSH and tocopherols are of special significance. Thus, ascorbic acid donates electrons in a number of enzymatic and nonenzymatic reactions, while GSH by reaction with $OH^{\bullet}$ or H_2O_2 can function directly as a free radical scavenger and protect proteins, lipids, or DNA either by the formation of adducts directly with reactive electrophiles or by acting as proton donor in the presence of RS. Tocopherols potently scavenge oxygen and protect lipids and lipid structures against peroxidation.

2.4 Neurotoxicity Induced by Apoptosis

Due to physiological as well as pathological conditions including intracellular rise in RS or Ca^{2+} levels as well as other strong stressors (e.g., hyperthermia, ionizing radiation, some toxic substances; (Bojes et al. 1999; Darzynkiewicz et al. 2009)), a programmed cell death (apoptosis) appears. Apoptosis is a multistep process characterized by biochemical events that lead to cell blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and, finally, cell death. The process of apoptosis is controlled by diverse signals, which may originate either extracellularly (e.g., toxins, cytokines) that must cross the plasma membrane or transduce a response or intracellularly (Darzynkiewicz et al. 2009).

Apoptosis is executed by either caspases or noncaspase proteases (Wyllie 2010). Caspases are cysteine-dependent aspartate-specific proteases forming a family of over a dozen caspases. They function as initiator caspases (caspase 2, 8, 9, 10 leading to activation of effector caspases) and effector caspases (caspase 3, 6, 7 that proteolytically degrade intracellular proteins to kill a cell). Two intracellular caspase pathways have been recognized; one is triggered by message from a death receptor (so-called the extrinsic pathway) and the other activated by an intracellular message such as DNA damage and microtubule disruption (so-called the intrinsic or mitochondrial pathway) (Gabai et al. 2002).

The extrinsic pathway for caspase activation engages apoptotic mechanisms through stimulation of the TNR family, i.e., TNF receptors and Fas at the cell surface. TNF1 receptors are activated by TNF, and such linkage results in the release of silencer of death domains (SODD) and formation of a receptor-proximal complex containing the important adaptor proteins TNF receptor-associated death domain (TRADD), TNF-R-associated factor 2 (TRAF2), receptor-interacting protein (RIP), and Fas-associated death domain (FADD). These adaptor proteins enhance activity of the TNF pathway-specific enzymes (e.g., caspase 8 and IKKβ) to the TNF-R1 complex. These enzymes, in turn, initiate the activation of c-Jun NH_2-terminal kinase (JNK), a kinase that phosphorylates c-Jun and increases its transcriptional activity, while the protein kinase RIP is critical to the activation of the transcription factor NF-κB.

Fas are also stimulated by another death activator, Fas ligand (FasL). The interaction between Fas and FasL results in the formation of the death-inducing signaling complex (DISC), which contains the FADD, caspase 8 and caspase 10. Activation of caspase 8 initiates a cascade of caspase activation leading to cell phagocytosis.

The internal/mitochondrial pathway for caspase activation is initiated by proapoptotic (Bax, Bak, Bid, or Bad) homodimers of the *Bcl-2* family that inhibit antiapoptotic (Bcl-Xl and Bcl-2) Bcl-2 members. The proapoptotic proteins are required to allow the release of caspase activators such as cytochrome c and small mitochondria-derived activator of caspases (SMAC) out the mitochondrial membrane. The released cytochrome c binds to the apoptotic protease activating factor-1 (Apaf-1) forming apoptosomes that later activate caspase 9. Caspase 9, in turn, activates effector caspases 3 and 7. Caspase 3 destroys poly-ADP ribose polymerase (PARP), a protein which takes part in DNA repair; caspase 3 also initiates DNA fragmentation. SMACs are mitochondrial proteins released into the cytosol to bind to the inhibitor of apoptosis proteins (IAPs) such as caspases and deactivate them.

The noncaspase-related execution of apoptosis is linked to activation of calpains. The calpains exist in two isoforms (I and II) and are Ca^{2+}-dependent proteases. They degrade membrane, cytoplasmic, and nuclear substrates, leading to the breakdown of cellular architecture. Among others, they release proapoptotic factor (AIF) (Breckenridge and Xue 2004) from the mitochondria into the cytosol. AIF migration into the nucleus is mediated by its nuclear localization signal (NSL); in the nucleus AIF binds with DNA strands inducing its fragmentation and cell death. Of note, another effector of caspase-independent cell death is endonuclease G (Endo-G) released from mitochondria (Singh et al. 2004).

2.5 Neurotoxicity Induced by Glial Cell Activation

Among several glial cells, microglia are preferentially exposed to toxic factors and are strongly engaged in brain toxicity.

Microglia constitute 20 % of the total glial cell population within the brain and are the first form of active immune defense in the CNS responsible for maintaining homeostasis. There are several factors that activate microglia, such as glutamate and glutamate receptor agonists, proinflammatory cytokines, cell necrosis factors, lipopolysaccharide, and extracellular potassium changes (Nakajima and Kohsaka 2001; Graeber and Streit 2010). In turn, activated microglia via hemichannels within cell membranes (Orellana et al. 2012) can secrete a variety of neurotoxic substances, among them are (i) large amounts of H_2O_2 and $NO^{\bullet}$ – the process known as "respiratory burst" leads to neuronal cell death; (ii) proteases (i.e., cathepsins B, L, and S; the matrix metalloproteinases (MMP)-1, MMP-2, MMP-3, and MMP-9; the metalloprotease-disintegrin ADAM8 plasminogen; and elastase) that catabolize various proteins causing cellular damage; (iii) nuclear factor κB, a transcription factor that starts proinflammatory actions in microglia; (iv) proinflammatory chemokines (MCP-1, MIP-1α, and MIP-1β); (v) proinflammatory cytokines (IL-1α, IL-1β) and TNF-α that initiate demyelination of neuronal axons and/or

apoptosis through activation of caspase 8 and caspase 3; and (vi) excitatory amino-acid glutamate and aspartate that injury neurons (Block et al. 2007; Graeber and Streit 2010).

Another glial cell type, oligodendroglia, expresses a variety of ligand- and voltage-gated neurotransmitter receptors/ion channels, including NMDA and AMPA (Belachew et al. 1999; Rogister et al. 1999). The presence of functional neurotransmitter receptors/ion channels in glia indicates that there is glutamate-mediated cross talk between neurons and glia (Bergles et al. 2000; Gallo and Ghiani 2000) as well as that which oligodendrocytes share with neurons – a high vulnerability to glutamate excitotoxicity.

On the other hand, oligodendrocytes (as well as astrocytes) have the ability to protect themselves from RS as they contain high concentrations of antioxidant enzymes and GSH (Makar et al. 1994). However, in conditions where protective functions of astrocytes are impaired, oligodendrocytes may be vulnerable to oxidative and RS-mediated damage (e.g., following increased metabolism of catecholamine neurotransmitters that generates H_2O_2) (Noble et al. 1994).

2.6 Neurotoxicity Induced by DNA Damage

DNA is a genetic material, and any DNA damage can result in malfunctions or complete inactivation of encoded proteins. Genomic instability is an important mechanism leading to death of neurons. Several environmental factors (e.g., UV radiation, plant and chemical toxic substances, viruses) or endogenous sources (e.g., RS) are responsible for DNA damage that may occur during the synthesis of DNA precursors, DNA repair, and during various methylation events, including DNA methylation (see below). Both DNA repair and DNA methylation are critical for maintaining genomic stability. Several forms of DNA damage may appear including mutations, deletions, gene amplification, and rearrangements (Matés et al. 2008).

There is a relationship between oxidative stress and genomic instability (Matés et al. 2008). In fact, ROS (especially $OH^{\bullet}$) are a major source of DNA sequence changes leading to deoxyribose oxidation, a generation of a deoxyribose radical that further initiates strand breakage, removal of nucleotides, a variety of modifications in the organic bases of the nucleotides, and DNA-protein cross-linkages. Of note, the formation of 8-hydroxy-2′-deoxyguanosine is regarded as an index of oxidative DNA damage (Frenkel et al. 1991).

DNA damage may result in the initiation of signaling leading to neurotoxic consequences such as cell death or to the activation of several proto-oncogenes and/or the inactivation of some tumor suppressor genes such as p53 (Hetman et al. 2010; Raj et al. 2011). p53 gene is a suppressive gene coding a p53 protein – a tumor suppressor protein and its level in unstressed cells are kept low through a continuous degradation, but DNA damage and other stress signals trigger the increase of p53 protein levels and its activity. The major functions of p53 protein are (i) a growth arrest (a stop signal for progression of cell cycle) during DNA damage and

(ii) induction of DNA repair, and when unsuccessful, it directs cells on the road to apoptosis via an upregulation of Bax, Fas, and Apaf- 1 expression (Hetman et al. 2010; Raj et al. 2011).

2.7 Neurotoxicity Induced by Epigenetic Mechanisms

Apart from genetic material, epigenetic regulation of gene expression (=epigenetic processes) can change dynamically in response to external factors, providing a key mechanism by which the environment can influence gene expression and, hence, phenotype (Taft et al. 2007; Archer et al. 2010). Epigenetic mechanisms reflect the regulation of gene expression, occurring independently of DNA sequence (Henikoff and Matzke 1997). There were recognized – at least – three forms of epigenetic mechanism: DNA methylation, histone tail modifications, and regulatory noncoding RNA (ncRNA).

DNA methylation occurs primarily at cytosine-guanine dinucleotides (CpG) and requires DNA methyltransferases (DNMTs). The addition of a methyl group to CpG sites displaces the binding of transcription factors and attracts methyl-binding proteins that instigate chromatin compaction of DNA and gene silencing.

Histones are the basic proteins around which DNA is wrapped to form nucleosome. Covalent histone tail modifications, including methylation, acetylation, phosphorylation, sumoylation, and ubiquitylation, modulate gene expression via alterations in chromatin structure. Acetylation, one of the most frequent posttranscriptional modifications of histones, depends on histone-modifying enzymes, such as histone acetyltransferases (HATs) and histone deacetylases (HDACs). Generally, histone acetylation belongs to the active chromatin marks; it increases the accessibility of transcription complexes to genomic DNA. Histone methylation, controlled by several families of histone methyltransferases (HMTs), has been associated with both transcriptional activation and repression, depending on the particular residue and the extent of methylation (Su and Tarakhovsky 2006; Maze and Nestler 2011); this reaction can be reversed by equally diverse histone demethylases (HDMs).

Regulatory ncRNAs (encoded by part of DNA noncoding proteins) are able to control transcription and translation in an organ- and cell-specific manner; the disturbance of their function could lead to perturbation in cellular homeostasis. These include, inter alia, microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), endogenous small interfering RNAs (endo-siRNAs, esiRNAs), and long noncoding RNAs (lncRNAs).

miRNAs represent a class of short (∼18–25 nucleotides in length), single-stranded molecules which cause epigenetic changes in gene transcription, mediated by both DNA methylation and histone modifications (Kim et al. 2007, 2009). miRNA are expressed in the mature mammalian brain (Landgraf et al. 2007) and are thought to be involved in long-term potentiation and in regulating structural and functional aspects of synaptic plasticity (i.e., neuronal morphogenesis, activity-dependent translation during synapse formation, memory and synapse development, and plasticity (Schratt 2009)). miRNAs in the vertebrate system predominantly inhibit productive translation

of mRNAs in the cytoplasm (Schratt et al. 2006) and may regulate transcriptomic networks via the expression of other types of lncRNAs in the nucleus (Zhou et al. 2010). On the other hand, the expression of miRNAs is modulated by DNA methylation and histone modifications.

piRNAs are a class of small noncoding RNA, 24–31 nucleotides in length that forms RNA-protein complexes with Piwi proteins (Dharap et al. 2011). piRNAs are present in the polysomal fraction, but surprisingly they were also found in the nucleus. piRNAs play an important role in silencing pathways that regulate mRNA stability, heterochromatin formation, and genome integrity (Brower-Toland et al. 2007). They may guide DNA methylation and control transposon activity (Xu et al. 2008; Zhou et al. 2010), and in turn, impaired epigenetic regulation of transposons can interfere with the expression of neighboring genes. Recent studies confirmed their expression in somatic tissues (Spadaro and Bredy 2012) and in the epigenetic facilitation (chromatin remodeling) of long-term memory and neuronal plasticity (Lee et al. 2011; Rajasethupathy et al. 2012; Spadaro and Bredy 2012).

esiRNAs represent one of several classes of small noncoding RNAs involved in RNA interference. esiRNA is derived from repeated DNA elements and transposons (Obbard and Finnegan 2008). In addition to their role as posttranscriptional regulators, esiRNAs function as epigenetic regulators through either RNA-directed DNA methylation or RNA-mediated heterochromatin formation (Verdel et al. 2009; Lejeune and Allshire 2011). In both cases, esiRNAs may act as guide molecules to direct epigenetic factors such as site-specific MNMT1 or HMTs to target specific genomic regions (Song et al. 2011). Chen et al. (2012) have demonstrated that esiRNAs by silencing LINE-1 activity through DNA methylation in human cells can function as a link between the expression of esiRNAs and LINE-1 retrotransposons. The recent study has also showed that adult mouse hippocampus expresses esiRNAs that possibly play more specific roles in regulating synaptic plasticity and learning (Smalheiser et al. 2011).

lncRNAs are mRNA-like transcripts ranging in length from 200 nucleotides to ~100 kilobases that do not have functional open reading frames. Most lncRNAs are localized in nucleus, display low expression compared to protein coding genes, and are composed of both polyA+ and polyA− transcripts (Kaikkonen et al. 2011). lncRNA can be divided into five categories based on their proximity to coding genes: intronic, intragenic, bidirectional, sense, and antisense (Qureshi and Mehler 2011). They have been associated with various cellular processes, such as transcription regulation (in *cis* or *trans* loci), alternative splicing, control of the subcellular localization of transcription regulatory factors and proteins, chromosomal dosage compensation, as well as chromatin remodeling (Johnson et al. 2012; Li et al. 2012). lncRNAs recruit histone modification complex to target sites in chromatin and thus can function as epigenetic regulatory factors (Rinn and Chang 2012). They exhibit cell-specific patterns of expression within brain, suggesting their role in the modulation of genes associated with neuronal connectivity and synaptic plasticity (Bernard et al. 2010; Li et al. 2012). Recently, lncRNAs were shown as factors engaged in GABA neuron neurogenesis (Mercer et al. 2010).

3 Conclusion

Among endogenous brain toxins, high concentrations of glutamate, calcium ions, and RS are well-known factors that potently disturb the normal physiology and function of the nervous system. It was also established that glial cells (i.e., astroglia, microglia, and oligodendrocytes) and their released factors (e.g., proinflammatory cytokines and chemokines, excitatory amino acids, cell necrosis factors, RS) also play a number of active roles in the brain and are sensitive indicators for pathology of the CNS. Recently, genomic instability due to DNA damage and epigenetic mechanisms (such as DNA methylation, histone tail modifications, and regulatory ncRNAs) were recognized as important mechanisms leading to disturbances of neurons and finally to their death.

References

Aksenov, M. Y., Aksenova, M. V., Carney, J. M., & Butterfield, D. A. (1997). Oxidative modification of glutamine synthetase by amyloid beta peptide. *Free Radical Research, 27*, 267–281.

Aloisi, F. (2001). Immune function of microglia. *Glia, 36*, 165–179.

Aloisi, F., Ambrosini, E., Columba-Cabezas, S., Magliozzi, R., & Serafini, B. (2001). Intracerebral regulation of immune responses. *Annals of Medicine, 33*, 510–515.

Archer, T., Beninger, R. J., Palomo, T., & Kostrzewa, R. M. (2010). Epigenetics and biomarkers in the staging of neuropsychiatric disorders. *Neurotoxicity Research, 18*, 347–366.

Bartosz, G. (2009). Reactive oxygen species: Destroyers or messengers? *Biochemical Pharmacology, 77*, 1303–1315.

Belachew, S., Rogister, B., Rigo, J. M., Malgrange, B., & Moonen, G. (1999). Neurotransmitter-mediated regulation of CNS myelination: A review. *Acta Neurologica Belgica, 99*, 21–31.

Ben Achour, S., & Pascual, O. (2012). Astrocyte-neuron communication: Functional consequences. *Neurochemical Research, 37*, 2464–2473.

Ben-Shachar, D., Zuk, R., & Glinka, Y. (1995). Dopamine neurotoxicity: Inhibition of mitochondrial respiration. *Journal of Neurochemistry, 64*, 718–723.

Bergles, D. E., Roberts, J. D., Somogyi, P., & Jahr, C. E. (2000). Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature, 405*, 187–191.

Bernard, D., Prasanth, K. V., Tripathi, V., Colasse, S., Nakamura, T., Xuan, Z., Zhang, M. Q., Sedel, F., Jourdren, L., Coulpier, F., Triller, A., Spector, D. L., & Bessis, A. (2010). A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression. *The EMBO Journal, 29*, 3082–3093.

Block, M. L., Zecca, L., & Hong, J.-S. (2007). Microglia-mediated neurotoxicity: Uncovering the molecular mechanisms. *Nature Reviews. Neuroscience, 8*, 57–69.

Bojes, H. K., Feng, X., Kehrer, J. P., & Cohen, G. M. (1999). Apoptosis in hematopoietic cells (FL5.12) caused by interleukin-3 withdrawal: Relationship to caspase activity and the loss of glutathione. *Cell Death and Differentiation, 6*(1), 61–70.

Breckenridge, D. G., & Xue, D. (2004). Regulation of mitochondrial membrane permeabilization by BCL-2 family proteins and caspases. *Current Opinion in Cell Biology, 16*, 647–652.

Brower-Toland, B., Findley, S. D., Jiang, L., Liu, L., Yin, H., Dus, M., Zhou, P., Elgin, S. C., & Lin, H. (2007). Drosophila PIWI associates with chromatin and interacts directly with HP1a. *Genes & Development, 21*, 2300–2311.

Chen, L., Dahlstrom, J. E., Lee, S. H., & Rangasamy, D. (2012). Naturally occurring endo-siRNA silences LINE-1 retrotransposons in human cells through DNA methylation. *Epigenetics, 7*, 758–771.

Clark, K. H., Wiley, C. A., & Bradberry, C. W. (2013). Psychostimulant abuse and neuroinflammation: Emerging evidence of their interconnection. *Neurotoxicity Research, 23*, 174–188.

Court, F. A., & Coleman, M. P. (2012). Mitochondria as a central sensor for axonal degenerative stimuli. *Trends in Neurosciences, 35*, 364–372.

Darzynkiewicz, Z., Traganos, F., & Wlodkowic, D. (2009). Impaired DNA damage response–an Achilles' heel sensitizing cancer to chemotherapy and radiotherapy. *European Journal of Pharmacology, 625*, 143–150.

Deng, W., & Poretz, R. D. (2003). Oligodendroglia in developmental neurotoxicity. *Neurotoxicology, 24*, 161–178.

Dharap, A., Nakka, V. P., & Vemuganti, R. (2011). Altered expression of PIWI RNA in the rat brain after transient focal ischemia. *Stroke, 42*, 1105–1109.

Duncan, A. J., & Heales, S. J. (2005). Nitric oxide and neurological disorders. *Molecular Aspects of Medicine, 26*, 67–96.

Frenkel, K., Zhong, Z., Wei, H., Karkoszka, J., Patel, U., Rashid, K., Georgescu, M., & Solomon, J. J. (1991). Quantitative high-performance liquid chromatography analysis of DNA oxidized in vitro and in vivo. *Analytical Biochemistry, 196*, 126–136.

Gabai, V. L., Mabuchi, K., Mosser, D. D., & Sherman, M. Y. (2002). Hsp72 and stress kinase c-jun N-terminal kinase regulate the bid-dependent pathway in tumor necrosis factor-induced apoptosis. *Molecular and Cellular Biology, 22*, 3415–3424.

Gallo, V., & Ghiani, C. A. (2000). Glutamate receptors in glia: New cells, new inputs and new functions. *Trends in Pharmacological Sciences, 21*, 252–258.

Gonzalez, F. J. (2005). Role of cytochromes P450 in chemical toxicity and oxidative stress: Studies with CYP2E1. *Mutation Research, 569*, 101–110.

Graeber, M. B., & Streit, W. J. (2010). Microglia: Biology and pathology. *Acta Neuropathology, 119*, 89–105.

Halliwell, B. (2006). Oxidative stress and neurodegeneration: Where are we now? *Journal of Neurochemistry, 97*, 1634–1658.

Halliwell, B., & Gutteridge, J. M. (1988). Free radicals and antioxidant protection: Mechanisms and significance in toxicology and disease. *Human Toxicology, 7*, 7–13.

Hanisch, U. K. (2002). Microglia as a source and target of cytokines. *Glia, 40*, 140–155.

Hardingham, G. E., & Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: Implications for neurodegenerative disorders. *Nature Reviews. Neuroscience, 11*, 682–696.

Henikoff, S., & Matzke, M. A. (1997). Exploring and explaining epigenetic effects. *Trends in Genetics, 13*, 293–295.

Hermida-Ameijeiras, A., Mendez-Alvarez, E., Sanchez-Iglesias, S., Sanmartin-Suarez, C., & Soto-Otero, R. (2004). Autoxidation and MAO-mediated metabolism of dopamine as a potential cause of oxidative stress: Role of ferrous and ferric ions. *Neurochemistry International, 45*, 103–116.

Hetman, M., Vashishta, A., & Rempala, G. (2010). Neurotoxic mechanisms of DNA damage: Focus on transcriptional inhibition. *Journal of Neurochemistry, 114*, 1537–1549.

Hua, X., Malarkey, E. B., Sunjara, V., Rosenwald, S. E., Li, W. H., & Parpura, V. (2004). Ca^{2+}-dependent glutamate release involves two classes of endoplasmic reticulum Ca^{2+} stores in astrocytes. *The Journal of Neuroscience, 76*, 86–97.

Johnson, R., Noble, W., Tartaglia, G. G., & Buckley, N. J. (2012). Neurodegeneration as an RNA disorder. *Progress in Neurobiology, 99*, 293–315.

Kaikkonen, M. U., Lam, M. T., & Glass, C. K. (2011). Non-coding RNAs as regulators of gene expression and epigenetics. *Cardiovascular Research, 90*, 430–440.

Kim, J., Inoue, K., Ishii, J., Vanti, W. B., Voronov, S. V., Murchison, E., Hannon, G., & Abeliovich, A. (2007). A microRNA feedback circuit in midbrain dopamine neurons. *Science, 317*, 1220–1224.

Kim, S., Hwang do, W., & Lee, D. S. (2009). A study of microRNAs in silico and in vivo: Bioimaging of microRNA biogenesis and regulation. *The FEBS Journal, 276*, 2165–2174.

Koppal, T., Drake, J., & Butterfield, D. A. (1999). In vivo modulation of rodent glutathione and its role in peroxynitrite-induced neocortical synaptosomal membrane protein damage. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1453*, 407–411.

Landgraf, P., Rusu, M., Sheridan, R., Sewer, A., Iovino, N., Aravin, A., Pfeffer, S., Rice, A., Kamphorst, A. O., Landthaler, M., Lin, C., Socci, N. D., Hermida, L., Fulci, V., Chiaretti, S., Foà, R., Schliwka, J., Fuchs, U., Novosel, A., Müller, R. U., Schermer, B., Bissels, U., Inman, J., Phan, Q., Chien, M., Weir, D. B., Choksi, R., De Vita, G., Frezzetti, D., Trompeter, H. I., Hornung, V., Teng, G., Hartmann, G., Palkovits, M., Di Lauro, R., Wernet, P., Macino, G., Rogler, C. E., Nagle, J. W., Ju, J., Papavasiliou, F. N., Benzing, T., Lichter, P., Tam, W., Brownstein, M. J., Bosio, A., Borkhardt, A., Russo, J. J., Sander, C., Zavolan, M., & Tuschl, T. (2007). A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell, 129*, 1401–1414.

Lee, M., McGeer, E. G., & McGeer, P. L. (2011). Mechanisms of GABA release from human astrocytes. *Glia, 59*, 1600–1611.

Lejeune, E., & Allshire, R. C. (2011). Common ground: Small RNA programming and chromatin modifications. *Current Opinion in Cell Biology, 23*, 258–265.

Li, X., Wu, Z., Fu, X., & Han, W. (2012). Long noncoding RNAs: Insights from biological features and functions to diseases. *Medicinal Research Reviews, 33*(3), 517–533. doi:10.1002/med.21254.

Lieb, K., Andrae, J., Reisert, I., & Pilgrim, C. (1995). Neurotoxicity of dopamine and protective effects of the NMDA receptor antagonist AP-5 differ between male and female dopaminergic neurons. *Experimental Neurology, 134*, 222–229.

Maciel, E. N., Vercesi, A. E., & Castilho, R. F. (2001). Oxidative stress in Ca2? -induced membrane permeability transition in brain mitochondria. *Journal of Neurochemistry, 79*, 1237–1245.

Makar, T. K., Nedergaard, M., Preuss, A., Gelbard, A. S., Perumal, A. S., & Cooper, A. J. (1994). Vitamin E, ascorbate, glutathione, glutathione disulfide, and enzymes of glutathione metabolism in cultures of chick astrocytes and neurons: Evidence that astrocytes play an important role in antioxidative processes in the brain. *Journal of Neurochemistry, 62*, 45–53.

Matés, J. M., Segura, J. A., Alonso, F. J., & Márquez, J. (2008). Intracellular redox status and oxidative stress: Implications for cell proliferation, apoptosis, and carcinogenesis. *Archives of Toxicology, 82*, 273–299.

Matés, J. M., Segura, J. A., Alonso, F. J., & Márquez, J. (2010). Roles of dioxins and heavy metals in cancer and neurological diseases using ROS-mediated mechanisms. *Free Radical Biology & Medicine, 49*, 1328–1341.

Mattson, M. P., & Chan, S. L. (2003). Neuronal and glial calcium signaling in Alzheimer's disease. *Cell Calcium, 34*, 385–397.

Maze, I., & Nestler, E. J. (2011). The epigenetic landscape of addiction. *Annals of the New York Academy of Sciences, 1216*, 99–113.

Mercer, T. R., Qureshi, I. A., Gokhan, S., Dinger, M. E., Li, G., Mattick, J. S., & Mehler, M. F. (2010). Long noncoding RNAs in neuronal-glial fate specification and oligodendrocyte lineage maturation. *BMC Neuroscience, 11*, 14.

Miksys, S., & Tyndale, R. F. (2004). The unique regulation of brain cytochrome P450 2 (CYP2) family enzymes by drugs and genetics. *Drug Metabolism Reviews, 36*, 313–333.

Nakajima, K., & Kohsaka, S. (2001). Microglia: Activation and their significance in the central nervous system. *Journal of Biochemistry, 130*, 169–175.

Noack, H., Lindenau, J., Rothe, F., Asayama, K., & Wolf, G. (1998). Differential expression of superoxide dismutase isoforms in neuronal and glial compartments in the course of excitotoxically mediated neurodegeneration: Relation to oxidative and nitrergic stress. *GLIA*, *23*, 285–297.

Noble, P. G., Antel, J. P., & Yong, V. W. (1994). Astrocytes and catalase prevent the toxicity of catecholamines to oligodendrocytes. *Brain Research, 633*, 83–90.

Obbard, D. J., & Finnegan, D. J. (2008). RNA interference: Endogenous siRNAs derived from transposable elements. *Current Biology, 18*, 561–563.

Orellana, J. A., von Bernhardi, R., Giaume, C., & Sáez, J. C. (2012). Glial hemichannels and their involvement in aging and neurodegenerative diseases. *Reviews in the Neurosciences, 23*, 163–177.

Qureshi, I. A., & Mehler, M. F. (2011). Non-coding RNA networks underlying cognitive disorders across the lifespan. *Trends in Molecular Medicine, 17*, 337–346.

Raj, L., Ide, T., Gurkar, A. U., Foley, M., Schenone, M., Li, X., Tolliday, N. J., Golub, T. R., Carr, S. A., Shamji, A. F., Stern, A. M., Mandinova, A., Schreiber, S. L., & Lee, S. W. (2011). Selective killing of cancer cells by a small molecule targeting the stress response to ROS. *Nature, 475*, 231–234.

Rajasethupathy, P., Antonov, I., Sheridan, R., Frey, S., Sander, C., Tuschl, T., & Kandel, E. R. (2012). A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. *Cell, 149*, 693–707.

Rego, A. C., & Oliveira, C. R. (2003). Mitochondrial dysfunction and reactive oxygen species in excitotoxicity and apoptosis: Implications for the pathogenesis of neurodegenerative diseases. *Neurochemistry Research, 28*, 1563–1574.

Rinn, J. L., & Chang, H. Y. (2012). Genome regulation by long noncoding RNAs. *Annual Review of Biochemistry, 81*, 145–166.

Rogister, B., Belachew, S., & Moonen, G. (1999). Oligodendrocytes: From development to demyelinated lesion repair. *Acta Neurologica Belgica, 99*, 32–39.

Santello, M., Calì, C., & Bezzi, P. (2012). Gliotransmission and the tripartite synapse. *Advances in Experimental Medicine and Biology, 970*, 307–331.

Schratt, G. (2009). microRNAs at the synapse. *Nature Reviews. Neuroscience, 10*, 842–849.

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kiebler, M., & Greenberg, M. E. (2006). A brain-specific microRNA regulates dendritic spine development. *Nature, 439*, 283–289.

Singh, I. N., Goody, R. J., Dean, C., Ahmad, N. M., Lutz, S. E., Knapp, P. E., Nath, A., & Hauser, K. F. (2004). Apoptotic death of striatal neurons induced by human immunodeficiency virus-1 Tat and gp120: Differential involvement of caspase-3 and endonuclease G. *Journal of Neurovirology, 10*, 141–151.

Smalheiser, N. R., Lugli, G., Thimmapuram, J., Cook, E. H., & Larson, J. (2011). Endogenous siRNAs and noncoding RNA-derived small RNAs are expressed in adult mouse hippocampus and are up-regulated in olfactory discrimination training. *RNA, 17*, 166–181.

Song, R., Hennig, G. W., Wu, Q., Jose, C., Zheng, H., & Yan, W. (2011). Male germ cells express abundant endogenous siRNAs. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 13159–13164.

Spadaro, P. A., & Bredy, T. W. (2012). Emerging role of non-coding RNA in neural plasticity, cognitive function, and neuropsychiatric disorders. *Frontiers in Genetics, 3*, 132.

Spencer, J. P., Jenner, P., Daniel, S. E., Lees, A. J., Marsden, D. C., & Halliwell, B. (1998). Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: Possible mechanisms of formation involving reactive oxygen species. *Journal of Neurochemistry, 71*, 2112–2122.

Su, I. H., & Tarakhovsky, A. (2006). Lysine methylation and signaling memory. *Current Opinion in Immunology, 18*, 152–157.

Trotti, D., Rossi, D., Gjesdal, O., Levy, L. M., Racagni, G., Danbolt, N. C., & Volterra, A. (1996). Peroxynitrite inhibits glutamate transporter subtypes. *The Journal of Biological Chemistry, 271*, 5976–5979.

Taft, R. J., Pheasant, M., & Mattick, J. S. (2007). The relationship between non-protein-coding DNA and eukaryotic complexity. *BioEssays, 29*, 288–299.

Verdel, A., Vavasseur, A., Le Gorrec, M., & Touat-Todeschini, L. (2009). Common themes in siRNA-mediated epigenetic silencing pathways. *The International Journal of Developmental Biology, 53*, 245–257.

Wyllie, A. H. (2010). "Where, O death, is thy sting?" A brief review of apoptosis biology. *Molecular Neurobiology, 42*, 4–9.

Xu, M., You, Y., Hunsicker, P., Hori, T., Small, C., Griswold, M. D., & Hecht, N. B. (2008). Mice deficient for a small cluster of Piwi-interacting RNAs implicate Piwi-interacting RNAs in transposon control. *Biology of Reproduction, 79*, 51–57.

Zhou, H., Hu, H., & Lai, M. (2010). Non-coding RNAs and their epigenetic regulatory mechanisms. *Biology of the Cell, 102*, 645–655.

Zorec, R., Araque, A., Carmignoto, G., Haydon, P. G., Verkhratsky, A., & Parpura, V. (2012). Astroglial excitability and gliotransmission: An appraisal of Ca^{2+} as a signalling route. *ASN Neuro, 4*(2), doi:pii:e00080. 10.1042/AN20110061

Quinolinate and Related Excitotoxins: Mechanisms of Neurotoxicity and Disease Relevance

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Abstract

There are many ways in which neuronal damage can be produced in the brain, including the overactivation of depolarizing receptors, exposure to high levels of pro-inflammatory proteins such as cytokines, or miscellaneous toxins, but the kynurenine pathway has emerged as a novel but potentially major factor in regulating neuronal viability or death. It is the major route for the metabolism of the essential amino acid tryptophan, which is oxidized by indoleamine-2,3-dioxygenase (IDO) to a series of compounds which can activate, block, or modulate conventional neurotransmitter receptors. Quinolinic acid is an agonist at *N*-methyl-D-aspartate receptors, kynurenic acid is an antagonist at these and other glutamate receptors, and other kynurenine metabolites are highly redox-active. Superimposed on the discovery of this neuromodulatory pathway have been observations that activity in the pathway is linked to neurological and psychiatric disorders, correlating with disease state (as in Huntington's disease) or cognitive function (as following bypass surgery). Together, the data accumulated to date make a strong case for this hitherto obscure pathway being a major factor in determining cell damage, death, or recovery in health and disease.

Keywords

Alzheimer's disease • Depression • Excitotoxicity • HIV-1-Associated neurocognitive disorder • Huntington's disease • Kynurenate • Kynurenine pathway • Multiple sclerosis • Neurodegeneration • Neuro-inflammation • Parkinson's disease • Quinolinic acid • Schizophrenia • Stroke

List of Abbreviations

3HA	3-Hydroxyanthranilic acid (3HA)
3HAO	3-Hydroxyanthranilic acid oxygenase
3HK	3-Hydroxkynurenine
AD	Alzheimer's disease
AIDS	Acquired immunodeficiency syndrome
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Gly	Glycine
HD	Huntington's disease
HIV	Human immunodeficiency virus
IDO	Indole amine 2,3-dioxygenase
IFN	Interferon
KAT	Kynurenine amino transferases
LPS	Lipopolysaccharides
MAPK	Mitogen-activated protein kinase
mGluR	Metabotropic glutamate receptor
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	Multiple sclerosis
nAChR	Nicotinic acetylcholine receptor
NAD^+	Nicotinamide adenine dinucleotide

NMDA	*N*-methyl-D-aspartate
PD	Parkinson's disease
TDO	Tryptophan 2,3-dioxygenase
TNF	Tumor necrosis factor

1 Introduction

Quinolinic acid is a highly selective agonist at the population of glutamate receptors that respond to the synthetic amino acid *N*-methyl-D-aspartate (NMDA). In contrast to NMDA, however, quinolinic acid is an endogenous compound, generated via the kynurenine pathway for the oxidation of another amino acid, tryptophan (Stone and Perkins 1981). The discovery that quinolinic acid is able to produce depolarization by activating NMDA receptors (NMDARs) led to the demonstration that it also causes neurodegeneration (Schwarcz et al. 1983), consistent with the knowledge that glutamate itself can induce neuronal damage by promoting depolarization and an excessive influx of calcium. These discoveries launched the concept that the brain damage associated with stroke or degenerative disease might be attributable not only to the accumulation of glutamate or hyperactivity of its receptors, but also to abnormal function of the kynurenine pathway.

The kynurenine pathway also produces redox-active compounds (3-hydroxykynurenine and 3-hydroxyanthranilic acid) which cause neuronal damage by generating reactive oxygen species (Eastman and Guilarte 1989; Giles et al. 2003). A branch of the pathway generates kynurenate which is an antagonist of glutamate (Perkins and Stone 1982) and nicotinic cholinergic receptors (Hilmas et al. 2001; Stone 2007). As a result, kynurenate exhibits neuroprotective properties. A number of diseases have been associated with altered levels of quinolinic acid and other kynurenines in the CNS, including the AIDS–dementia complex, Alzheimer's disease, Huntington's disease, and Parkinson's disease. In addition, as the relevance of neuronal viability and damage become clear in the etiology of psychiatric disorders such as schizophrenia and depression, and inflammatory diseases such as multiple sclerosis, the pathological and therapeutic importance of kynurenine metabolism continues to expand, as indicated in the sections that follow.

2 Components of the Kynurenine Pathway

2.1 Tryptophan Cleavage and Immune System

L-tryptophan, the least abundant of the essential amino acids used for protein synthesis, is also a substrate for synthesizing the neurotransmitter serotonin via the methoxyindole pathway, or vitamin B3 (nicotinamide) and quinolinic acid via the kynurenine pathway (Fig. 1).

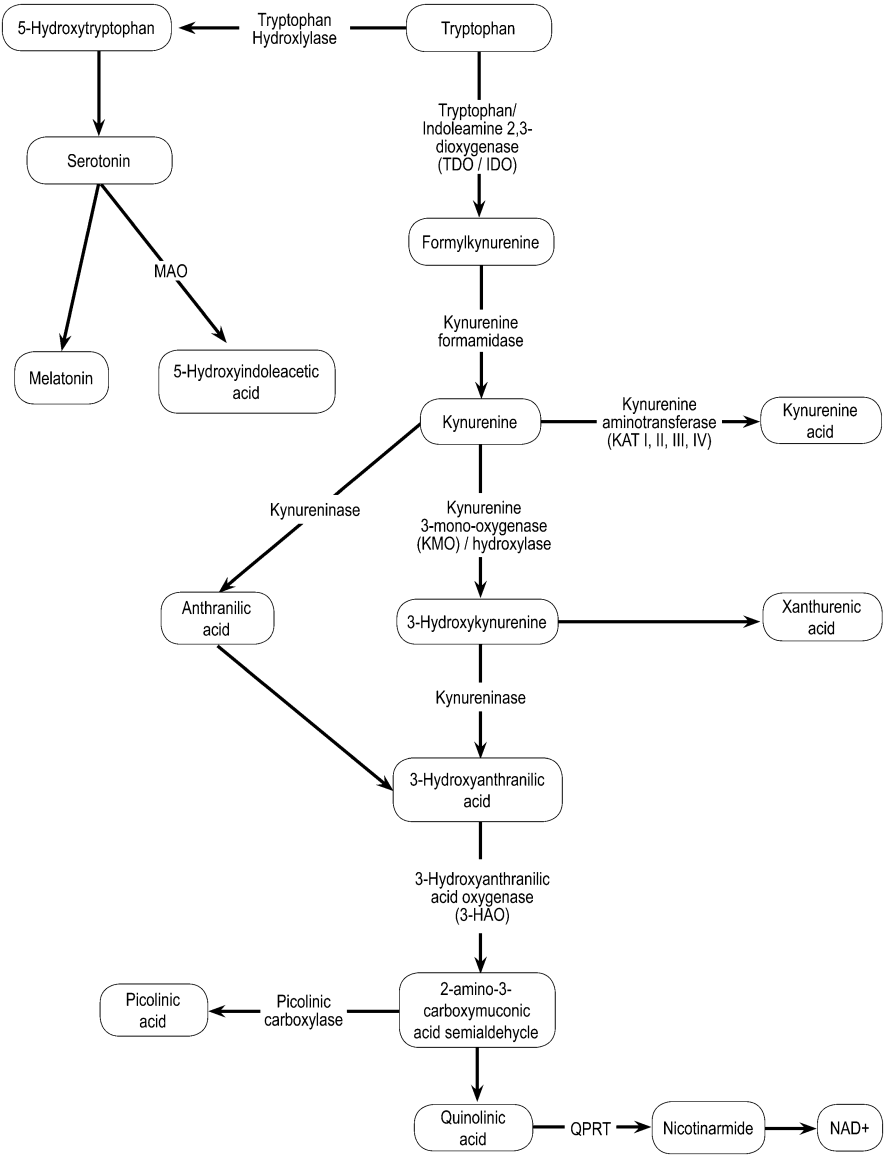

Fig. 1 A summary of the major compounds in the kynurenine pathway of tryptophan oxidation

Most of the ingested tryptophan (over 95 %) is catabolized in the kynurenine pathway (Stone 1993a; Takikawa 2007) and generates nicotinamide adenine dinucleotide (NAD^+) under physiological conditions. L-tryptophan is metabolized to kynurenine by oxidative cleavage of the indole ring by the enzymes tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO), the activities of which may act as rate-limiting steps under some conditions (Takikawa et al. 1986;

Rafice et al. 2009). IDO, a heme-containing inducible enzyme, is found in the lungs, blood, and brain (Heyes et al. 1993; Banks et al. 1994) and has a higher affinity for tryptophan (Km = ~10 μM) than does TDO (Km = ~200 μM). IDO has recently been shown to exist in at least two isoforms, though little is yet known about IDO-2 (Ball et al. 2009).

A key aspect of the kynurenine pathway that is becoming increasingly recognized for its importance in neurodegeneration is its intimate relationship with the immune system. The IDO promoter has one interferon-γ (IFN-γ) activation site and two nonspecific IFN-stimulated response elements that respond to IFN-α, IFN-β, or IFN-γ, with IFN-γ being the most potent inducer (Puccetti 2007). Pro-inflammatory interleukins such as IL-10 activate T cells and natural killer cells to produce IFN-γ which acts synergistically with tumor necrosis factor-α (TNF-α) to increase expression of IDO (Robinson et al. 2003). During immune system activation, this sequence serves as a form of defense against infection by causing tryptophan depletion (Takikawa 2007). IDO can be activated also by some pro-inflammatory cytokines (IL-18, IL-12) independently of IFN-γ (Liebau et al. 2002).

In the CNS, IDO is found in microglia, macrophages, astrocytes, neurons, and microvascular endothelial cells (Guillemin et al. 2001, 2005, 2007). Activated microglia produce pro-inflammatory cytokines IL-1βb, IL-2, IL-6, TNF-α, and IFN-γ which cause further microglial activation to amplify the inflammatory response.

TDO is found mainly in the liver but is also present in several brain areas including the cerebral cortex, cerebellum, hypothalamus, and brainstem (Haber et al. 1993; Kanai et al. 2009). The enzyme is readily activated by IFN-γ or bacterial endotoxins (Yoshida et al. 1981).

2.2 Formation of 3-Hydroxy-Kynurenine, 3-Hydroxy-Anthranilic Acid, and Quinolinic Acid

Kynurenine is transported easily into the CNS across the blood–brain barrier by the large neutral amino acid transporter; hence, changes in the plasma level of kynurenine are reflected in CSF levels (Fukui et al. 1991; Jauch et al. 1993). Approximately 60 % of the brain kynurenine is from the systemic circulation (Gal and Sherman 1978). Brain immune cells take up kynurenine from the extracellular space via the Na^+-independent large neutral amino acid transporter (Speciale et al. 1989).

Kynurenine 3-monoxygenase (KMO) [also known as kynurenine 3-hydroxylase], which uses kynurenine as a substrate, is present mainly in the liver. It is also present in the brain albeit with a much lower activity than in peripheral tissues. KMO is located in the outer membrane of mitochondria and is not saturated under physiological conditions. The production of 3-hydroxkynurenine (3HK) can, therefore, be increased when there is increased supply of kynurenine, as, for example, from the periphery (Schwarcz et al. 2010). The cytokine IFN-γ activates KMO; hence, inflammatory states stimulate the expression of KMO and production of quinolinic acid (Heyes and Morrison 1997; Connor et al. 2008).

In the CNS, resident microglia and invading macrophages from the periphery express far more KMO and 3-hydroxyanthranilic acid oxygenase (3HAO) enzymes than astrocytes, and therefore are the major source of quinolinic acid (Heyes et al. 1996; Lehrmann et al. 2001).

2.3 Formation of Kynurenate

In mammalian brain, kynurenine amino transferases (KAT), the enzymes that convert kynurenine to kynurenate, are found mainly not only in astrocytes but also in neurons to some extent (Guillemin et al. 2000; Guidetti et al. 2007b). Kynurenate is produced by the irreversible transamination of kynurenine by four mammalian pyridoxal-5′-phosphate dependent enzymes – KAT I (glutamine transaminase or cysteine conjugate beta-lyase I), KAT II (alpha aminoadipate aminotransferase), KAT III (cysteine conjugate beta-lyase II), and KAT IV (glutamic-oxaloacetic transaminase or mitochondrial aspartate aminotransferase). KAT II accounts for most (50–60 %) of kynurenate formation in human and rat brains, whereas KAT IV, a more recent addition, is the dominant enzyme (60 %) in mouse brain (Guidetti et al. 2007a; Han et al. 2010).

Once formed, kynurenate cannot be metabolized further and is released into the extracellular compartment by a non-Ca^{2+} -dependent process (Turski et al. 1989; Kiss et al. 2003). Kynurenate is removed slowly from the brain by the probenecid-sensitive, nonspecific acid transporter and by passive diffusion (Moroni et al. 1988; Schwarcz and Pellicciari 2002). Although KMO is in the pathway that produces 3HK and quinolinic acid, its activity regulates the levels of kynurenine in the brain, and, hence, indirectly affects the rate of formation of kynurenate (Wonodi and Schwarcz 2010).

3 Neurodegeneration and Kynurenine Metabolites

Excitotoxicity was first described by Olney et al. (1975) as the damage to neurons that results from excessive stimulation of glutamate receptors (Olney et al. 1975). The neuronal damage caused by stimulation of glutamate receptors does not affect the axons of passage or the afferent nerve terminals. Endogenously released glutamate does not cause much excitotoxicity due to the very efficient uptake systems (Danbolt 2001); hence, quinolinic acid, being an endogenous excitotoxin with no efficient removal mechanism, has been of particular interest for several years.

The concentration of quinolinic acid in the brain is normally too low to cause acute toxicity although maintained high nanomolar concentrations may damage or kill particular groups of neurons (Giulian et al. 1990). During inflammation, quinolinic acid may increase from the normal nM levels to micromolar levels which can cause overt and rapid neurotoxicity (Heyes et al. 1998). Nonetheless, quinolinic acid has been reported to be toxic to neurons even at concentrations as low as 150 nM (Braidy et al. 2009).

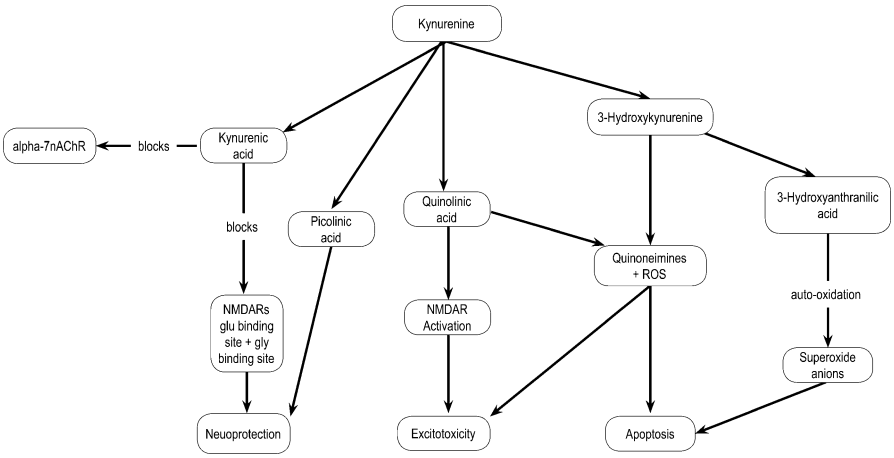

Fig. 2 A diagrammatic representation of how the role of the kynurenine pathway in regulating neuronal death or survival depends critically on the ratio between compounds such as quinolinic acid, which can be neurotoxic in excessive concentrations, and kynurenic acid, which is protective by blocking NMDA and nicotinic receptors. The contributions of redox-active compounds such as 3-hydroxykynurenine and 3-hydroxyanthranilic acid depend on their relative concentrations as well as the ambient redox state of the tissue

Quinolinic acid produced by microglia and infiltrating macrophages acts on NMDARs (Addae and Stone 1987; Stone 1993a), particularly those with NR1/NR2A or NR1/NR2B subunits (Perkins and Stone 1983; Stone 1993b; de Carvalho et al. 1996), and causes neurotoxicity partly via overactivation of these receptors (Fig. 2). Excessive stimulation of NMDARs causes influx of Ca^{2+} which overcomes cellular Ca^{2+} sequestration processes and leads to activation of proteases, endonucleases, generation of reactive oxygen species and nitric oxide that eventually cause neuronal death by lipid peroxidation or apoptosis. In addition, quinolinic acid stimulates hypertrophy and proliferation of astrocytes which then release increased amount of cytokines, leading to astrogliosis and inhibition of glutamate reuptake to cause further excitation of NMDARs (Tavares et al. 2002; Ting et al. 2009).

Two other products in the kynurenine pathway, 3-hydroxykynurenine (3HK) and 3-hydroxyanthranilic acid (3HA), cause neurodegeneration by generating superoxide and hydrogen peroxide under appropriate redox conditions (Goldstein et al. 2000). The axon-sparing excitotoxicity of quinolinic acid is potentiated by the presence of 3HK and reactive oxygen species; this synergistic effect is unique to quinolinic acid, because there is no such effect between NMDA and 3HK (Guidetti and Schwarcz 1999; Behan and Stone 2002). This synergistic effect explains why, although the potency of quinolinic acid on NMDARs is less than that of NMDA itself, the concentration of quinolinic acid that causes damage in the CNS is about the same as that of NMDA.

The activity of 3HAO, the oxygenase enzyme that converts 3-hydroxyanthranilic acid to quinolinic acid, is iron (Fe^{2+}) dependent. Hence,

excessive iron levels in the brain dramatically increase the levels of quinolinic acid which may contribute to the neurodegeneration caused by iron (Stachowski and Schwarcz 2011). Quinolinic acid itself may also cause neurotoxicity via formation of iron complexes, leading to lipid peroxidation (Platenik et al. 2001).

4 Neuroprotective Kynurenine Metabolites and Their Control

Kynurenate is the only known endogenous antagonist of NMDARs that preferentially blocks the glycine co-agonist binding site (Gly_2 or Gly_B) where it has a higher affinity for the glycine site than glutamate (Birch et al. 1988; Kessler et al. 1989). Kynurenate is normally present in the brain at average levels that are in the high nanomolar range (200–1,500 nM) (Turski et al. 1988), but at higher micromolar concentrations, which are likely to exist near the sites of production and cellular efflux, kynurenate blocks competitively all ionotropic glutamate receptors (Perkins and Stone 1982; Stone 1993a). This effect of kynurenate on non-NMDA glutamate receptors at synaptic sites enables it to effectively block thalamic projections to neocortical neurons (Addae and Stone 1988). Kynurenate also blocks noncompetitively the α7-nicotinic acetylcholine receptor (nAChR), and inhibits release of glutamate from presynaptic terminals by acting on this type of receptor (Hilmas et al. 2001; Carpenedo et al. 2001).

One consequence of the antagonistic activity of kynurenate at glutamate receptors is that a reduction in the levels of brain kynurenate promotes NMDAR-mediated excitotoxicity (Sapko et al. 2006). Such a reduction may occur when there are changes in extracellular ionic concentrations (e.g., high potassium) (Gramsbergen et al. 1997) or during a state of reduced cellular energy generation (e.g., hypoglycemia) which reduces the energy-dependent transport systems for kynurenine. Kynurenate formation is reduced also by compounds, such as the metabotropic glutamate receptor (mGluR) agonists and the sulfur-containing endogenous amino acid cysteine sulfinate, which inhibit KAT II (Battaglia et al. 2000; Kocki et al. 2003). Also, L-α-aminoadipate, a gliotoxic metabolite of the amino acid lysine, decreases the transamination of kynurenine to kynurenate and exerts its neuropathological effects partly by such an action on astrocytes (Wu et al. 1995). In contrast to the effects of the above compounds, pyruvate and other 2-oxoacids increase kynurenate levels by serving as co-substrates for the transamination process (Hodgkins et al. 1999).

Another important neuroprotective metabolite in the kynurenine pathway is picolinic acid, which is produced in neurons from 2-amino-3-carboxymuconic acid semialdehyde. The level of picolinic acid in the CSF declines with age which would contribute to the increasing prevalence of neurodegenerative diseases with age (Coggan et al. 2009). The neuroprotective properties of picolinic acid are achieved in part through its ability to promote chelation of iron and other metals (Testa et al. 1985; Jhamandas et al. 1990; Guillemin et al. 2007).

5 Diseases Associated with Kynurenines

5.1 HIV-1-Associated Neurocognitive Disorder (HAND)

Human immunodeficiency virus (HIV-1)-induced neuropathology (ranging from the most severe AIDS–dementia complex to mild cognitive impairment) does not result from direct infection of neurons by the virus but indirectly from the release of neurotoxins from activated immune cells (Glass et al. 1995; Adle-Biassette et al. 1999).

HIV-1 infection causes intestinal mucosal damage and elevated levels of lipopolysaccharides (LPS) in the blood arising from enteric bacteria (Brenchely et al. 2006) Infected monocytes in the blood cross through the blood–brain barrier which is breached in part by the LPS and pro-inflammatory mediators (Zhou et al. 2006). In the CNS, the monocytes not only release viruses that infect other monocytes, but also differentiate into macrophages and release viral proteins, for example, gp120 and Tat that activate microglia and, to a lesser degree, astrocytes. The chemokines and cytokines (e.g., TNF-α, IL-1β and monocyte chemo-attractant protein-1) produced by the activated brain immune cells promote further migration of monocytes across the blood–brain barrier (Boven et al. 2000; Fantuzzi et al. 2003; Persidsky and Gendelman 2003). Additionally, Tat activates IDO in peripheral and brain macrophages – a process that is mediated by p38 MAPK (mitogen-activated protein kinase) and enhanced by IFN-γ (Heyes et al. 1992; Sardar and Reynolds 1995; Smith et al. 2001; Fu et al. 2011). The increased expression of IDO eventually results in elevated quinolinic acid levels and neurotoxicity (Fig. 2). Additionally, the released quinolinic acid induces astrocytes to produce more chemokines, thus amplifying the neuropathology (Croitoru-Lamoury et al. 2003). Also, the neuronal damage is exacerbated by increased levels of glutamate due to the latter's impaired reuptake by the damaged astrocytes.

5.2 Alzheimer's Disease

The severity of Alzheimer's disease (AD) depends on the accumulation of two groups of molecules in the CNS – amyloid-β fragments and tau proteins. However, there are other pathological processes that contribute to the disorder including excitotoxicity and free radical damage caused by kynurenine pathway metabolites. For example, the kynurenine/tryptophan ratio is increased in AD (Widner et al. 2000), while increased levels of circulating IgA antibodies to IDO have been detected in the blood of AD patients, suggesting an increase in the production of the enzyme (Duleu et al. 2010). Also, amyloid-β activates microglia and induces IDO expression, leading to increased quinolinic acid production (Guillemin and Brew 2002; Guillemin et al. 2003a, b) and an associated decreased level of kynurenate (Hartai et al. 2007). NMDAR overactivation by quinolinic acid increases amyloid-β production, thus providing a positive feedback for disease progression (Lesne et al. 2005). In rodents, the level of quinolinic acid in the cortex increases with age, providing another mechanism for age-related predisposition to AD.

5.3 Huntington's Disease

Huntington's disease (HD) is an autosomal-dominant disease associated with neuronal loss in the striatum and neocortex. Beal et al. (1986) were the first to report that injection of quinolinic acid to the striatum of rodents produces a pattern of neuronal damage similar to that seen in human postmortem HD tissue. In HD, the evidence points strongly to an over-expression of the abnormal huntingtin gene and consequent neuronal damage by the huntingtin protein (Carter et al. 1999; Usdin et al. 1999). Interestingly, quinolinic acid has also been shown to induce expression of huntingtin in the striatum (Tatter et al. 1995). Additionally, huntingtin increases expression of the NR1/NR2 subunit combination of NMDA receptors in some types of neurons in the striatum, and the resulting increase in cellular sensitivity to quinolinic acid and glutamate (Fan et al. 2007) promotes disease progression.

The huntingtin protein triggers microglial activation (Haass and Selkoe 2007; Thakur et al. 2009) and in the R6/2 mouse, a well-established animal model of HD, the levels of quinolinic acid and 3HK are increased in the cortex and striatum (Guidetti et al. 2006). The increased level of 3HK is due to the combined increased activity of the synthesizing enzyme, KMO, and decreased activity of its degradative enzyme, kynureninase (Giorgini et al. 2005; Sathyasaikumar et al. 2010). Increased 3HK and quinolinic acid levels have also been observed in the striatum and cortex of other animal models of HD, for example, YAC128 and Hdh^{Q92}/Hdh^{Q111} mice (Guidetti et al. 2006).

In humans, there is an increased plasma kynurenine:tryptophan ratio in HD patients (Stoy et al. 2005; Forrest et al. 2010). Also, the levels of quinolinic acid and 3HK are increased in the brain of low-grade HD patients, but the levels tend to be similar to those of age-matched controls as the disease progresses (Pearson and Reynolds 1992; Guidetti et al. 2004). The severity of HD is linked to the number of glutamine residues (coded by CAG nucleotide triplets) close to the N-terminal of the huntingtin protein. The increased conversion of tryptophan to kynurenine by IDO in HD patients is further supported by the finding of a positive association between the plasma kynurenine:tryptophan ratio and the severity of HD, as determined by the clinical state or the degree of CAG triplet repeats (Stoy et al. 2005; Forrest et al. 2010). The correlation between kynurenine pathway activity and both disease severity and CAG repeat length represents one of the few known instances of a biochemical parameter which can act as a reliable marker of the disease and, potentially a predictor of disease progression. It also remains a strong possibility that these correlations reflect a causative role for the kynurenine pathway in the severity of the disease (Stone et al. 2012a, b). Thus, one consequence of the extended glutamine repeat sequences in huntingtin may be a change in one or more of the kynurenine pathway enzymes, as a result of which there is an altered susceptibility to neuronal damage.

A more detailed discussion of kynurenines and Huntington's disease, together with some of the issues associated with viewing the kynurenine pathway as

a potential therapeutic target for the prevention, delay, and treatment of the disorder, may be found in recent reviews (Stone et al. 2012a, b).

5.4 Parkinson's Disease

Parkinson's disease (PD) is associated with neuro-inflammation: Microglial activation forms part of the pathobiology of PD induced in rodents by 1-methyl-4-phenyl-tetrahydroyridine (MPTP) or LPS (Wu et al. 2002; Liu and Hong 2003). Also, PD patients exhibit microglial activation in the basal ganglia and cortex (Gerhard et al. 2006), with a resulting increase in 3HK and quinolinic acid and subsequent neuronal damage (Ogawa et al. 1992; Zinger et al. 2011). In addition, the antagonist activity of kynurenate at glutamate receptors is reduced in PD, since there is decreased expression of KAT1 in the substantia nigra of a rodent model of PD (Knyihar-Csillik et al. 2004) and there are lowered levels of kynurenate in the basal ganglia and cortex of PD patients (Ogawa et al. 1992). Overall, kynurenine pathway activation in PD seems to be such that the production of neurotoxic metabolites (quinolinic acid, 3HK) by microglia and infiltrating microphages outweighs the production of neuroprotective metabolites kynurenate (by astrocytes) and picolinic acid (by neurons) (Zinger et al. 2011) (Fig. 2).

5.5 Schizophrenia

Patients with schizophrenia have increased plasma levels of the pro-inflammatory cytokines IFN-γ, TNF-α, and IL-6 (Kim et al. 2009) and an associated activation of the kynurenine pathway (Barry et al. 2009). There is increased expression of IDO which is accompanied by a relatively greater activation of astrocytes than microglia, thus leading to the observed increase in levels of kynurenate in the cortex and CSF (Schwarcz et al. 2001; Erhardt et al. 2001; Muller et al. 2011). The blockage of NMDARs by kynurenate in the cortex could account for the glutamatergic hypofunction that is now believed to be a major factor in creating the established dopaminergic dysfunction in schizophrenia. For example, since glutamate and nicotinic acetylcholine receptors (nAChRs) are essential for cognitive processing, their blockage by kynurenate explains the cognitive deficit in schizophrenics (Zmarowski et al. 2009; Wu et al. 2010). Additionally, the sensory gating deficits in schizophrenia can be explained by kynurenate decreasing excitability of CA1 stratum radiatum interneurons via its blockade of NMDARs and nAChRs (Alkondon et al. 2011).

5.6 Depression

Depression is associated with an activated immune system (Maes et al. 1995; Maes 1999). For example, pro-inflammatory cytokines produced by activated

macrophages induce symptoms of depression when given to volunteers (Smith 1991; Dantzer et al. 2011) and patients receiving IFN-α for hepatitis C infection develop symptoms of depression. Also, depression is associated with increased IDO activity (which is reflected in an increased plasma kynurenine:tryptophan ratio), and a reduced level of the neuroprotective compound kynurenate (Wichers et al. 2005; Myint et al. 2007). The activity of TDO also is increased by pro-inflammatory cytokines, and by cortisol (Leonard and Myint 2006). Hence, the increased levels of cortisol during depression augment the activation of the kynurenine pathway by the cytokines to cause the neurodegenerative changes in susceptible brain regions – such as the subgenual anterior cingulate cortex, hippocampus, and amygdala – that are linked to the symptoms of depression.

Depression has an established association with low levels of serotonin for synaptic transmission, a link which has led to the widespread use of serotonin uptake inhibitors for treating depression. However, the diversion of tryptophan to the kynurenine pathway following immune system activation is likely to reduce the availability of tryptophan for serotonin synthesis. Changes of serotonergic neurotransmission may, therefore, be secondary consequences of kynurenine pathway activity, a hypothesis that could partly explain the association between activation of the kynurenine pathway and depression (Mackay et al. 2009).

5.7 Stroke

Cerebral ischemia induced by 10 min of bilateral carotid occlusion in gerbils results in increased levels of brain quinolinic acid at 4 days post ischemia (Heyes and Morrison 1997). In stroke, there is increased production of pro-inflammatory cytokines including IFN-γ, IL-1β, IL-8, IL-6, and IL-17 (Tarkowski et al. 1995; Kostulas et al. 1999; Hoshi et al. 2009); a subsequent localized increase in IDO expression (Taguchi et al. 2008; Hoshi et al. 2009); and increased kynurenine metabolites in the brain (Saito et al. 1993).

In humans, activation of the kynurenine pathway in stroke is supported by the finding of an association between stroke and an early onset decrease in plasma tryptophan levels and 3-hydroxyanthranilic acid:anthranilic acid ratio. Stroke also causes increases in the plasma kynurenine:tryptophan ratio (a measure of IDO expression), neopterin (a major inflammation marker), S100B protein (a marker of brain injury), and lipid peroxidation products (malondialdehyde and 4-hydroxynonenal). The levels of S100 and peroxidation products, as well as aspects of kynurenine metabolism, correlate with the infarct volume, as determined by CT scans (Darlington et al. 2007). Furthermore, the degree of IDO expression is well correlated with mortality risk following a stroke (Darlington et al. 2007) and with the subsequent degree of cognitive impairment following acute stroke (Gold et al. 2011). The latter observation is consistent with data showing very strong correlations between kynurenine pathway activity and cognitive function following cardiac bypass or thoracic surgery, to the extent that the biochemical changes may be used to predict cognitive outcome (Forrest et al. 2011).

5.8 Multiple Sclerosis

In multiple sclerosis (MS), there are parenchymal infiltrates associated with the multifocal areas of demyelination, macrophages and microglia being the main cell types found in the infiltrates. The activated macrophages and microglia release cytokines (IL-6, TNF-α, IL-1β, IL-12 and IL-23) that are capable of activating the kynurenine pathway.

Patients with MS have low tryptophan levels in serum and CSF (Sandyk 1996; Rudzite et al. 1996), suggesting activation of the kynurenine pathway. There have also been reports of decreased kynurenate levels in the CSF (Rejdak et al. 2002) and increased levels of plasma kynurenate (Hartai et al. 2005). The difference is unlikely to be due to the sample used since kynurenate easily crosses the blood–brain barrier. However, the difference might be due to the stage of the disease with increased kynurenate production during the active phase and a decreased production during the chronic non-active phase (Lim et al. 2010).

It seems that activation of microglia and perivascular macrophages in MS causes increased amounts of quinolinic acid that overwhelm the neuroprotective actions of kynurenate and contribute to the axonal demyelination. The oligodendrocytes that are damaged in MS lack IDO-1 activity and hence play an insignificant role in the kynurenine pathway; however, they do have NMDAR on their processes and AMPA/kainate receptors on the cell bodies (McDonald et al. 1998), making them susceptible to quinolinic acid– and glutamate-mediated excitotoxicity.

6 Therapeutic Possibilities

Acyclovir, an established drug against herpes virus infection, has been shown to inhibit quinolinic acid–induced lipid peroxidation in rat brain (Muller et al. 2005a), making it a potential treatment for quinolinic acid–mediated neurodegenerative diseases even when viral infection is not a primary factor.

A number of other compounds such as Necorostatin-1, Annulin, and Exigamine A have been shown to be effective IDO inhibitors in experimental models (Muller et al. 2005b; Brastianos et al. 2006; Pereira et al. 2006). That activity may make the compounds suitable for use as anticancer therapy or to inhibit the production of quinolinic acid, 3HK, and 3-hydroxyanthranilic acid in order to reduce excitotoxicity. However, they have not yet been shown to effective and safe enough to enter general clinical use (Takikawa 2007).

Because kynurenate production takes place in astrocytes, whereas 3HK and quinolinic acid are produced in microglia, the two branches of the kynurenine pathway are anatomically segregated. Additionally, these two branches are functionally segregated since the enzymes KAT II and KMO can be blocked selectively: Blocking of KAT II has no effect on 3HK/quinolinic acid levels, while blocking KMO decreases 3HK/quinolinic acid levels but increases kynurenate levels (Amori et al. 2009). Selective KMO inhibitors have been shown to produce neuroprotection against neuronal cell death in hippocampal slices (Carpenedo et al. 2002), brain

ischemia in gerbils (Cozzi et al. 1999), and dystonia in hamsters (Hamann et al. 2008). The therapeutic use of KMO inhibitors as neuroprotectants may be particularly effective when immune system activity is responsible for the changes in kynurenine pathway activation. Examples of this situation include parasitic disorders with CNS involvement. Infection of appropriate strains of mice with *Plasmodium* species, able to produce cerebral involvement similar to that encountered in humans, leads to ataxia and death within a few days; KMO inhibition reduces symptom severity and prevents mortality (Clark et al. 2005). Protection against brain damage has also been shown in a murine model of cerebral trypanosomiasis ("sleeping sickness"), a disease which is almost invariably fatal in humans (Rodgers et al. 2009). Despite these successes, the compounds used to date as KMO inhibitors penetrate the blood–brain barrier only poorly, and even greater success is likely to be achieved with the development of compounds that effectively cross this barrier more effectively (Zwilling et al. 2011).

6.1 Diet

Finally, it should be noted that in the fields of neurodegeneration and neuroprotection, as with several other major areas of central and peripheral pathology, dietary and nutritional factors may not only play a part in determining the extent of brain damage, but may also point toward potential therapeutic strategies for the reduction of damage. For example, there is accumulating evidence that a ketogenic diet (high in fat, low in carbohydrate) can increase plasma levels of acetoacetate, β-hydroxybutyrate, and acetone. The high levels of these ketone bodies may then provide neuroprotection against a number of CNS disorders in which brain damage occurs (in animal models or patients) including epilepsy, Alzheimer's disease, Parkinson's diseases, stroke, and traumatic brain injury (Gasior et al. 2006). Mice on a 4-week ketogenic diet exhibited smaller increases in brain TNF-α induced by the potent exogenous excitotoxin kainate (Jeong et al. 2011); the dietary effect would lead to reduced production of endogenous excitotoxins. Other studies have reported that ketone bodies induce the expression of KAT I and II in glial cells (Chmiel-Perzynska et al. 2011), leading to increased kynurenate levels in areas such as the hippocampus and striatum (Zmarowski et al. 2009).

Since some lifestyle activities including fasting or strenuous exercise can mimic ketogenic diets by promoting the formation of ketone bodies, it is likely that these or similar interventions will be examined closely as potential means by which the incidence and severity of CNS damage can be minimized, or recovery enhanced.

7 Conclusion

The key message from the work described above is that pharmacological interference with the kynurenine pathway should have importance in the treatment of a wide range of neuro-inflammatory and neurodegenerative disorders, including

those with primarily neurological or psychiatric symptomatology. This strategy would represent a major conceptual advance over the use of general receptor antagonists, since glutamate is the most widely used excitatory neurotransmitter in the mammalian CNS. A general blocker of glutamate receptors (or the major subdivisions of NMDA, AMPA, and metabotropic receptors) is, as a result, likely to induce a plethora of unwanted side effects that may include neurological manifestations such as paralysis, loss of memory, and compromised function of controlling output systems to the cardiovascular and respiratory systems. Interference with the kynurenine pathway should only affect those neurons and glia affected by the brain insult and thus should produce a locally directed modification of dysfunction with correspondingly fewer associated problems – one of the gold standard objectives of modern drug development.

References

Addae, J. I., & Stone, T. W. (1987). Interactions between topically applied excitatory amino acids on rat cerebral cortex: Discrimination by pentobarbitone. *Experimental Brain Research, 68*, 613–618.

Addae, J. I., & Stone, T. W. (1988). Purine receptors and kynurenic acid modulate the somatosensory evoked potential in rat cerebral cortex. *Electroencephalography and Clinical Neurophysiology, 69*, 186–189.

Adle-Biassette, H., Chretien, F., Wingertsmann, L., Hery, C., Ereau, T., Scaravilli, F., Tardieu, M., & Gray, F. (1999). Neuronal apoptosis does not correlate with dementia in HIV infection but is related to microglial activation and axonal damage. *Neuropathology and Applied Neurobiology, 25*, 123–133.

Alkondon, M., Pereira, E. F., & Albuquerque, E. X. (2011). Endogenous activation of nAChRs and NMDA receptors contributes to the excitability of CA1 stratum radiatum interneurons in rat hippocampal slices: Effects of kynurenic acid. *Biochemical Pharmacology, 82*, 842–851.

Amori, L., Guidetti, P., Pellicciari, R., Kajii, Y., & Schwarcz, R. (2009). On the relationship between the two branches of the kynurenine pathway in the rat brain in vivo. *Journal of Neurochemistry, 109*, 316–325.

Ball, H. J., Yuasa, H. J., Austin, C. J., Weiser, S., & Hunt, N. H. (2009). Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. *The International Journal of Biochemistry & Cell Biology, 41*, 467–471.

Banks, W. A., Kastin, A. J., & Ehrensing, C. A. (1994). Blood-borne interleukin-1 alpha is transported across the endothelial blood-spinal cord barrier of mice. *The Journal of Physiology, 479*, 257–264.

Barry, S., Clarke, G., Scully, P., & Dinan, T. G. (2009). Kynurenine pathway in psychosis: Evidence of increased tryptophan degradation. *Journal of Psychopharmacology, 23*, 287–294.

Battaglia, G., Rassoulpour, A., Wu, H. Q., Hodgkins, P. S., Kiss, C., Nicoletti, F., & Schwarcz, R. (2000). Some metabotropic glutamate receptor ligands reduce kynurenate synthesis in rats by intracellular inhibition of kynurenine aminotransferase II. *Journal of Neurochemistry, 75*, 2051–2060.

Beal, M. F., Kowall, N. W., Ellison, D. W., Mazurek, M. F., Swartz, K. J., & Martin, J. B. (1986). Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature, 321*, 168–171.

Behan, W. M., & Stone, T. W. (2002). Enhanced neuronal damage by co-administration of quinolinic acid and free radicals, and protection by adenosine A2A receptor antagonists. *British Journal of Pharmacology, 135*, 1435–1442.

Birch, P. J., Grossman, C. J., & Hayes, A. G. (1988). Kynurenic acid antagonises responses to NMDA via an action at the strychnine-insensitive glycine receptor. *European Journal of Pharmacology, 154*, 85–87.

Boven, L. A., Middel, J., Breij, E. C., Schotte, D., Verhoef, J., Soderland, C., & Nottet, H. S. (2000). Interactions between HIV-infected monocyte-derived macrophages and human brain microvascular endothelial cells result in increased expression of CC chemokines. *Journal of Neurovirology, 6*, 382–389.

Braidy, N., Grant, R., Adams, S., Brew, B. J., & Guillemin, G. J. (2009). Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons. *Neurotoxicity Research, 16*, 77–86.

Brastianos, H. C., Vottero, E., Patrick, B. O., Van Soest, R., Matainaho, T., Mauk, A. G., & Andersen, R. J. (2006). Exiguamine A, an indoleamine-2,3-dioxygenase (IDO) inhibitor isolated from the marine sponge Neopetrosia exigua. *Journal of the American Chemical Society, 128*, 16046–16047.

Brenchely, J. M., Price, D. A., & Douek, D. C. (2006). HIV disease: fallout from a mucosal catastrophe? *Nature Immunology, 7*, 235–239.

Carpenedo, R., Meli, E., Peruginelli, F., Pellegrini-Giampietro, D. E., & Moroni, F. (2002). Kynurenine 3-mono-oxygenase inhibitors attenuate post-ischemic neuronal death in organotypic hippocampal slice cultures. *Journal of Neurochemistry, 82*, 1465–1471.

Carpenedo, R., Pittaluga, A., Cozzi, A., Attucci, S., Galli, A., Raiteri, M., & Moroni, F. (2001). Presynaptic kynurenate-sensitive receptors inhibit glutamate release. *European Journal of Neuroscience, 13*, 2141–2147.

Carter, R. J., Lione, L. A., Humby, T., Mangiarini, L., Mahal, A., Bates, G. P., Dunnett, S. B., & Morton, A. J. (1999). Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. *The Journal of Neuroscience, 19*, 3248–3257.

Chmiel-Perzynska, I., Kloc, R., Perzynski, A., Rudzki, S., & Urbanska, E. M. (2011). Novel aspect of ketone action: Beta-hydroxybutyrate increases brain synthesis of kynurenic acid in vitro. *Neurotoxicity Research, 20*, 40–50.

Clark, C. J., Mackay, G. M., Smythe, G. A., Bustamante, S., Stone, T. W., & Phillips, R. S. (2005). Prolonged survival of a murine model of cerebral malaria by kynurenine pathway inhibition. *Infection and Immunity, 73*, 5249–5251.

Coggan, S. E., Smythe, G. A., Bilgin, A., & Grant, R. S. (2009). Age and circadian influences on picolinic acid concentrations in human cerebrospinal fluid. *Journal of Neurochemistry, 108*, 1220–1225.

Connor, T. J., Starr, N., O'Sullivan, J. B., & Harkin, A. (2008). Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: A role for IFN-gamma? *Neuroscience Letters, 441*, 29–34.

Cozzi, A., Carpenedo, R., & Moroni, F. (1999). Kynurenine hydroxylase inhibitors reduce ischemic brain damage: Studies with (*m*-nitrobenzoyl)-alanine (mNBA) and 3,4-dimethoxy-[−*N*-4-(nitrophenyl)thiazol-2yl]-benzenesulfonamide (Ro 61–8048) in models of focal or global brain ischemia. *Journal of Cerebral Blood Flow and Metabolism, 19*, 771–777.

Croitoru-Lamoury, J., Guillemin, G. J., Dormont, D., & Brew, B. J. (2003). Quinolinic acid up-regulates chemokine production and chemokine receptor expression in astrocytes. *Advances in Experimental Medicine and Biology, 527*, 37–45.

Danbolt, N. C. (2001). Glutamate uptake. *Progress in Neurobiology, 65*, 1–105.

Dantzer, R., O'Connor, J. C., Lawson, M. A., & Kelley, K. W. (2011). Inflammation-associated depression: From serotonin to kynurenine. *Psychoneuroendocrinology, 36*, 426–436.

Darlington, L. G., Mackay, G. M., Forrest, C. M., Stoy, N., George, C., & Stone, T. W. (2007). Altered kynurenine metabolism correlates with infarct volume in stroke. *European Journal of Neuroscience, 26*, 2211–2221.

de Carvalho, L. P., Bochet, P., & Rossier, J. (1996). The endogenous agonist quinolinic acid and the non endogenous homoquinolinic acid discriminate between NMDAR2 receptor subunits. *Neurochemistry International, 28*, 445–452.

Duleu, S., Mangas, A., Sevin, F., Veyret, B., Bessede, A., & Geffard, M. (2010). Circulating antibodies to IDO/THO pathway metabolites in Alzheimer's disease. *International Journal of Alzheimer's Disease, 2010*, PMID 20721333. doi:10.4061/2010/501541.

Eastman, C. L., & Guilarte, T. R. (1989). Cytotoxicity of 3-hydroxykynurenine in a neuronal hybrid cell line. *Brain Research, 495*, 225–231.

Erhardt, S., Blennow, K., Nordin, C., Skogh, E., Lindstrom, L. H., & Engberg, G. (2001). Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neuroscience Letters, 313*, 96–98.

Fan, M. M., Fernandes, H. B., Zhang, L. Y., Hayden, M. R., & Raymond, L. A. (2007). Altered NMDA receptor trafficking in a yeast artificial chromosome transgenic mouse model of Huntington's disease. *Journal of Neuroscience, 27*, 3768–3779.

Fantuzzi, L., Belardelli, F., & Gessani, S. (2003). Monocyte/macrophage-derived CC chemokines and their modulation by HIV-1 and cytokines: A complex network of interactions influencing viral replication and AIDS pathogenesis. *Journal of Leukocyte Biology, 74*, 719–725.

Forrest, C. M., Mackay, G. M., Oxford, L., Millar, K., Darlington, L. G., Higgins, M. J., & Stone, T. W. (2011). Kynurenine metabolism predicts cognitive function in patients following cardiac bypass and thoracic surgery. *Journal of Neurochemistry, 119*, 136–152.

Forrest, C. M., Mackay, G. M., Stoy, N., Spiden, S. L., Taylor, R., Stone, T. W., & Darlington, L. G. (2010). Blood levels of kynurenines, interleukin-23 and soluble human leucocyte antigen-G at different stages of Huntington's disease. *Journal of Neurochemistry, 112*, 112–122.

Fu, X., Lawson, M. A., Kelley, K. W., & Dantzer, R. (2011). HIV-1 Tat activates indoleamine 2,3 dioxygenase in murine organotypic hippocampal slice cultures in a p38 mitogen-activated protein kinase-dependent manner. *Journal of Neuroinflammation, 8*, 88.

Fukui, S., Schwarcz, R., Rapoport, S. I., Takada, Y., & Smith, Q. R. (1991). Blood–brain barrier transport of kynurenines: Implications for brain synthesis and metabolism. *Journal of Neurochemistry, 56*, 2007–2017.

Gal, E. M., & Sherman, A. D. (1978). Synthesis and metabolism of L-kynurenine in rat brain. *Journal of Neurochemistry, 30*, 607–613.

Gasior, M., Rogawski, M. A., & Hartman, A. L. (2006). Neuroprotective and disease-modifying effects of the ketogenic diet. *Behavioural Pharmacology, 17*, 431–439.

Gerhard, A., Pavese, N., Hotton, G., Turkheimer, F., Es, M., Hammers, A., Eggert, K., Oertel, W., Banati, R. B., & Brooks, D. J. (2006). In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiology of Disease, 21*, 404–412.

Giles, G. I., Collins, C. A., Stone, T. W., & Jacob, C. (2003). Electrochemical and in vitro evaluation of the redox-properties of kynurenine species. *Biochemical and Biophysical Research Communications, 300*, 719–724.

Giorgini, F., Guidetti, P., Nguyen, Q., Bennett, S. C., & Muchowski, P. J. (2005). A genomic screen in yeast implicates kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. *Nature Genetics, 37*, 526–531.

Giulian, D., Vaca, K., & Noonan, C. A. (1990). Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. *Science, 250*, 1593–1596.

Glass, J. D., Fedor, H., Wesselingh, S. L., & McArthur, J. C. (1995). Immunocytochemical quantitation of human immunodeficiency virus in the brain: Correlations with dementia. *Annals of Neurology, 38*, 755–762.

Gold, A. B., Herrmann, N., Swardfager, W., Black, S. E., Aviv, R. I., Tennen, G., Kiss, A., & Lanctot, K. L. (2011). The relationship between indoleamine 2,3-dioxygenase activity and post-stroke cognitive impairment. *Journal of Neuroinflammation, 8*, 17.

Goldstein, L. E., Leopold, M. C., Huang, X., Atwood, C. S., Saunders, A. J., Hartshorn, M., Lim, J. T., Faget, K. Y., Muffat, J. A., Scarpa, R. C., Chylack, L. T., Jr., Bowden, E. F., Tanzi, R. E., & Bush, A. I. (2000). 3-Hydroxykynurenine and 3-hydroxyanthranilic acid generate hydrogen peroxide and promote alpha-crystallin cross-linking by metal ion reduction. *Biochemistry, 39*, 7266–7275.

Gramsbergen, J. B., Hodgkins, P. S., Rassoulpour, A., Turski, W. A., Guidetti, P., & Schwarcz, R. (1997). Brain-specific modulation of kynurenic acid synthesis in the rat. *Journal of Neurochemistry, 69*, 290–298.

Guidetti, P., Amori, L., Sapko, M. T., Okuno, E., & Schwarcz, R. (2007a). Mitochondrial aspartate aminotransferase: A third kynurenate-producing enzyme in the mammalian brain. *Journal of Neurochemistry, 102*, 103–111.

Guidetti, P., Bates, G. P., Graham, R. K., Hayden, M. R., Leavitt, B. R., MacDonald, M. E., Slow, E. J., Wheeler, V. C., Woodman, B., & Schwarcz, R. (2006). Elevated brain 3-hydroxykynurenine and quinolinate levels in Huntington disease mice. *Neurobiology of Disease, 23*, 190–197.

Guidetti, P., Hoffman, G. E., Melendez-Ferro, M., Albuquerque, E. X., & Schwarcz, R. (2007b). Astrocytic localization of kynurenine aminotransferase II in the rat brain visualized by immunocytochemistry. *Glia, 55*, 78–92.

Guidetti, P., Luthi-Carter, R. E., Augood, S. J., & Schwarcz, R. (2004). Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. *Neurobiology of Disease, 17*, 455–461.

Guidetti, P., & Schwarcz, R. (1999). 3-Hydroxykynurenine potentiates quinolinate but not NMDA toxicity in the rat striatum. *European Journal of Neuroscience, 11*, 3857–3863.

Guillemin, G. J., & Brew, B. J. (2002). Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease. *Redox Report, 7*, 199–206.

Guillemin, G. J., Cullen, K. M., Lim, C. K., Smythe, G. A., Garner, B., Kapoor, V., Takikawa, O., & Brew, B. J. (2007). Characterization of the kynurenine pathway in human neurons. *Journal of Neuroscience, 27*, 12884–12892.

Guillemin, G. J., Kerr, S. J., Smythe, G. A., Smith, D. G., Kapoor, V., Armati, P. J., Croitoru, J., & Brew, B. J. (2001). Kynurenine pathway metabolism in human astrocytes: A paradox for neuronal protection. *Journal of Neurochemistry, 78*, 842–853.

Guillemin, G. J., Smith, D. G., Kerr, S. J., Smythe, G. A., Kapoor, V., Armati, P. J., & Brew, B. J. (2000). Characterisation of kynurenine pathway metabolism in human astrocytes and implications in neuropathogenesis. *Redox Report, 5*, 108–111.

Guillemin, G. J., Smythe, G., Takikawa, O., & Brew, B. J. (2005). Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. *Glia, 49*, 15–23.

Guillemin, G. J., Smythe, G. A., Veas, L. A., Takikawa, O., & Brew, B. J. (2003a). A beta 1–42 induces production of quinolinic acid by human macrophages and microglia. *Neuroreport, 14*, 2311–2315.

Guillemin, G. J., Williams, K. R., Smith, D. G., Smythe, G. A., Croitoru-Lamoury, J., & Brew, B. J. (2003b). Quinolinic acid in the pathogenesis of Alzheimer's disease. *Advances in Experimental Medicine and Biology, 527*, 167–176.

Haass, C., & Selkoe, D. J. (2007). Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid beta-peptide. *Nature Reviews. Molecular Cell Biology, 8*, 101–112.

Haber, R., Bessette, D., Hulihan-Giblin, B., Durcan, M. J., & Goldman, D. (1993). Identification of tryptophan 2,3-dioxygenase RNA in rodent brain. *Journal of Neurochemistry, 60*, 1159–1162.

Hamann, M., Sander, S. E., & Richter, A. (2008). Effects of the kynurenine 3-hydroxylase inhibitor Ro 61–8048 after intrastriatal injections on the severity of dystonia in the dt sz mutant. *European Journal of Pharmacology, 586*, 156–159.

Han, Q., Cai, T., Tagle, D. A., & Li, J. (2010). Structure, expression, and function of kynurenine aminotransferases in human and rodent brains. *Cellular and Molecular Life Sciences, 67*, 353–368.

Hartai, Z., Juhasz, A., Rimanoczy, A., Janaky, T., Donko, T., Dux, L., Penke, B., Toth, G. K., Janka, Z., & Kalman, J. (2007). Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease. *Neurochemistry International, 50*, 308–313.

Hartai, Z., Klivenyi, P., Janaky, T., Penke, B., Dux, L., & Vecsei, L. (2005). Kynurenine metabolism in multiple sclerosis. *Acta Neurologica Scandinavica, 112*, 93–96.

Heyes, M. P., Achim, C. L., Wiley, C. A., Major, E. O., Saito, K., & Markey, S. P. (1996). Human microglia convert L-tryptophan into the neurotoxin quinolinic acid. *The Biochemical Journal, 320*, 595–597.

Heyes, M. P., & Morrison, P. F. (1997). Quantification of local de novo synthesis versus blood contributions to quinolinic acid concentrations in brain and systemic tissues. *Journal of Neurochemistry, 68*, 280–288.

Heyes, M. P., Saito, K., Crowley, J. S., Davis, L. E., Demitrack, M. A., Der, M., Dilling, L. A., Elia, J., Kruesi, M. J. P., Lackner, A., Larsen, S. A., Lee, K., Leonard, H. L., Markey, S. P., Martin, A., Milstein, S., Mouradian, M. M., Pranzatelli, M. R., Quearry, B. J., Salazar, A., Smith, M., Straus, S. E., Sunderland, T., Swedo, S. E., & Tourtellotte, W. W. (1992). Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain, 115*, 1249–1273.

Heyes, M. P., Saito, K., Lackner, A., Wiley, C. A., Achim, C. L., & Markey, S. P. (1998). Sources of the neurotoxin quinolinic acid in the brain of HIV-1-infected patients and retrovirus-infected macaques. *FASEB Journal, 12*, 881–896.

Heyes, M. P., Saito, K., Major, E. O., Milstien, S., Markey, S. P., & Vickers, J. H. (1993). A mechanism of quinolinic acid formation by brain in inflammatory neurological disease. Attenuation of synthesis from L-tryptophan by 6-chlorotryptophan and 4-chloro-3-hydroxyanthranilate. *Brain, 116*, 1425–1450.

Hilmas, C., Pereira, E. F., Alkondon, M., Rassoulpour, A., Schwarcz, R., & Albuquerque, E. X. (2001). The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: Physiopathological implications. *Journal of Neuroscience, 21*, 7463–7473.

Hodgkins, P. S., Wu, H. Q., Zielke, H. R., & Schwarcz, R. (1999). 2-Oxoacids regulate kynurenic acid production in the rat brain: Studies in vitro and in vivo. *Journal of Neurochemistry, 72*, 643–651.

Hoshi, M., Saito, K., Murakami, Y., Taguchi, A., Fujigaki, H., Tanaka, R., Takemura, M., Ito, H., Hara, A., & Seishima, M. (2009). Marked increases in hippocampal neuron indoleamine 2, 3-dioxygenase via IFN-gamma-independent pathway following transient global ischemia in mouse. *Neuroscience Research, 63*, 194–198.

Jauch, D. A., Sethy, V. H., Weick, B. G., Chase, T. N., & Schwarcz, R. (1993). Intravenous administration of L-kynurenine to rhesus monkeys: Effect on quinolinate and kynurenate levels in serum and cerebrospinal fluid. *Neuropharmacology, 32*, 467–472.

Jeong, E. A., Jeon, B. T., Shin, H. J., Kim, N., Lee, D. H., Kim, H. J., Kang, S. S., Cho, G. J., Choi, W. S., & Roh, G. S. (2011). Ketogenic diet-induced peroxisome proliferator-activated receptor-gamma activation decreases neuroinflammation in the mouse hippocampus after kainic acid-induced seizures. *Experimental Neurology, 232*, 195–202.

Jhamandas, K., Boegman, R. J., Beninger, R. J., & Bialik, M. (1990). Quinolinate-induced cortical cholinergic damage: Modulation by tryptophan metabolites. *Brain Research, 529*, 185–191.

Kanai, M., Nakamura, T., & Funakoshi, H. (2009). Identification and characterization of novel variants of the tryptophan 2,3-dioxygenase gene: Differential regulation in the mouse nervous system during development. *Neuroscience Research, 64*, 111–117.

Kessler, M., Terramani, T., Lynch, G., & Baudry, M. (1989). A glycine site associated with *N*-methyl-D-aspartic acid receptors: Characterization and identification of a new class of antagonists. *Journal of Neurochemistry, 52*, 1319–1328.

Kim, Y. K., Myint, A. M., Verkerk, R., Scharpe, S., Steinbusch, H., & Leonard, B. (2009). Cytokine changes and tryptophan metabolites in medication-naive and medication-free schizophrenic patients. *Neuropsychobiology, 59*, 123–129.

Kiss, C., Ceresoli-Borroni, G., Guidetti, P., Zielke, C. L., Zielke, H. R., & Schwarcz, R. (2003). Kynurenate production by cultured human astrocytes. *Journal of Neural Transmission, 110*, 1–14.

Knyihar-Csillik, E., Csillik, B., Pakaski, M., Krisztin-Peva, B., Dobo, E., Okuno, E., & Vecsei, L. (2004). Decreased expression of kynurenine aminotransferase-I (KAT-I) in the substantia nigra

of mice after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment. *Neuroscience, 126*, 899–914.

Kocki, T., Luchowski, P., Luchowska, E., Wielosz, M., Turski, W. A., & Urbanska, E. M. (2003). L-cysteine sulphinate, endogenous sulphur-containing amino acid, inhibits rat brain kynurenic acid production via selective interference with kynurenine aminotransferase II. *Neuroscience Letters, 346*, 97–100.

Kostulas, N., Pelidou, S. H., Kivisakk, P., Kostulas, V., & Link, H. (1999). Increased IL-1beta, IL-8, and IL-17 mRNA expression in blood mononuclear cells observed in a prospective ischemic stroke study. *Stroke, 30*, 2174–2179.

Lehrmann, E., Molinari, A., Speciale, C., & Schwarcz, R. (2001). Immunohistochemical visualization of newly formed quinolinate in the normal and excitotoxically lesioned rat striatum. *Experimental Brain Research, 141*, 389–397.

Leonard, B. E., & Myint, A. (2006). Changes in the immune system in depression and dementia: Causal or coincidental effects? *Dialogues in Clinical Neuroscience, 8*, 163–174.

Lesne, S., Ali, C., Gabriel, C., Croci, N., MacKenzie, E. T., Glabe, C. G., Plotkine, M., Marchand-Verrecchia, C., Vivien, D., & Buisson, A. (2005). NMDA receptor activation inhibits alpha-secretase and promotes neuronal amyloid-beta production. *Journal of Neuroscience, 25*, 9367–9377.

Liebau, C., Baltzer, A. W., Schmidt, S., Roesel, C., Karreman, C., Prisack, J. B., Bojar, H., & Merk, H. (2002). Interleukin-12 and interleukin-18 induce indoleamine 2,3-dioxygenase (IDO) activity in human osteosarcoma cell lines independently from interferon-gamma. *Anticancer Research, 22*, 931–936.

Lim, C. K., Brew, B. J., Sundaram, G., & Guillemin, G. J. (2010). Understanding the roles of the kynurenine pathway in multiple sclerosis progression. *International Journal of Tryptophan Research, 3*, 157–167.

Liu, B., & Hong, J. S. (2003). Role of microglia in inflammation-mediated neurodegenerative diseases: Mechanisms and strategies for therapeutic intervention. *The Journal of Pharmacology and Experimental Therapeutics, 304*, 1–7.

Mackay, G. M., Forrest, C. M., Christofides, J., Bridel, M. A., Mitchell, S., Cowlard, R., Stone, T. W., & Darlington, L. G. (2009). Kynurenine metabolites and inflammation markers in depressed patients treated with fluoxetine or counselling. *Clinical and Experimental Pharmacology & Physiology, 36*, 425–435.

Maes, M. (1999). Major depression and activation of the inflammatory response system. *Advances in Experimental Medicine and Biology, 461*, 25–46.

Maes, M., Smith, R., & Scharpe, S. (1995). The monocyte-T-lymphocyte hypothesis of major depression. *Psychoneuroendocrinology, 20*, 111–116.

McDonald, J. W., Althomsons, S. P., Hyrc, K. L., Choi, D. W., & Goldberg, M. P. (1998). Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. *Nature Medicine, 4*, 291–297.

Moroni, F., Russi, P., Carla, V., & Lombardi, G. (1988). Kynurenic acid is present in the rat brain and its content increases during development and aging processes. *Neuroscience Letters, 94*, 145–150.

Muller, A. C., Maharaj, H., Maharaj, D. S., & Daya, S. (2005a). Aciclovir protects against quinolinic-acid-induced oxidative neurotoxicity. *Journal of Pharmacy and Pharmacology, 57*, 883–888.

Muller, A. J., Malachowski, W. P., & Prendergast, G. C. (2005b). Indoleamine 2,3-dioxygenase in cancer: Targeting pathological immune tolerance with small-molecule inhibitors. *Expert Opinion on Therapeutic Targets, 9*, 831–849.

Muller, N., Myint, A. M., & Schwarz, M. J. (2011). Kynurenine pathway in schizophrenia: Pathophysiological and therapeutic aspects. *Current Pharmaceutical Design, 17*, 130–136.

Myint, A. M., Kim, Y. K., Verkerk, R., Scharpe, S., Steinbusch, H., & Leonard, B. (2007). Kynurenine pathway in major depression: Evidence of impaired neuroprotection. *Journal of Affective Disorders, 98*, 143–151.

Ogawa, T., Matson, W. R., Beal, M. F., Myers, R. H., Bird, E. D., Milbury, P., & Saso, S. (1992). Kynurenine pathway abnormalities in Parkinson's disease. *Neurology, 42*, 1702–1706.

Olney, J. W., Misra, C. H., & de Gubareff, T. (1975). Cysteine-*S*-sulfate: Brain damaging metabolite in sulfite oxidase deficiency. *Journal of Neuropathology and Experimental Neurology, 34*, 167–177.

Pearson, S. J., & Reynolds, G. P. (1992). Increased brain concentrations of a neurotoxin, 3-hydroxykynurenine, in Huntington's disease. *Neuroscience Letters, 144*, 199–201.

Pereira, A., Vottero, E., Roberge, M., Mauk, A. G., & Andersen, R. J. (2006). Indoleamine 2,3-dioxygenase inhibitors from the Northeastern Pacific Marine Hydroid Garveia annulata. *Journal of Natural Products, 69*, 1496–1499.

Perkins, M. N., & Stone, T. W. (1982). An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Research, 247*, 184–187.

Perkins, M. N., & Stone, T. W. (1983). Quinolinic acid: Regional variations in neuronal sensitivity. *Brain Research, 259*, 172–176.

Persidsky, Y., & Gendelman, H. E. (2003). Mononuclear phagocyte immunity and the neuropathogenesis of HIV-1 infection. *Journal of Leukocyte Biology, 74*, 691–701.

Platenik, J., Stopka, P., Vejrazka, M., & Stipek, S. (2001). Quinolinic acid-iron(ii) complexes: Slow autoxidation, but enhanced hydroxyl radical production in the Fenton reaction. *Free Radical Research, 34*, 445–459.

Puccetti, P. (2007). On watching the watchers: IDO and type I/II IFN. *European Journal of Immunology, 37*, 876–879.

Rafice, S. A., Chauhan, N., Efimov, I., Basran, J., & Raven, E. L. (2009). Oxidation of L-tryptophan in biology: A comparison between tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase. *Biochemical Society Transactions, 37*, 408–412.

Rejdak, K., Bartosik-Psujek, H., Dobosz, B., Kocki, T., Grieb, P., Giovannoni, G., Turski, W. A., & Stelmasiak, Z. (2002). Decreased level of kynurenic acid in cerebrospinal fluid of relapsing-onset multiple sclerosis patients. *Neuroscience Letters, 331*, 63–65.

Robinson, C. M., Shirey, K. A., & Carlin, J. M. (2003). Synergistic transcriptional activation of indoleamine dioxygenase by IFN-gamma and tumor necrosis factor-alpha. *Journal of Interferon & Cytokine Research, 23*, 413–421.

Rodgers, J., Stone, T. W., Barrett, M. P., Bradley, B., & Kennedy, P. G. (2009). Kynurenine pathway inhibition reduces central nervous system inflammation in a model of human African trypanosomiasis. *Brain, 132*, 1259–1267.

Rudzite, V., Berzinsh, J., Grivane, I., Fuchs, D., Baier-Bitterlich, G., & Wachter, H. (1996). Serum tryptophan, kynurenine, and neopterin in patients with Guillain-Barre-syndrome (GBS) and multiple sclerosis (MS). *Advances in Experimental Medicine and Biology, 398*, 183–187.

Saito, K., Nowak, T. S., Jr., Suyama, K., Quearry, B. J., Saito, M., Crowley, J. S., Markey, S. P., & Heyes, M. P. (1993). Kynurenine pathway enzymes in brain: Responses to ischemic brain injury versus systemic immune activation. *Journal of Neurochemistry, 61*, 2061–2070.

Sandyk, R. (1996). Tryptophan availability and the susceptibility to stress in multiple sclerosis: A hypothesis. *International Journal of Neuroscience, 86*, 47–53.

Sapko, M. T., Guidetti, P., Yu, P., Tagle, D. A., Pellicciari, R., & Schwarcz, R. (2006). Endogenous kynurenate controls the vulnerability of striatal neurons to quinolinate: Implications for Huntington's disease. *Experimental Neurology, 197*, 31–40.

Sardar, A. M., & Reynolds, G. P. (1995). Frontal cortex indoleamine-2,3-dioxygenase activity is increased in HIV-1-associated dementia. *Neuroscience Letters, 187*, 9–12.

Sathyasaikumar, K. V., Stachowski, E. K., Amori, L., Guidetti, P., Muchowski, P. J., & Schwarcz, R. (2010). Dysfunctional kynurenine pathway metabolism in the R6/2 mouse model of Huntington's disease. *Journal of Neurochemistry, 113*, 1416–1425.

Schwarcz, R., Guidetti, P., Sathyasaikumar, K. V., & Muchowski, P. J. (2010). Of mice, rats and men: Revisiting the quinolinic acid hypothesis of Huntington's disease. *Progress in Neurobiology, 90*, 230–245.

Schwarcz, R., & Pellicciari, R. (2002). Manipulation of brain kynurenines: Glial targets, neuronal effects, and clinical opportunities. *The Journal of Pharmacology and Experimental Therapeutics, 303*, 1–10.

Schwarcz, R., Rassoulpour, A., Wu, H. Q., Medoff, D., Tamminga, C. A., & Roberts, R. C. (2001). Increased cortical kynurenate content in schizophrenia. *Biological Psychiatry, 50*, 521–530.

Schwarcz, R., Whetsell, W. O., Jr., & Mangano, R. M. (1983). Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. *Science, 219*, 316–318.

Smith, D. G., Guillemin, G. J., Pemberton, L., Kerr, S., Nath, A., Smythe, G. A., & Brew, B. J. (2001). Quinolinic acid is produced by macrophages stimulated by platelet activating factor, Nef and Tat. *Journal of Neurovirology, 7*, 56–60.

Smith, R. S. (1991). The macrophage theory of depression. *Medical Hypotheses, 35*, 298–306.

Speciale, C., Hares, K., Schwarcz, R., & Brookes, N. (1989). High-affinity uptake of L-kynurenine by a Na^+−independent transporter of neutral amino acids in astrocytes. *Journal of Neuroscience, 9*, 2066–2072.

Stachowski, E. K., & Schwarcz, R. (2011). Regulation of quinolinic acid neosynthesis in mouse, rat and human brain by iron and iron chelators in vitro. *Journal of Neural Transmission, 119*, 123–131.

Stone, T. W. (1993a). Neuropharmacology of quinolinic and kynurenic acids. *Pharmacological Reviews, 45*, 309–379.

Stone, T. W. (1993b). Subtypes of NMDA receptors. *General Pharmacology, 24*, 825–832.

Stone, T. W. (2007). Kynurenic acid blocks nicotinic synaptic transmission to hippocampal interneurons in young rats. *European Journal of Neuroscience, 25*, 2656–2665.

Stone, T. W., Forrest, C. M., & Darlington, L. G. (2012a). Kynurenine pathway inhibition as a therapeutic strategy for neuroprotection. *FEBS Journal, 279*, 1386–1397.

Stone, T. W., Forrest, C. M., Stoy, N., & Darlington, L. G. (2012b). Involvement of kynurenines in Huntington's disease and stroke-induced brain damage. *Journal of Neural Transmission, 119*, 261–274.

Stone, T. W., & Perkins, M. N. (1981). Quinolinic acid: A potent endogenous excitant at amino acid receptors in CNS. *European Journal of Pharmacology, 72*, 411–412.

Stoy, N., Mackay, G. M., Forrest, C. M., Christofides, J., Egerton, M., Stone, T. W., & Darlington, L. G. (2005). Tryptophan metabolism and oxidative stress in patients with Huntington's disease. *Journal of Neurochemistry, 93*, 611–623.

Taguchi, A., Hara, A., Saito, K., Hoshi, M., Niwa, M., Seishima, M., & Mori, H. (2008). Localization and spatiotemporal expression of IDO following transient forebrain ischemia in gerbils. *Brain Research, 1217*, 78–85.

Takikawa, O. (2007). Clinical aspects of indoleamine 2,3-dioxygenase (IDO)-initiated tryptophan metabolism: IDO is a target of drug discovery for various diseases. *International Congress Series, 1304*, 290–297.

Takikawa, O., Yoshida, R., Kido, R., & Hayaishi, O. (1986). Tryptophan degradation in mice initiated by indoleamine 2,3-dioxygenase. *Journal of Biological Chemistry, 261*, 3648–3653.

Tarkowski, E., Rosengren, L., Blomstrand, C., Wikkelso, C., Jensen, C., Ekholm, S., & Tarkowski, A. (1995). Early intrathecal production of interleukin-6 predicts the size of brain lesion in stroke. *Stroke, 26*, 1393–1398.

Tatter, S. B., Galpern, W. R., Hoogeveen, A. T., & Isacson, O. (1995). Effects of striatal excitotoxicity on huntingtin-like immunoreactivity. *Neuroreport, 6*, 1125–1129.

Tavares, R. G., Tasca, C. I., Santos, C. E., Alves, L. B., Porciuncula, L. O., Emanuelli, T., & Souza, D. O. (2002). Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochemistry International, 40*, 621–627.

Testa, U., Louache, F., Titeux, M., Thomopoulos, P., & Rochant, H. (1985). The iron-chelating agent picolinic acid enhances transferrin receptors expression in human erythroleukaemic cell lines. *British Journal of Haematology, 60*, 491–502.

Thakur, A. K., Jayaraman, M., Mishra, R., Thakur, M., Chellgren, V. M., Byeon, I. J., Anjum, D. H., Kodali, R., Creamer, T. P., Conway, J. F., Gronenborn, A. M., & Wetzel, R. (2009).

Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. *Nature Structural & Molecular Biology, 16*, 380–389.

Ting, K. K., Brew, B. J., & Guillemin, G. J. (2009). Effect of quinolinic acid on human astrocytes morphology and functions: Implications in Alzheimer's disease. *Journal of Neuroinflammation, 6*, 36.

Turski, W. A., Gramsbergen, J. B., Traitler, H., & Schwarcz, R. (1989). Rat brain slices produce and liberate kynurenic acid upon exposure to L-kynurenine. *Journal of Neurochemistry, 52*, 1629–1636.

Turski, W. A., Nakamura, M., Todd, W. P., Carpenter, B. K., Whetsell, W. O., Jr., & Schwarcz, R. (1988). Identification and quantification of kynurenic acid in human brain tissue. *Brain Research, 454*, 164–169.

Usdin, M. T., Shelbourne, P. F., Myers, R. M., & Madison, D. V. (1999). Impaired synaptic plasticity in mice carrying the Huntington's disease mutation. *Human Molecular Genetics, 8*, 839–846.

Wichers, M. C., Koek, G. H., Robaeys, G., Verkerk, R., Scharpe, S., & Maes, M. (2005). IDO and interferon-alpha-induced depressive symptoms: A shift in hypothesis from tryptophan depletion to neurotoxicity. *Molecular Psychiatry, 10*, 538–544.

Widner, B., Leblhuber, F., Walli, J., Tilz, G. P., Demel, U., & Fuchs, D. (2000). Tryptophan degradation and immune activation in Alzheimer's disease. *Journal of Neural Transmission, 107*, 343–353.

Wonodi, I., & Schwarcz, R. (2010). Cortical kynurenine pathway metabolism: A novel target for cognitive enhancement in schizophrenia. *Schizophrenia Bulletin, 36*, 211–218.

Wu, D. C., Jackson-Lewis, V., Vila, M., Tieu, K., Teismann, P., Vadseth, C., Choi, D. K., Ischiropoulos, H., & Przedborski, S. (2002). Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *Journal of Neuroscience, 22*, 1763–1771.

Wu, H. Q., Pereira, E. F., Bruno, J. P., Pellicciari, R., Albuquerque, E. X., & Schwarcz, R. (2010). The astrocyte-derived alpha7 nicotinic receptor antagonist kynurenic acid controls extracellular glutamate levels in the prefrontal cortex. *Journal of Molecular Neuroscience, 40*, 204–210.

Wu, H. Q., Ungerstedt, U., & Schwarcz, R. (1995). L-alpha-aminoadipic acid as a regulator of kynurenic acid production in the hippocampus: A microdialysis study in freely moving rats. *European Journal of Pharmacology, 281*, 55–61.

Yoshida, R., Imanishi, J., Oku, T., Kishida, T., & Hayaishi, O. (1981). Induction of pulmonary indoleamine 2,3-dioxygenase by interferon. *Proceedings of the National Academy of Sciences of the United States of America, 78*, 129–132.

Zhou, H., Lapointe, B. M., Clark, S. R., Zbytnuik, L., & Kubes, P. (2006). A requirement for microglial TLR4 in leukocyte recruitment into brain in response to lipopolysaccharide. *Journal of Immunology, 177*, 8103–8110.

Zinger, A., Barcia, C., Herrero, M. T., & Guillemin, G. J. (2011). The involvement of neuroinflammation and kynurenine pathway in Parkinson's disease. *Parkinson's Disease, 2011*.

Zmarowski, A., Wu, H. Q., Brooks, J. M., Potter, M. C., Pellicciari, R., Schwarcz, R., & Bruno, J. P. (2009). Astrocyte-derived kynurenic acid modulates basal and evoked cortical acetylcholine release. *European Journal of Neuroscience, 29*, 529–538.

Zwilling, D., Huang, S. Y., Sathyasaikumar, K. V., Notarangelo, F. M., Guidetti, P., Wu, H. Q., Lee, J., Truong, J., Andrews-Zwilling, Y., Hsieh, E. W., Louie, J. Y., Wu, T., Scearce-Levie, K., Patrick, C., Adame, A., Giorgini, F., Moussaoui, S., Laue, G., Rassoulpour, A., Flik, G., Huang, Y., Muchowski, J. M., Masliah, E., Schwarcz, R., & Muchowski, P. J. (2011). Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. *Cell, 145*, 863–874.

Role of Neurotoxicity in Depression

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Abstract

Despite years of research, the pathophysiology of depression and the mechanism of action in antidepressant drugs largely remain unknown. The subsequent hypotheses examining depression witnessed attention being paid to the decrease of the serotonin and noradrenaline synaptic concentrations, adaptive changes in the neurotransmitters receptor, activation of the immune system, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, and enhanced glutamatergic activity. Current research suggests that disturbed neuronal plasticity and the degeneration of neurons and glia cells may be involved in the pathogenesis of depression. In fact, in depressed patients, structural changes are observed in several brain regions, mainly in the hippocampus. Also, in various animal models of depression, morphological alteration in neurons and glial cells is present besides depression-like behavioral changes. A lot of evidence indicates that an increased amount or activity of glucocorticoids, glutamate, and proinflammatory cytokines is the reason for these changes. In both animal models of depression and postmortem studies, a reduced neurogenesis in the dentate gyrus of the hippocampus; a decrease in the total number of neurons and astrocytes; a reduction of the dendritic length, branching density, or the number of synapses; and a decrease in the brain-derived neurotrophic factor were observed. Moreover, activation of microglia, a source of proinflammatory cytokines and reactive oxygen species, has a disadvantageous effect on neurons and astrocytes. However, it should be noted that not all of the studies demonstrate neurodegenerative changes in depression, or the glucocorticoids' detrimental action, so further studies are needed in order to clearly determine the contribution of neurotoxic agents in the pathogenesis of depression and also the importance of the neuroprotective properties of some antidepressant drugs in their therapeutic effect.

Keywords

Animal models of depression • Antidepressant • Depression • Neurotoxicity

List of Abbreviations

5HT	Serotonin
ACTH	Adrenocorticotropic hormone
BDNF	Brain-derived neurotrophic factor
CMS	Chronic mild stress
CREB	Cyclic AMP response element-binding protein
CRF	Corticotropin-releasing factor

CRP	C-reactive protein
CUS	Chronic unpredictable stress
GFAP	Glial fibrillary acidic protein
HPA	Hypothalamic-pituitary-adrenal
LPS	Lipopolysaccharide
mPFC	Medial prefrontal cortex
NA	Noradrenaline
NMDA	*N*-methyl-D-aspartate
NOS	Nitric oxide synthase
PFC	Prefrontal cortex
ROS	Reactive oxygen species
SSRI	Selective serotonin reuptake inhibitors
TCA	Tricyclic antidepressant

1 Introduction

The first effective antidepressant drugs were introduced around 50 years ago, although their mode of action remained unclear (Charney et al. 1981). The investigation into the mechanism of action of antidepressants (iproniazid, imipramine) showed that these substances increase the central serotonin and norepinephrine transmission. Using these results as the basis, it was hypothesized that the cause of depression is the decrease in the level of monoamines such as serotonin or norepinephrine in the synaptic space, as well as a reduction in nerve conduction (Berton and Nestler 2006). It has been also shown that antihypertensive drugs such as reserpine, which cause monoamine depletion, sometimes lead to depression (Freis 1954). However, the hypothesis concerning monoamine has been recently questioned. It has been observed that not all drugs that increase monoamine concentration exert an antidepressant effect, and drugs such as monoamine oxidase inhibitors (tranylcypromine), and inhibitors of selective serotonin reuptake (fluoxetine), act immediately through the enhancement of monoaminergic conduction, yet their therapeutic effect in the improvement of mood is observed after a few weeks of therapy (Ruhé et al. 2007). Both the clinical and etiological heterogeneities of depression make it hard to define its pathophysiology (Hasler 2010). The last few years have paid witness to a variety of theories explaining the etiology of this disease. A decrease in the synaptic levels of monoamines, changes in neurotransmitter receptors, protein kinases, proinflammatory cytokines, neurotrophic factors, and hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis in the pathophysiology of depression were postulated as being potentially involved. Studies conducted in recent years have provided abundant evidence that the disturbance of molecular and cellular regulation of neuronal plasticity may be involved in the pathogenesis of depressive disorders (Nestler et al. 2002; Pittenger and Duman 2008). A considerable amount of data has suggested that degeneration of neurons and glia cells evoked by

a disturbance in neurotrophic factor pathways and enhanced glutamate, glucocorticoids, and proinflammatory cytokines' action play an important role in the pathogenesis of this disease. However, it has not presently been conclusively established whether morphological and functional brain changes observed in depression are one of the causes of disease or if they are the result of depression.

2 The Evidence for Brain Structures Changes in Depression: Clinical Data

Several changes have been noted in the structures anatomically linked with depression, such as the prefrontal cortex, hippocampus, amygdala, or nucleus accumbens. First of all, the structure which is the most vulnerable to atrophy is the hippocampus. The hippocampus is an area of the brain responsible for learning, memory, mood, and emotions. It plays an important role in the consolidation of information from short-term memory to long-term memory and spatial orientation. Volume reduction of this structure in depression, observed in postmortem studies, might be the result of many processes, including the remodeling of cellular elements, retraction of dendrites, reduction of neurogenesis in the dentate gyrus, and loss of glial cells (McEwen 1999; Rajkowska 2000; Czéh et al. 2001). In depressed patients, it was observed that the size of pyramidal neurons decreased, whereas the packing density of glial cells, pyramidal neurons, and granule cell neurons was significantly greater (Stockmeier et al. 2004; Tsopelas et al. 2011). The hippocampus is the most extensively studied region in depression by neuroimaging, and the resulting findings seem to suggest that hippocampal volumetric reductions are associated with depression (Bremner et al. 2000; Frodl et al. 2002b; MacQueen et al. 2003).

There were also reports that the volume of other brain structures have also decreased in postmortem research. Patients with depression had a significantly smaller medial orbitofrontal cortical volume, without smaller volumes of other frontal regions. These findings are consistent with other studies where the reduced volume of the orbitofrontal cortex, as well as the reduction of the entire frontal lobe, was shown (Bremner et al. 2002; Shah et al. 2002; Frodl et al. 2006). This change occurs in a more severely ill patient group, but not in those who were less severely ill (Bremner et al. 2000; Lacerda et al. 2004). Similar to the hippocampus, the decreased volume of the orbitofrontal cortex is caused by an increased density of glial cells and neurons (Rajkowska 2000). Depression was also associated with neuronal loss in some of the subcortical structures investigated (nucleus basalis, substantia nigra, raphe nucleus) (Tsopelas et al. 2011).

Examination of the brain of depressives showed an enlargement of the cerebral ventricles (Kellner et al. 1986). With this increase in ventricular volume, it was noted that the surrounding tissue shrinks (mammillary bodies, thalamus) and there are changes in formations that are connected to structures that pass through the third ventricle, such as the hippocampus (via the fornix) and the amygdala, which is connected via the stria terminals (Baumann et al. 1997; Dasari et al. 1999; Bernstein et al. 2008).

There were also noted changes in the volume of the amygdala. The amygdala is a structure that contains an extensive system of neuronal connections. It is involved in the analysis and processing of incoming information and with then comparing them to the stored patterns of emotional memory. Patients with depression have impaired functioning in emotional tasks involving the amygdala and, furthermore, they have an abnormal resting amygdala blood flow (Ledoux 2000; Phan et al. 2002). Depressed patients had reduced amygdala core nuclei volumes, without any differences in the total amygdala volumes. Studies indicate that reduction of the hippocampal volume was often accompanied by the volume reduction of amygdala (Caetano et al. 2004). It seems that glucocorticoid-induced neurotoxicity has been mediated by the glutamatergic connections in these closely cooperating structures (Sheline et al. 1998). It was noted that the initial stage of the disease was characterized by increased volume of this structure (Frodl et al. 2002a, b; Lange and Irle 2004; Weniger et al. 2006), and during the development of the depression, the volume reduction was observed, especially in females (Bremner et al. 2000; Caetano et al. 2004; Hastings et al. 2004).

3 The Evidence for Changes in Brain Structures in Depression: Experimental Data

Depression is a very diverse disease, with mostly different symptoms amongst individuals, and its etiology is still poorly recognized despite extensive investigation (Table 1). More importantly, it is not possible to reproduce some of the symptoms characteristic of major depression in animals, such as suicidal thoughts, a depressed mood, or the feeling of guilt. This is the reason why it is hard to delineate the most accurate animal model of depression.

The majority of the presently applied models are based on different stress paradigms and in different stress patterns (chronic, acute, unpredictable). Chronic mild stress (CMS), which consists of the long-term exposure of animals to a series of unpredictable, mild stressors, produces depression-like behavioral changes, including anhedonia. In this model of depression, a decrease in the total number of granule cells in the ventral hippocampus, an enhanced blood corticosterone level, and an increase in the number of caspase-3 positive neurons in the cerebral cortex were observed (Jayatissa et al. 2006; Bachis et al. 2008; Nowak et al. 2010). In chronic unpredictable stress (CUS), which has been conducted on rabbits, glial atrophy in the PFC has been confirmed (Banasr and Duman 2008). In prenatal restraint stress, which, similar to CMS, exhibits face, predictive and construct validity, a decrease in hippocampal cell neurogenesis in the dentate gyrus and impairment in the hippocampal-related spatial tasks were indicated (Lemaire et al. 2000). A decrease in hippocampal neurogenesis has also been observed in the olfactory bulbectomy (Jaako-Movits et al. 2006). In another examination, with chronic or acute restraint stress, there were a decrease in the total number of neurons and the structure volume and a decrease of the neurogenesis of the CA3 field dentate gyrus (Pham et al. 2003). In chronic, social stress in tree shrews,

Table 1 Summary of structural alterations related to neurodegenerative processes demonstrated in animal models of depression

Animal model of depression	Structural changes	References
Chronic mild stress	▼Granular cell number in hippocampus	(Bachis et al. 2008; Nowak et al. 2010)
	▲Caspase cascade in cerebral cortex cells	
Chronic unpredictable stress	▲Atrophy of the glial cells in prefrontal cortex	(Banasr and Duman 2008; Nowak et al. 2010)
	▼Granular cell number in hippocampus	
Tree shrews	▼Hippocampal volume	(Czéh et al. 2001; Lucassen et al. 2001)
	▼Neurogenesis in hippocampus	
	▲Apoptosis in the temporal cortex	
Restraint stress	▼Neurogenesis in the region CA3 of the hippocampus	(Pham et al. 2003)
	▼Total neuron number in the hippocampus	
	▼Hippocampal volume	
Maternal separation	▲Apoptosis in the hippocampus	(Lee et al. 2001)
	▼Cell proliferation in the hippocampus	
Learned helplessness	▼Cell proliferation in dentate gyrus	(Vollmayr et al. 2003)
Olfactory bulbectomy	▼Hippocampal neurogenesis	(Jaako-Movits et al. 2006)
Prenatal stress	▼Hippocampal cell neurogenesis	(Lemaire et al. 2000; Murmu et al. 2006)
	▼Dendritic length and complexity in prefrontal cortex	

a model with high validity for depression, a reduced hippocampal volume, a decreased hippocampal neurogenesis, and an increased number of apoptotic cells in the temporal cortex occur (Czéh et al. 2001; Lucassen et al. 2001). A decrease in cell proliferation and enhanced apoptosis in the dentate gyrus of the hippocampus were reported in the maternal separation rat model (Lee et al. 2001). The expression of antiapoptotic proteins (Bcl-2, Bcl-xL) was lower in the cingulate and frontal cortex, hippocampus, and central nucleus of the amygdala in CUS (an animal model of depression) (Kosten et al. 2008). In the restraint model of psychological stress in mice, the increased proliferation of microglia cells was observed (Nair and Bonneau 2006).

4 The Involvement of the HPA Axis in Neurodegenerative Processes in Depression

Amongst the possible mechanisms involved in the brain's morphological changes observed in depression, hyperactivity of the HPA axis, itself leading to prolonged hypercortisolemia, has been considered. This mechanism may play an important role in the volumetric and neurodegenerative alteration of several brain areas, especially in those with a high concentration of glucocorticoid receptors, such as

the hippocampus (Herman et al. 2003). It has been observed that, on the one hand, stress-induced neurotoxicity can cause volumetric changes in different brain structures, while, on the other hand, these changes may in turn induce an increase in HPA axis activity (Cerqueira et al. 2005). This results from the fact that stress and glucocorticoids induce neuronal loss mainly in the hippocampus, a structure which exerts an inhibitory effect on HPA axis activity. Thus, hippocampal damage disinhibits the HPA axis and, as a result, additionally enhances glucocorticoid synthesis and release. This "glucocorticoid cascade hypothesis," formulated by Sapolsky et al. (1986), was proposed as an explanation of hippocampal damage in both some neurodegenerative disorders and depression.

Psychological studies conducted in patients affected by depression at the end of the 1970s revealed the role of psychological stress as a factor contributing to the development of depression (Brown and Harris 1978). At the same time, HPA axis abnormalities, including an increased cortisol serum level, were shown to be one of depression's features. In addition, it has been observed that dexamethasone administration suppresses the cortisol level in the blood of depressed patients less potently than in healthy people (Carroll 1982). The participation of the HPA axis dysfunction in the pathogenesis of depression has been also supported by the observations that patients suffering from Cushing's syndrome, characterized by elevated levels of serum cortisol, often exhibit symptoms of depression (Sonino et al. 1993). Similarly, it has been shown that the administration of chronic glucocorticoids evoked depression-like symptoms in rodents (Gourley et al. 2007). A HPA axis dysfunction in depression can manifest in a variety of ways, including excessive sensitivity of the adrenal gland to the adrenocorticotropic hormone (ACTH) (Parker et al. 2003), increased secretion of the corticotropin-releasing factor (CRF) (Nemeroff and Owens 2002), or the reduction in negative feedback exerted via the glucocorticoids' receptor (Brown et al. 2004).

Glucocorticoids regulate metabolism and immune system activity and are essential for maintenance, homeostasis, and adaptation to stress, but their long-term increased level or disturbance in the circadian rhythm can have a deleterious effect on the brain's morphology and function. Glucocorticoid receptors are present in many brain regions which are affected by depression, such as the prefrontal cortex (PFC), hippocampus, nucleus accumbens, striatum, limbic system, and ventral midbrain (Herman 1993; Van Craenenbroeck et al. 2005). It was discovered that when corticosterone concentration increases for a long time, it causes neuronal damage in the CA1 and CA3 hippocampal region and enhances neurodegenerative processes evoked by excitatory amino acids, hypoxia, hypoglycemia, seizures, or age. One of the long established changes caused by chronic stress or long-term administration of glucocorticoids is the reorganization of the apical dendrites of pyramidal cells in the CA3 region of the hippocampus. Stress or glucocorticoid treatment induces an extensive reduction of the total dendritic length, a reduction in the distal dendritic branching density, and a reduction of the number of synapses in the hippocampal CA3 region in rats (Magariños et al. 1999; Sousa et al. 2000). Apart from the hippocampus, atrophy of the dendrites was also witnessed in the prefrontal cortex in experimental animals after chronic stress (Magariños and McEwen 1995; Wellman 2001; Duman 2002).

It seems that one of the reasons for hippocampal volume reduction observed in depression is an impairment in the process of neurogenesis. A lot of data indicated that glucocorticoids, stress, and glutamate inhibited neurogenesis in the dentate gyrus of the hippocampus, while physical and mental activity, insulin-like growth factor, serotonin, estradiol, and adrenalectomy enhanced this process in experimental animals. It seems that glucocorticoids and glutamate regulate neurogenesis by a common mechanism, because *N*-methyl-D-aspartate (NMDA) receptor antagonists inhibit corticosterone action, while NMDA activation blocks the effect of adrenalectomy (Cameron et al. 1998). Since glucocorticoids enhance extracellular glutamate concentration in the hippocampus, their inhibitory effect on the process of neurogenesis may result from the enhancement of glutamate activities.

The mechanisms of glucocorticoids' deleterious action on brain cells are unclear, but most probably are connected with enhancing glutamate action, inhibition of astrocyte glucose uptake, and decreasing the synthesis of neurotrophic factors. For example, stress or glucocorticoids reduce synthesis of the brain-derived neurotrophic factor (BDNF), a compound responsible for the growth, maturation and survival of nerve cells as well as for the processes of arborization and brain plasticity. It has been shown that chronic stress decreases the synthesis of BDNF in the hippocampus, whereas antidepressants lead to an increase in its production in the hippocampus and PFC (Shimizu et al. 2003). It has also been found that stress reduces the BDNF and tyrosine kinase B (*TrkB*) (BDNF-specific receptor) genes' expression and phosphorylation of the cyclic AMP response element-binding protein (CREB), which is the transcription factor that regulates the expression of BDNF (Nibuya et al. 1995, 1996; Duman et al. 1997, 2000).

The role of glucocorticoids in the pathogenesis of depression is evidenced by the data indicating the antidepressant effect of GR antagonists and glucocorticoid synthesis inhibitors in animal models (Baez and Volosin 1994; Healy et al. 1999). Clinical data also showed the antidepressant action of glucocorticoid synthesis inhibitors, and clinical trials with specific glucocorticoid and CRF receptor antagonists as potential antidepressants are in progress (Wolkowitz and Reus 1999; Mathew et al. 2008).

5 The Involvement of Enhanced Glutamate Transmission in Neurodegenerative Processes in Depression

The hypothesis that dysfunction of the glutamatergic system may be involved in the pathophysiology and treatment of depression was proposed in 1996 (Skolnick et al. 1996), following several discoveries concerning the importance of the NMDA receptor in antidepressant action (the first article by Trullas and Skolnick evidenced that NMDA receptor functional antagonists exhibit antidepressant-like activity, Trullas and Skolnick 1990). Many studies demonstrated that patients with depression have an elevated glutamate level in the blood (Kim et al. 1982; Altamura et al. 1993) and a positive correlation between the blood glutamate level and the severity of depressive symptoms was discovered (Mauri et al. 1998; Mitani et al. 2006). Similarly, it was

demonstrated that the glutamate level in the cerebrospinal fluid of patients with depression is higher than in the healthy controls (Levine et al. 2000). The disturbance of glutamatergic neurotransmission in depression is also supported by postmortem studies. Alterations in [^{3}H]CGP39653 (an antagonist of the glutamate binding site of the NMDA receptor) binding and its modulation by glycine were demonstrated in both the frontal cortex and hippocampus in suicide/depressed humans (Nowak et al. 1995; Beneyto et al. 2007). Furthermore, a decrease of [^{3}H]L-689,560 binding (an antagonist of the NMDA receptor glycine site), as well as the reduction in the NR1 subunit of NMDA receptor immunoreactivity in the superior temporal cortex of depressed patients was reported (Nudmamud-Thanoi and Reynolds 2004). Also, changes in mRNA/protein concentrations of the glutamate receptor subunits (NMDA, metabotropic) in the brain regions of depressed subjects were described (e.g., Law and Deakin 2001; Feyissa et al. 2009; Deschwanden et al. 2011).

Elevated concentration of extracellular glutamate is considered to be the main reason for the degeneration of neurons and/or cell death observed in neurodegenerative diseases (Hardingham and Bading 2010). Environmental stress, which increases both glutamate and glucocorticoids, has a huge impact on the morphology of the brain tissue and is also a risk factor for mood/anxiety disorders (de Kloet et al. 2005). Studies in rats using the microdialysis technique indicated an increase in extracellular levels of excitatory neurotransmitters (glutamate and aspartate) within the striatum, hippocampus, nucleus accumbens, and medial prefrontal cortex (mPFC) after acute restraint stress (for 20 min). The highest glutamate level increase was observed in the mPFC, when compared to other structures. The same result was obtained in swimming stress, conducted in the same period of time. Glucocorticoids, which inhibit glutamate uptake into glia cells, are probably responsible for an enhanced intracellular glutamate level and, consequently, the increase of excitotoxicity processes. It has been reported that an elevated level of glucocorticoids in the hippocampus and mPFC increased glutamate concentration and that both of these compounds are responsible for the retraction of dendrites in the CA3 region of the hippocampus (McEwen 2005; Pittenger and Duman 2008; Gorman and Docherty 2010). In glutamatergic synapses, the typical mechanisms of the synaptic plasticity affecting their activity are changes in the shape and size of the synaptic shafts and spines (Sorra and Harris 2000). Stress can affect the processes of the synaptic plasticity of various brain structures, and different forms of stress may induce atrophy, retraction, and remodeling of dendrites of the pyramidal neurons in the hippocampal CA3 region (Watanabe et al. 1992; Magariños and McEwen 1995). It has been demonstrated that certain intracellular processes, associated with glutamatergic receptor stimulation, may be involved in the pathophysiology of depression and neuronal degeneration. Stimulation of the NMDA receptor leads to an increase in intracellular calcium concentration in postsynaptic neurons. Ca^{2+} bind to and stimulate the Ca^{2+}/calmodulin-dependent protein kinase, which via action on the nitric oxide synthase (NOS) may then enhance the synthesis of the nitric oxide (II) (Southam and Garthwaite 1993; Li and Poulos 2005). Nitric oxide is a membrane-permeable molecule associated with the processes of cellular signaling and communication in various systems. In the brain, nitric oxide regulates

many physiological processes, including neurotransmission, synaptic plasticity, neuromorphogenesis, gene expression, and the perception of pain and learning (Esplugues 2002), suggesting that its enhanced level may contribute to the etiology of depression. Clinical studies have shown increased immunoreactivity for nNOS in the hippocampal CA1 and subiculum region in patients suffering from major depression and bipolar disorder (Oliveira et al. 2008). Administration of 7-nitroindazole, an inhibitor of neuronal nitric oxide synthase (nNOS), into the dorsal hippocampus, induces an antidepressant-like effect in rats (Joca and Guimarães 2006). Moreover, paroxetine, a selective serotonin reuptake inhibitor, inhibits the activity of constitutive nitric oxide synthase (cNOS) (Finkel 1996).

6 The Involvement of the Immune System in Neurodegenerative Processes in Depression

Patients suffering from depression have a higher incidence of immune system disorders when compared to the total population, and also depression often occurs as a side effect of cytokine therapies that are used in the treatment of hepatitis C, multiple sclerosis, Kaposi's sarcoma, and many other cancer types. Depression occurs most frequently in therapies using IFN-α and IL-2, less frequently during IFN-β administration, and has not been recorded in the case of IFN-γ (Valentine et al. 1998; Gohier et al. 2003). Patients with depression often exhibit increased activity of the immune system, which is reflected in the increase of the level of "positive" acute phase proteins – C-reactive protein (CRP), haptoglobin, ceruloplasmin – as well as in the reduction of the "negative" acute phase proteins' concentration (albumin, transferrin, retinol-binding protein). Depression is also more common in patients suffering from disease connected with immune component disturbances (Kronfol 2002). Studies conducted in experimental animals have shown that administration of lipopolysaccharide (LPS) or IL-1 evoked "sickness behavior" syndrome, which has symptoms that are similar to those observed in depression (Kent et al. 1994). Similarly, administration of LPS to healthy volunteers resulted in the appearance of symptoms of anxiety and depression (Reichenberg et al. 2001). A lot of data especially indicates the important role of TNF-α, IL-1, and IL-6 in the pathogenesis of affective disorders. Under physiological conditions, these cytokines positively affect the central nervous system, through neurogenesis enhancement and trophic action on neurons. However, excessive stimulation of signal transduction pathways through the proinflammatory cytokines in the central nervous system can have a disadvantageous effect (Miller et al. 2009). Peripheral administration of LPS causes an increase in the concentration of TNF-α and IL-1 in the hippocampus, which is associated with a decreased expression of BDNF and TrkB in this brain structure. This leads to a decrease in the neurogenesis processes, impairs cognitive function and may also cause an increase in glutamatergic transmission, enhances oxidative stress, and induces apoptosis of astrocytes and oligodendrocytes, thus, the processes that are most likely involved in the pathogenesis of depression (Wu et al. 2007; Miller et al. 2009). Apart from enhancing neurodegenerative processes, proinflammatory

cytokines can modify the level of neurotransmitters. Animal studies suggest that proinflammatory cytokines alter the turnover of serotonin (5HT) in different brain regions (Dunn et al. 1999), lead to the downregulation of serotonin receptor $5HT_{1A}$ (Cai et al. 2005), and inhibit serotonin synthesis through activation of the indoleamine 2,3-dioxygenase enzyme that converts the tryptophan (precursor of serotonin) into kynurenine (Schwarcz and Pellicciari 2002). Additionally, proinflammatory cytokines, especially IFN-α, increase the kynurenine-3-monooxygenase enzyme and in this way shift kynurenine metabolism toward the synthesis of 3-hydroxykynurenin and quinolinic acid, NMDA receptor agonists which can contribute to the neurotoxic processes observed in depression (Zunszain et al. 2012). Cytokines also affect dopamine neurotransmission by upregulation of the dopamine receptor and an increase of the nitric oxide (II), which in turn inhibits the synthesis of dopamine (Kitagami et al. 2003; Moron et al. 2003). Proinflammatory cytokines were also indicated as modulating the function of the HPA axis. For example, administration of IL-1 and IL-6 enhances CRF secretion and consequently activates the HPA axis (Besedovsky and del Ray 1996). Cytokines can activate the HPA axis acting on the hypothalamus, pituitary, or adrenal cortex and can also modulate glucocorticoid receptor action (Pariante et al. 1999).

7 The Disturbed Glucose Metabolism in Depression

Functional neuroimaging research of depressed patients showed anatomical differences in the glucose metabolism in the brain. In 1985, and for the first time, scientists described reduced glucose metabolism in the prefrontal cortex in bipolar depressives (Baxter et al. 1985). These findings were confirmed in other investigations, which then also demonstrated hypometabolism in unipolar patients (Baxter et al. 1989; Hurwitz et al. 1990; Martinot et al. 1990). Further reports indicate the coexistence of reduced blood flow and impaired glucose metabolism in the prefrontal cortex in patients with a reduced cortical volume (Drevets et al. 1997). Moreover, alterations of brain glucose metabolism are stronger in patients with hyperactivity of the HPA axis, which suggests that the impaired glucose metabolism can result from elevated glucocorticoid action.

On the other hand, numerous studies have shown a correlation between an increased severity of depressive symptoms and increased use of glucose in the subgenual anterior cingulate cortex (Drevets et al. 1999; Osuch et al. 2000), although the opposite result has also been reported (Price and Drevets 2010). It is probable that the increase of glucose metabolism in this brain region is the compensatory mechanism acting at the initial stage of this disease and in unmedicated patients. In another research, an increased perfusion in the medial orbitofrontal cortex was observed during the induction of sadness in healthy controls (Liotti et al. 2002). Glucose hypermetabolism has also been used as a potential indicator of treatment response during the antidepressant therapy (Mayberg et al. 1997, 2000). Most frequently in antidepressant respondents, the increased dorsolateral and decreased ventrolateral prefrontal metabolism have been reported (Kennedy et al. 2007).

Interestingly, researchers have also found a correlation between the amygdala volume and glucose metabolism in the human brain in vivo (Johansen-Berg et al. 2008). Reduction in gray matter volume in the amygdala is probably compensated by an increased glucose metabolism at the beginning of a depressive episode. This prolonged compensation could provoke neuroplastic changes in the brain structure.

8 The Role of Glial Cells in Neurodegenerative Processes in Depression

Changes in the structure or function of neurons may be the result of disturbed action of glial cells (astrocytes, oligodendrocytes, and microglia). Astrocytes regulate many brain functions, including extracellular concentration of ions and neurotransmitters, glucose uptake, synthesis of neurotransmitters, synaptic function, neuronal metabolism, and migration. Astrocytes and microglia are also a source of many neurotrophic factors and cytokines.

Many studies have indicated the role of glial elements in the pathophysiology of various neurodegenerative disorders, including depression (Rajkowska and Miguel-Hidalgo 2007). It has been shown that glial ablation in the PFC evoked by an astrocyte-specific toxin, L-alpha-aminoadipic acid, can sufficiently induce depressive-like behaviors in rodents (Banasr and Duman 2008). Numerous studies reported a decrease of the glial density in various brain structures in depression. For instance, it has been revealed in layers 3 and 5 of the dorsolateral prefrontal cortex and in the deeper layers of the caudal orbitofrontal cortex (Rajkowska et al. 1999). Reduction of glial cell density was also observed in the subgenual part of the anterior cingulate cortex and in the supracallosal part of the anterior cingulate cortex in depressive subjects (Ongur et al. 1998; Cotter et al. 2001). By contrast, in hippocampal CA subfields and in the granule cell layer of the dentate gyrus, the glial cell density was increased in subjects with depression (Stockmeier et al. 2004).

Experimental data indicated that glucocorticoids, which appear to be elevated in depression, reduce the activation of astrocytes in the hippocampus (Sonino et al. 1993; Laping et al. 1994). According to this, concentration of glial fibrillary acidic protein (GFAP), a protein expressed in astrocytes, is also decreased in the hippocampus after chronic psychosocial stress (Czéh et al. 2006). Since a decreased number or function of astrocytes attenuates synaptic glutamate uptake and in the end enhances synaptic glutamate concentration, it may be one of the mechanisms responsible for neurodegenerative changes observed in depression. Glial cells are the main source of proinflammatory cytokines, which then regulate the kynurenine pathway, the main pathway for brain tryptophan metabolism (Schwarcz and Pellicciari 2002). Microglia cells, macrophages, and monocytes produce quinolinic acid, which is an agonist of the NMDA receptor, while astrocytes synthesize kynurenic acid, which is its antagonist (Guillemin et al. 2000, 2001, 2005). Increased quinolinic acid levels were found in blood and cerebrospinal fluid in major depression and in the cytokine-induced animal model of this disease

(Myint et al. 2007; Dantzer et al. 2008; Raison et al. 2010), while higher kynurenic acid production was linked to schizophrenia (Erhardt et al. 2001; Nilsson et al. 2005; Linderholm et al. 2012). In this context, quinolinic acid may be the link between immune and neurotransmitter changes that are specific to depression and involved in neurodegenerative changes (Steiner et al. 2011). Moreover, glial cells are also the source and the target of various cytokines, e.g., interleukins, tumor necrosis factor, whose level appears to be elevated in depression (Müller and Ackenheil 1998; Howren et al. 2009; Dowlati et al. 2010).

An important function of astrocytes is glucose metabolism and providing energy to neurons. The majority of research with positron emission tomography and functional magnetic resonance, which is based on the activities of glucose uptake, glycolysis, and oxidative phosphorylation, indicated hypometabolism in the prefrontal cortex in depression patients. As such, these changes might be evoked by either a reduced number or weaker activation of astrocytes.

Properly functioning glial cells are necessary for the appropriate function of the neurons, including resistance to damage. It has been found that brain injury is associated with activation of astrocytes and manifested by the increase of the GFAP expression (Krohn et al. 1999). Activated astrocytes may promote neuronal survival by enhanced release of trophic factors but, conversely, they may also inhibit the process of regeneration by forming a glial scar.

Microglia, similar to astrocytes, regulate the extracellular concentration of ions and neurotransmitters and via the release of the trophic factor act on neuroprotective/neurodegenerative processes. However, overstimulation of microglia seems to be harmful for brain cells. Microglia is activated mainly in response to immunity stimulation and brain damage, and this process is associated with the transition of microglia cells into the amoeboid form, which promotes phagocytosis and mobility (Streit et al. 1988; Kreutzberg 1996; Streit et al. 1999; Liu and Hong 2003). Activation of microglia, in response to proinflammatory triggers such as LPS (Pei et al. 2007; Qin et al. 2005a, b), β-amyloid (Aβ) (Bamberger and Landreth 2001), and HIV-Tat (Turchan-Cholewo et al. 2009), is associated with the liberation of cytotoxic agents, including TNF-α (Sawada et al. 1989; Lee et al. 1993), inflammatory prostaglandins (Wang et al. 2005), or reactive oxygen species (ROS) such as superoxide ($O2^{\bullet -}$) (Colton and Gilbert 1987) and NO (Moss and Bates 2001; Liu et al. 2002), for the neutralization of pathogens (Oehmichen and Gencic 1975; Graeber et al. 1988). These compounds, besides the hydrogen peroxidase and hydroxyl radicals also produced by microglia, may injure neurons and glial cells (Chao et al. 1992; Boje and Arora 1992). Several studies revealed that antidepressant drugs (selective serotonin reuptake inhibitors, SSRI) modulate the ability of microglia to produce TNF-α and NO in mice (Hashioka et al. 2007, 2009; Hwang et al. 2008; Horikawa et al. 2010). Fluoxetine, sertraline, paroxetine, fluvoxamine, and citalopram have been reported to strongly inhibit the production of TNF-α and NO in LPS-stimulated microglial cells (Tynan et al. 2012). The above reports support the hypothesis of the role of activated microglia in the pathogenesis of depression. Furthermore, activation of microglia and an increased corticosterone level can appear together. In the restraint model of psychological stress in mice, the elevated corticosterone level was accompanied by an increased proliferation of

microglia cells. The inhibition of glucocorticoid synthesis or antagonists of either the glucocorticoid receptor or NMDA receptor resulted in suppression of the stress-induced microglia cell proliferation (Nair and Bonneau 2006).

9 Effects of Antidepressant Drugs on Neurodegenerative Processes in Depression

Currently used antidepressants show a large variation in the mechanism of their action. Most registered drugs are based on the monoaminergic hypothesis of depression. According to this, the effect is acquired by increasing noradrenergic and/or serotonergic transmission through inhibition of monoamine's reuptake, the influence on adrenergic and serotonergic receptors, or inhibition of enzymatic degradation of monoamines. Tricyclic antidepressants, being the oldest group of drugs with a particularly broad spectrum of activity, have a number of adverse effects. The pharmacological action of antidepressants, as well as their toxicity, should be considered always in terms of two processes: changes after a single dose of the substance and the activity of the drug after prolonged use, due to changes in quantity/activity of the receptors, the intracellular molecular changes, and the process of plasticity. In this chapter, we focus on the effect and mechanism of action of antidepressant drugs on neuronal maintenance, the process of neurogenesis, and their influence on the morphological changes that occur during the state of depression. Although some antidepressant drugs exerted neuroprotective action on neurons and glial cells, some of these drugs, especially at higher doses, may exert a cytotoxic effect. For example, amitriptyline, desipramine, and fluoxetine induced in in vitro studies at μM concentrations cell death in hippocampal neurons and PC12 pheochromocytoma cells, and some antidepressants from the TCA and SSRI group induce apoptosis in neurons and glia cells (Španová et al. 1997; Post et al. 2000; Bartholomä et al. 2002).

9.1 Effects of Antidepressants on Proinflammatory Cytokines

The neuropharmacology of tricyclic antidepressants (TCA) cannot be described only in the example of noradrenaline (NA) and 5-HT reuptake inhibition because it has transpired that the profile of these drugs is much broader. The neuroprotective effect of tricyclic antidepressants has been described in the case of imipramine and clomipramine in in vitro studies. It has been shown that these drugs decrease the concentration of nitric oxide, TNFα, and IL-1β, as well as neurotoxic changes, observed in microglia cells and astrocytes stimulated with LPS (Hwang et al. 2008). This data is in line with the inflammatory and neurodegenerative theory of depression, whereby an increased level of proinflammatory cytokines and reactive oxygen species is purportedly involved in the pathogenesis of depression and the inhibition of microglia activation in the brain has a neuroprotective effect (e.g., Hwang et al. 2008; Maes et al. 2012). Similarly, it was reported that fluvoxamine, reboxetine, and imipramine inhibited the production of

NO and IL-6 by microglial cells activated with IFN-γ (Hashioka et al. 2007). In the in vivo experiment, it has been found that a 2-week administration of paroxetine inhibited, when stimulated by IFNα, IL-1β and IL-10 concentrations in the hypothalamus, while desipramine decreased the IL-1β and TNFα concentration in the rat cortex after LPS administration (Myint et al. 2007; O'Sullivan et al. 2009). When stimulated by LPS, the production of proinflammatory cytokines was also inhibited by an atypical antidepressant – tianeptine, but only in the hypothalamus and not in the hippocampus (Castanon et al. 2004). These studies indicated that antidepressant drugs showing a different action on monoamines inhibit the synthesis of proinflammatory cytokines.

9.2 Effects of Antidepressant Drugs on Neurogenesis

Recently, a lot of data has indicated that depression is associated not only with the process of neurodegeneration but also with reduced neurogenesis in the brain. Although the function of new neurons generated in the adult mammalian brain, especially in the dentate gyrus of the hippocampus, is poorly established, studies in animals suggest a relation between neurogenesis and some hippocampus-dependent learning task. Experimental data indicated that the administration of long-term antidepressants increases hippocampal neurogenesis and prevents the fall in this process induced by different factors, most often by various kinds of stress. For example, in chronic mild stress – an animal model of depression – a decrease in the total number of granule cells in the ventral hippocampus was observed, and long-term treatment with escitalopram blocked this reduction (Jayatissa et al. 2006). Also, in the olfactory bulbectomy model of depression, citalopram reversed inhibition of the new cells' creation in the dentate gyrus of the hippocampus (Jaako-Movits et al. 2006). Different classes of antidepressants (selective serotonin reuptake inhibitors, selective noradrenaline reuptake inhibitors, monoamine oxidase inhibitors, atypical antidepressants) and electroconvulsive seizures have been found to increase neurogenesis acting on the process of proliferation or survival of newborn neurons.

9.3 Effects of Antidepressant Drugs on the Brain Derived Neurotrophic Factor

The BDNF regulates the growth, survival, and function of brain cells and is involved in both the formation of memory traces and learning process. Some data has evidenced the involvement of BDNF in the pathogenesis of depression and in the action of antidepressant drugs. Chronic stress and glucocorticoids decreased BDNF expression in the hippocampus, whereas antidepressant drugs increase its concentration or prevent the stress effect. For example, it has been shown that in an animal model of chronic unpredictable stress, venlafaxine, mirtazapine, and fluoxetine raised the level of the BDNF in the hippocampus (Zhang et al. 2010). Also, desipramine, sertraline, and tianeptine, in some experimental models, increase the

levels of BDNF. Chronic treatment with various antidepressant drugs also increases the expression or activity of TrkB (BDNF receptor), and BDNF/TrkB-signaling seems to be employed not only in the survival and differentiation of neurons but also in depression's etiology and the action of antidepressant drugs. It was revealed that in the TrkB knockout animals, antidepressant therapy has no effect, whereas application of BDNF into the hippocampus exerted an antidepressant-like effect (Shirayama et al. 2002; Saarelainen et al. 2003). In patients with depression, decreased levels of BDNF in the blood were normalized after long-term escitalopram treatment (Serra-Millàs et al. 2011). Also, postmortem studies showed decreased brain BDNF concentration in unmedicated depressed subjects (Pittenger and Duman 2008). A lot of data, but not all, indicated that BDNF is an important factor in the prevention of neuronal degeneration, the inhibition of neurogenesis, and may be essential for the action of antidepressants.

9.4 Effects of Antidepressant Drugs on the Atrophy of Hippocampal Neurons

Postmortem studies show that patients with depression have a significant atrophy of neuronal cells in the hippocampus (particularly area CA3 pyramidal neurons) and in the amygdala. In experimental animals, neurodegeneration is reversed after the application of antidepressant drugs belonging to different classes, such as fluoxetine, imipramine, tranylcypromine, rolipram, and reboxetine, plus also following electroconvulsive treatment or transcranial magnetic stimulation (Madsen et al. 2000; Mayberg et al. 2000; Czéh et al. 2002; Duman et al. 2004). In the inhibition of stress- or glucocorticoid-induced hippocampal neurodegeneration, tianeptine especially exerts a protective function. This drug inhibits stress- or glucocorticoid-induced atrophy of apical dendrites of the hippocampal Ca3 pyramidal neurons (Magariños and McEwen 1995). Also, a stress-related decrease in the hippocampal volume, inhibition of cell proliferation, and a decreased level of *N*-acetylaspartate in the hippocampus and amygdala were prevented by tianeptine (Czéh et al. 2001; McEwen and Chattarji 2004). Tianeptine is an atypical antidepressant drug, which in contrast to other antidepressants increases serotonin uptake, and its neuroprotective action perhaps results from both the inhibition of NMDA receptor function and the increasing serotonin reuptake in the hippocampus, monoamine, whose release in the hippocampus is increased under many types of stress (Kole et al. 2002).

10 Zinc Deficiency-Induced Neurodegeneration in Depression

Recently, much attention has been paid to the role of zinc in the pathophysiology and treatment of depression (Szewczyk et al. 2010; Szewczyk et al. 2011b). The first strong evidence indicating hypozincemia in human depression was demonstrated by Maes and co-workers (Maes et al. 1994). On the other hand, zinc exhibits

antidepressant activity in preclinical and clinical studies (Szewczyk et al. 2010, 2011a, b). Zinc deficiency is currently examined in experimentally induced depression in rodents (by a zinc deficient diet) and assessed in humans (e.g., Szewczyk et al. 2011a, b). Zinc deficiency induces depressive-like behavior and biochemical alterations similar to that observed in the animal models of depression (resembling human depression). The biochemical changes include reductions of brain BDNF, NGF concentrations, enhanced activity of the NMDA receptor, increased glucocorticoid and glutamate actions, and reduced neurogenesis processes (e.g., Szewczyk et al. 2011a). Examinations of zinc deficiency as a model of depression are in progress.

11 Conclusion

Major depression is one of the most common psychiatric disorders whose etiology, despite long and intensive studies, remains unclear. Because about 30 % of patients do not respond to the therapy with currently available antidepressants, there is a great need to investigate new hypotheses explaining the cause of depression, plus create new drugs. Previous research on depression was focused on changes in the concentration of neurotransmitters and their receptors. Actually, increasing evidence indicates that an impaired neuronal plasticity, especially observed in the hippocampus and frontal cortex, plays a role in the pathogenesis of depression. In turn, the disruption of cellular mechanisms regulating neuronal plasticity is most often connected with prolonged, damaging effects of the glucocorticoids, glutamate, and proinflammatory cytokines. Thus, it seems that in the pathophysiology of depression, all of the main systems involved in homeostasis maintenance, namely, neurotransmitters, glucocorticoids, and cytokines, interact in a complex way. To study the role of one factor in the pathogenesis of depression is relatively easy, while the influence of several compounds and the determination of their interactions remain difficult. For example, even the interaction between glucocorticoids and proinflammatory cytokines is complex and depends on the duration of action and their actual concentration, plus involves the inhibitory action of glucocorticoids on cytokine synthesis, modulation of the cytokine receptor function, the stimulatory effect of some cytokines on cortisol synthesis, or their influence on glucocorticoid receptors. Moreover, it must be noted that the role of glucocorticoids, cytokines, and glutamate in the pathogenesis of depression has not yet been fully determined and that other factors may be also involved, while, furthermore, the pathogenesis of depression should not be connected only with cell neurodegeneration/death.

References

Altamura, C. A., Mauri, M. C., Ferrara, A., Moro, A. R., D'Andrea, G., & Zamberian, F. (1993). Plasma and platelet excitatory amino acids in psychiatric disorders. *American Journal of Psychiatry, 150*, 1713–1731.

Bachis, A., Cruz, M. I., Nosheny, R. L., & Mocchetti, I. (2008). Chronic unpredictable stress promotes neuronal apoptosis in the cerebral cortex. *Neuroscience Letters, 442*, 104–108.

Baez, M., & Volosin, M. (1994). Corticosterone influence forced swim-induced immobility. *Pharmacology, Biochemistry and Behavior, 49*, 729–736.

Bamberger, M. E., & Landreth, G. E. (2001). Microglial interaction with beta-amyloid: Implications for the pathogenesis of Alzheimer's disease. *Microscopy Research and Technique, 54*, 59–70.

Banasr, M., & Duman, R. S. (2008). Glial loss in the prefrontal cortex is sufficient to induce depressive- like behaviors. *Biological Psychiatry, 64*, 863–870.

Bartholomä, P., Erlandsson, N., Kaufmann, K., Rössler, O. G., Baumann, B., Wirth, T., Giehl, K. M., & Thiel, G. (2002). Neuronal cell death induced by antidepressants: Lack of correlation with Egr-1, NF-κB and extracellular signal-regulated protein kinase activation. *Biochemical Pharmacology, 63*, 1507–1516.

Baumann, B., Bornschlegl, C., Krell, D., & Bogerts, B. (1997). Changes in CSF spaces differ in endogenous and neurotic depression a planimetric CT scan study. *Journal of Affective Disorders, 45*, 179–188.

Baxter, L. R., Jr., Phelps, M. E., Mazziotta, J. C., Schwartz, J. M., Selin, C. E., & Sumida, R. M. (1985). Cerebral metabolic rates for glucose in mood disorders. Studies with positron emission tomography and fluorodeoxyglucose F 18. *Archives of General Psychiatry, 42*, 441–447.

Baxter, L. R., Jr., Schwartz, J. M., Phelps, M. E., Mazziotta, J. C., Guze, B. H., Selin, C. E., Gerner, R. H., & Sumida, R. M. (1989). Reduction of prefrontal cortex glucose metabolism common to three types of depression. *Archives of General Psychiatry, 46*, 243–250.

Beneyto, M., Kristiansen, L. V., Oni-Orisan, A., McCullumsmith, R. E., & Meador-Woodruff, J. H. (2007). Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. *Neuropsychopharmacology, 32*, 1888–1902.

Bernstein, H. G., Krause, S., Klix, M., Steiner, J., Dobrowolny, H., & Bogerts, B. (2008). Structural changes of mammillary bodies are different in schizophrenia and bipolar disorder. *Schizophrenia Research, 98*, 7–8.

Berton, O., & Nestler, E. J. (2006). New approaches to antidepressant drug discovery: Beyond monoamines. *Nature Reviews Neuroscience, 7*, 137–151.

Besedovsky, H. O., & del Ray, A. (1996). Immune-neuro-endocrine interactions: Facts and hypothesis. *Endocrine Reviews, 17*, 64–102.

Boje, K. M., & Arora, P. K. (1992). Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. *Brain Research, 587*, 250–256.

Bremner, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., & Charney, D. S. (2000). Hippocampal volume reduction in major depression. *American Journal of Psychiatry, 157*, 115–118.

Bremner, J. D., Vythilingam, M., Vermetten, E., Nazeer, A., Adil, J., Khan, S., Staib, L. H., & Charney, D. S. (2002). Reduced volume of orbitofrontal cortex in major depression. *Biological Psychiatry, 51*, 273–279.

Brown, E. S., Varghese, F. P., & McEwen, B. S. (2004). Association of depression with medical illness: Does cortisol play a role? *Biological Psychiatry, 55*, 1–9.

Brown, G. W., & Harris, T. (1978). *Social origin of depression: A study of psychiatric disorder in women*. London: Tavistock.

Caetano, S. C., Hatch, J. P., Brambilla, P., Sassi, R. B., Nicoletti, M., Mallinger, A. G., Frank, E., Kupfer, D. J., Keshavan, M. S., & Soares, J. C. (2004). Anatomical MRI study of hippocampus and amygdala in patients with current and remitted major depression. *Psychiatry Research, 132*, 141–147.

Cai, W., Khaoustov, V. I., Xie, Q., Pan, T., Le, W., & Yoffe, B. (2005). Interferon-alpha-induced modulation of glucocorticoid and serotonin receptors as a mechanism of depression. *Journal of Hepatology, 42*, 880–887.

Cameron, H. A., Tanapat, P., & Gould, E. (1998). Adrenal steroids and *N*-methyl-D-aspartate receptor activation regulate neurogenesis in the dentate gyrus of adult rats through a common pathway. *Neuroscience, 82*, 349–354.

Carroll, B. J. (1982). The Dexamethasone suppression test for melancholia. *British Journal of Psychiatry, 140*, 292–304.

Castanon, N., Médina, C., Mormède, C., & Dantzer, R. (2004). Chronic administration of tianeptine balances lipopolysaccharide-induced expression of cytokines in the spleen and hypothalamus of rats. *Psychoneuroendocrinology, 29*, 778–790.

Cerqueira, J. J., Catania, C., Sotiropoulos, I., Schubert, M., Kalish, R., Almeida, O. F. X., Auer, D. P., & Sousa, N. (2005). Corticosteroid status influences the volume of the rat cingulate cortex – a magnetic resonance imaging study. *Journal of Psychiatric Research, 39*, 451–460.

Chao, C. C., Hu, S., Molitor, T. W., Shaskan, E. G., & Peterson, P. K. (1992). Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. *Journal of Immunology, 149*, 2736–2741.

Charney, D. S., Menkes, D. B., & Heninger, G. R. (1981). Receptor sensitivity and the mechanism of action of antidepressant treatment. Implications for the etiology and therapy of depression. *Archives of General Psychiatry, 38*, 1160–1180.

Colton, C. A., & Gilbert, D. L. (1987). Production of superoxide anions by a CNS macrophage, the microglia. *FEBS Letters, 223*, 284–288.

Cotter, D., Mackay, D., Landau, S., Kerwin, R., & Everall, I. (2001). Reduced glial cell density and neuronal volume in major depression in the anterior cingulate cortex. *Archives of General Psychiatry, 58*, 545–553.

Czéh, B., Michaelis, T., Watanabe, T., Frahm, J., de Biurrun, G., van Kampen, M., Bartolomucci, A., & Fuchs, E. (2001). Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. *Proceedings of the National Academy of Sciences, 98*, 12796–12801.

Czéh, B., Welt, T., Fischer, A. K., Erhardt, A., Schmitt, W., Müller, M. B., Toschi, N., Fuchs, E., & Keck, M. E. (2002). Chronic psychosocial stress and concomitant repetitive transcranial magnetic stimulation: Effects on stress hormone levels and adult hippocampal neurogenesis. *Biological Psychiatry, 52*, 1057–1065.

Czéh, B., Simon, M., Schmelting, B., Hiemke, C., & Fuchs, E. (2006). Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology, 31*, 1616–1626.

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: When the immune system subjugates the brain. *Nature Reviews Neuroscience, 9*, 46–56.

Dasari, M., Friedman, L., Jesberger, J., Stuve, T. A., Findling, F. L., Swales, T. P., & Schulz, S. C. (1999). A magnetic resonance imaging study of thalamic area in adolescent patients with either schizophrenia or bipolar disorder as compared to healthy controls. *Psychiatry Research, 91*, 155–162.

de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience, 6*, 463–475.

Deschwanden, A., Karolewicz, B., Feyissa, A. M., Treyer, V., Ametamey, S. M., Johayem, A., Burger, C., Auberson, Y. P., Sovago, J., Stockmeier, C. A., Buck, A., & Hasler, G. (2011). Reduced metabotropic glutamate receptor 5 density in major depression determined by [(11)C] ABP688 PET and postmortem study. *American Journal of Psychiatry, 168*, 727–734.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., & Lanctot, K. L. (2010). A meta-analysis of cytokines in major depression. *Biological Psychiatry, 67*, 446–457.

Drevets, W. C., Price, J. L., Simpson, J. R., Todd, R. D., Reich, T., Vannier, M., & Raichle, M. E. (1997). Subgenual prefrontal cortex abnormalities in mood disorders. *Nature, 386*, 824–827.

Drevets, W. C., Gadde, K., & Krishnan, R. (1999). *Neuroimaging studies of depression*. New York: Oxford University Press.

Duman, R. S. (2002). Pathophysiology of depression: The concept of synaptic plasticity. *European Psychiatry, 17*, 306–310.

Duman, R. S., Heninger, G. R., & Nestler, E. J. (1997). A molecular and cellular theory of depression. *Archives of General Psychiatry, 54*, 597–606.

Duman, R. S., Malberg, J., Nakagawa, S., & D'Sa, C. (2000). Neuronal plasticity and survival in mood disorders. *Biological Psychiatry, 48*, 732–739.

Duman, R. S., Kodama, M., & Fujioka, T. (2004). Chronic olanzapine or fluoxetine administration increases cell proliferation in hippocampus and prefrontal cortex of adult rat. *Biological Psychiatry, 56*, 570–580.

Dunn, A. J., Wang, J., & Ando, T. (1999). Effects of cytokines on cerebral neurotransmission. Comparison with the effects of stress. *Advances in Experimental Medicine and Biology, 46*, 1117–1127.

Erhardt, S., Blennow, K., Nordin, C., Skogh, E., Lindstrom, L. H., & Engberg, G. (2001). Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neuroscience Letters, 313*, 96–98.

Esplugues, J. V. (2002). NO as a signalling molecule in the nervous system. *British Journal of Pharmacology, 135*, 1079–1095.

Feyissa, A. M., Chandran, A., Stockmeier, C. A., & Karolewicz, B. (2009). Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 33*, 70–75.

Finkel, M. S. (1996). Paroxetine is a novel nitric oxide synthase inhibitor. *Psychopharmacology Bulletin, 32*, 653–658.

Freis, E. (1954). Mental depression in hypertensives treated for long periods with large doses of reserpine. *New England Journal of Medicine, 251*, 1006–1008.

Frodl, T., Meisenzahl, E. M., Zetzsche, T., Born, C., Groll, C., Jäger, M., Leinsinger, G., Bottlender, R., Hahn, K., & Möller, H. G. (2002a). Hippocampal changes in patients with a first episode of major depression. *American Journal of Psychiatry, 159*, 1112–1118.

Frodl, T., Meisenzahl, E., Zetzsche, T., Bottlender, R., Born, C., Groll, C., Jäger, M., Leinsinger, G., Hahn, K., & Möller, H. G. (2002b). Enlargement of the amygdala in patients with a first episode of major depression. *Biological Psychiatry, 51*, 708–714.

Frodl, T., Schaub, A., Banac, S., Charypar, M., Jäger, M., Kümmler, P., Bottlender, R., Zetzsche, T., Born, C., Leisinger, G., Reiser, M., Möller, H. J., & Meisenzahal, E. M. (2006). Reduced hippocampal volume correlates with executive dysfunctioning in major depression. *Journal of Psychiatry and Neuroscience, 31*, 316–325.

Gohier, B., Goeb, J. L., Rannou-Dubas, K., Fouchard, I., Cales, P., & Garre, J. B. (2003). Hepatitis C, alpha interferon, anxiety and depression disorders: A prospective study of 71 patients. *World Journal of Biological Psychiatry, 4*, 115–118.

Gorman, J. M., & Docherty, J. P. (2010). A hypothesized role for dendritic remodeling in the etiology of mood and anxiety disorders. *Journal of Neuropsychiatry and Clinical Neurosciences, 22*, 256–264.

Gourley, S. L., Wu, F. J., Kiraly, D. D., Ploski, J. E., Kedves, A. T., Duman, R. S., & Taylor, J. R. (2007). Regionally specific regulation of ERK MAP kinase in a model of antidepressant – sensitive chronic depression. *Biological Psychiatry, 63*, 353–359.

Graeber, M. B., Streit, W. J., & Kreutzberg, G. W. (1988). The microglial cytoskeleton: Vimentin is localized within activated cells in situ. *Journal of Neurocytology, 17*, 573–580.

Guillemin, G. J., Smith, D. G., Kerr, S. J., Smythe, G. A., Kapoor, V., Armati, P. J., & Brew, B. J. (2000). Characterisation of kynurenine pathway metabolism in human astrocytes and implications in neuropathogenesis. *Redox Report, 5*, 108–111.

Guillemin, G. J., Kerr, S. J., Smythe, G. A., Smith, D. G., Kapoor, V., Armati, P. J., Croitoru, J., & Brew, B. J. (2001). Kynurenine pathway metabolism in human astrocytes: A paradox for neuronal protection. *Journal of Neurochemistry, 78*, 842–853.

Guillemin, G. J., Smythe, G., Takikawa, O., & Brew, B. J. (2005). Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. *Glia, 49*, 15–23.

Hardingham, G. E., & Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signaling; implications for neurodegenerative disorders. *Nature Reviews Neuroscience, 11*, 682–696.

Hashioka, S., Klegeris, A., Monji, A., Kato, T., Sawada, M., McGeer, P. L., & Kanba, S. (2007). Antidepressants inhibit interferon-gamma-induced microglial production of IL-6 and nitric oxide. *Experimental Neurology, 206*, 33–42.

Hashioka, S., McGeer, P. L., Monji, A., & Kanba, S. (2009). Anti-inflammatory effects of antidepressants: Possibilities for preventives against Alzheimer's disease. *Central Nervous System Agents in Medicinal Chemistry, 9*, 12–19.

Hasler, G. (2010). Pathophysiology of depression: Do we have any solid evidence of interest to clinicians? *World Psychiatry, 9*, 155–161.

Hastings, R. S., Parsey, R. V., Oquendo, M. A., Arango, V., & Mann, J. (2004). Volumetric analysis of the prefrontal cortex, amygdala, and hippocampus in major depression. *Neuropsychopharmacology, 29*, 952–959.

Healy, D. G., Harkin, A., Cryan, J. F., Kelly, J. P., & Leonard, B. E. (1999). Metyrapone displays antidepressant-like properties in preclinical paradigms. *Psychopharmacology, 145*, 303–308.

Herman, J. P. (1993). Regulation of adrenocorticosteroid receptor mRNA expression in the central nervous system. *Cellular and Molecular Neurobiology, 13*, 349–372.

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., & Cullinan, W. E. (2003). Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Frontiers in Neuroendocrinology, 24*, 151–180.

Horikawa, H., Kato, T. A., Mizoguchi, Y., Monji, A., Seki, Y., Ohkuri, T., Gotoh, L., Yonaha, M., Ueda, T., Hashioka, S., & Kanba, S. (2010). Inhibitory effects of SSRIs on IFN gamma induced microglial activation through the regulation of intracellular calcium. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 34*, 1306–1316.

Howren, M. B., Lamkin, D. M., & Suls, J. (2009). Associations of depression with C-reactive protein, IL-1, and IL-6: A meta-analysis. *Psychosomatic Medicine, 71*, 171–186.

Hurwitz, T. A., Clark, C., Murphy, E., Klonoff, H., Martin, W. R., & Pate, B. D. (1990). Regional cerebral glucose metabolism in major depressive disorder. *Canadian Journal of Psychiatry, 35*, 684–688.

Hwang, J., Zheng, L. T., Ock, J., Lee, M. G., Kim, S. H., Lee, H. W., Lee, W. H., Park, H. C., & Suk, K. (2008). Inhibition of glial inflammatory activation and neurotoxicity by tricyclic antidepressants. *Neuropharmacology, 55*, 826–834.

Jaako-Movits, K., Zharkovsky, T., Pedersen, M., & Zharkovsky, A. (2006). Decreased hippocampal neurogenesis following olfactory bulbectomy is reversed by repeated Citalopram administration. *Cellular and Molecular Neurobiology, 26*, 1559–1570.

Jayatissa, M. N., Bisgaard, C., Tingström, A., Papp, M., & Wiborg, O. (2006). Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. *Neuropsychopharmacology, 31*, 2395–2404.

Joca, S. R., & Guimarães, F. S. (2006). Inhibition of neuronal nitric oxide synthase in the rat hippocampus induces antidepressant-like effects. *Psychopharmacology, 185*, 298–305.

Johansen-Berg, H., Gutman, D. A., Behrens, T. E., Matthews, P. M., Rushworth, M. F., Katz, E., Lozano, A. M., & Mayberg, H. S. (2008). Anatomical connectivity of the subgenual cingulate region targeted with deep brain stimulation for treatment-resistant depression. *Cerebral Cortex, 18*, 1374–1383.

Kellner, C. H., Rubinow, D. R., & Post, R. M. (1986). Cerebral ventricular size and cognitive impairment in depression. *Journal of Affective Disorders, 10*, 215–219.

Kennedy, S. H., Konarski, J. Z., Segal, Z. V., Lau, M. A., Bieling, P. J., McIntyre, R. S., & Mayberg, H. S. (2007). Differences in brain glucose metabolism between responders to CBT and venlafaxine in a 16-week randomized controlled trial. *American Journal of Psychiatry, 164*, 778–788.

Kent, S., Rodriguez, F., Kelley, K. W., & Dantzer, R. (1994). Reduction in food and water intake induced by microinjection of interleukin-1β in the ventromedial hypothalamus of the rat. *Physiology and Behavior, 56*, 1031–1036.

Kim, J. S., Schmid-Burgk, W., Claus, D., & Kornhuber, H. H. (1982). Increased serum glutamate in depressed patients. *Archives of Psychiatry and Neurological Sciences, 232*, 299–304.

Kitagami, T., Yamada, K., Miura, H., Hashimoto, R., Nabeshima, T., & Ohta, T. (2003). Mechanism of systemically injected interferon-alpha impeding monoamine biosynthesis in rats: Role of nitric oxide as a signal crossing the blood–brain barrier. *Brain Research, 978*, 104–114.

Kole, M. H. P., Swan, L., & Fuchs, E. (2002). The antidepressant tianeptine persistently modulates glutamate receptor currents of the hippocampal CA3 commissural associational synapse in chronically stressed rats. *European Journal of Neuroscience, 16*, 807–816.

Kosten, T. A., Galloway, M. P., Duman, R. S., Russell, D. S., & D'Sa, C. (2008). Repeated unpredictable stress and antidepressants differentially regulate expression of the Bcl-2 family of apoptotic genes in rat cortical, hippocampal, and limbic brain structures. *Neuropsychopharmacology, 33*, 1545–1558.

Kreutzberg, G. W. (1996). Microglia: A sensor for pathological events in the CNS. *Trends in Neurosciences, 19*, 312–318.

Krohn, K., Rozovsky, I., Wals, P., Teter, B., Anderson, C. P., & Finch, C. E. (1999). Glial fibrillary acidic protein transcription responses to transforming growth factor-b1 and interleukin-1b Are mediated by a nuclear factor-1-like site in the near-upstream promoter. *Journal of Neurochemistry, 72*, 1353–1361.

Kronfol, Z. (2002). Immune dysregulation in major depression: A critical review of existing evidence. *International Journal of Neuropsychopharmacology, 5*, 333–343.

Lacerda, A. L. T., Keshavan, M. S., Hardan, A. Y., Yorbik, O., Brambilla, P., Sassi, R. B., Nicoletti, M. A., Mallinger, A. G., Frank, E., Kupfer, D. J., & Soares, J. C. (2004). Anatomic evaluation of the orbitofrontal cortex in major depressive disorder. *Biological Psychiatry, 55*, 353–358.

Lange, C., & Irle, E. (2004). Enlarged amygdala volume and reduced hippocampal volume in young women with major depression. *Psychological Medicine, 34*, 1059–1064.

Laping, N. J., Teter, B., Nichols, N. R., Rozovbsky, I., & Finch, C. E. (1994). Glial fibrillary acidic protein: Regulation by hormones, cytokines and growth factors. *Brain Pathology, 1*, 259–275.

Law, A. J., & Deakin, J. F. (2001). Asymmetrical reductions of hippocampal NMDAR1 glutamate receptor mRNA in the psychoses. *NeuroReport, 12*, 2971–2974.

Ledoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155–184.

Lee, S. C., Liu, W., Dickson, D. W., Brosnan, C. F., & Berman, J. W. (1993). Cytokine production by human fetal microglia and astrocytes, differential induction by lipopolysaccharide and IL-1 beta. *Journal of Immunology, 150*, 2659–2667.

Lee, H. J., Kim, J. W., Yim, S. V., Kim, M. J., Kim, S. A., Kim, Y. J., Kim, C. J., & Chung, J. H. (2001). Fluoxetine enhances cell proliferation and prevents apoptosis in dentate gyrus of maternally separated rats. *Molecular Psychiatry, 6*, 725–728.

Lemaire, V., Koehl, M., Le Moal, M., & Abrous, D. N. (2000). Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 11032–11037.

Levine, J., Panchalingam, K., Rapoport, A., Gershon, S., McClure, R. J., & Pettegrew, J. W. (2000). Increased cerebrospinal fluid glutamine levels in depressed patients. *Biological Psychiatry, 47*, 586–593.

Li, H., & Poulos, T. L. (2005). Structure-function studies on nitric oxide synthases. *Journal of Inorganic Biochemistry, 99*, 293–305.

Linderholm, K. R., Skogh, E., Olsson, S. K., Dahl, M. L., Holtze, M., Engberg, G., Samuelsson, M., & Erhardt, S. (2012). Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. *Schizophrenia Bulletin, 38*, 426–432.

Liotti, M., Mayberg, H. S., McGinnis, S., Brannan, S. L., & Jerabek, P. (2002). Unmasking disease-specific cerebral blood flow abnormalities: Mood challenge in patients with remitted unipolar depression. *American Journal of Psychiatry, 159*, 1830–1840.

Liu, B., & Hong, J. S. (2003). Role of microglia in inflammation-mediated neurodegenerative diseases:Mechanisms and strategies for therapeutic intervention. *Journal of Pharmacology and Experimental Therapeutics, 304*, 1–7.

Liu, B., Gao, H. M., Wang, J. Y., Jeohn, G. H., Cooper, C. L., & Hong, J. S. (2002). Role of nitric oxide in inflammation mediated neurodegeneration. *Annals of the New York Academy of Sciences, 962*, 318–331.

Lucassen, P. J., Vollmann-Honsdorf, G. K., Gleisberg, M., Czéh, B., De Kloet, E. R., & Fuchs, E. (2001). Chronic psychosocial stress differentially affects apoptosis in hippocampal subregions and cortex of the adult tree shrew. *European Journal of Neuroscience, 14*, 161–166.

MacQueen, G. M., Campbell, S., McEwen, B. S., MacDonald, K., Amano, S., Joffe, R. T., Nahmias, C., & Young, L. T. (2003). Course of illness, hippocampal function, and. Hippocampal volume in major depression. *Proceedings of the National Academy of Sciences of the United States of America, 3*, 1387–1392.

Madsen, T. M., Greisen, M. H., Nielsen, S. M., Bolwig, T. G., & Mikkelsen, J. D. (2000). Electroconvulsive stimuli enhance both Neuropeptide Y receptor Y1 and Y2 messenger RNA expression and levels of binding in the rat hippocampus. *Neuroscience, 98*, 33–39.

Maes, M., D'Haese, P. C., Scharpé, S., D'Hondt, P., Cosyns, P., & De Broe, M. E. (1994). Hypozincemia in depression. *Journal of Affective Disorders, 31*, 135–140.

Maes, M., Fisar, Z., Medina, M., Scapagnini, G., Nowak, G., & Berk, M. (2012). New drug targets in depression: Inflammatory, cell-mediated immune, oxidative and nitrosative stress, mitochondrial, antioxidant, and neuroprogressive pathways. And new drug candidates – Nrf2 activators and GSK-3 inhibitors. *Inflammopharmacology, 20*, 127–150.

Magariños, A. M., & McEwen, B. S. (1995). Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience, 69*, 89–98.

Magariños, A. M., Deslandes, A., & McEwen, B. S. (1999). Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. *European Journal of Pharmacology, 371*, 113–122.

Martinot, J. L., Hardy, P., Feline, A., Huret, J. D., Mazoyer, B., Attar-Levy, D., Pappata, S., & Syrota, A. (1990). Left prefrontal glucose hypometabolism in the depressed state: A confirmation. *American Journal of Psychiatry, 147*, 1313–1317.

Mathew, S. J., Manji, H. K., & Charney, D. S. (2008). Novel drugs and therapeutic targets for severe mood disorders. *Neuropsychopharmacology, 33*, 2080–2092.

Mauri, M. C., Ferrara, A., Boscati, L., Bravin, S., Zamberlan, F., Alecci, M., & Invernizzi, G. (1998). Plasma and platelet amino acid concentrations in patients affected by major depression and under fluvoxamine treatment. *Neuropsychobiology, 37*, 124–129.

Mayberg, H. S., Brannan, S. K., Mahurin, R. K., Jerabek, P. A., Brickman, J. S., Tekell, J. L., Silva, J. A., McGinnis, S., Glass, T. G., Martin, C. C., & Fox, P. T. (1997). Cingulate function in depression: A potential predictor of treatment response. *Neuroreport, 8*, 1057–1061.

Mayberg, H. S., Brannan, S. K., Tekell, J. L., Silva, J. A., Mahurin, R. K., McGinnis, S., & Jerabek, P. A. (2000). Regional metabolic effects of fluoxetine in major depression: Serial changes and relationship to clinical response. *Biological Psychiatry, 48*, 830–843.

McEwen, B. S. (1999). Stress and hippocampal plasticity. *Annual Review of Neuroscience, 22*, 105–122.

McEwen, B. S. (2005). Glucocorticoids, depression, and mood disorders: Structural remodeling in the brain. *Metabolism, 54*, 20–23.

McEwen, B. S., & Chattarji, S. (2004). Molecular mechanisms of neuroplasticity and pharmacological implications: The example of tianeptine. *European Neuropsychopharmacology, 14*, 497–502.

Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and its discontents: The role of cytokines in the pathophysiology of major depression. *Biological Psychiatry, 65*, 732–741.

Mitani, H., Shirayama, Y., Yamada, T., Maeda, K., Ashby, C. R., Jr., & Kawahara, R. (2006). Correlation between plasma levels of glutamate, alanine and serine with severity of depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 30*, 1155–1158.

Moron, J. A., Zakharova, I., Ferrer, J. V., Merrill, G. A., Hope, B., Lafer, E. M., Lin, Z. C., Wang, J. B., Javitch, J. A., Galli, A., & Shippenberg, T. S. (2003). Mitogen-activated protein kinase regulates dopamine transporter surface expression and dopamine transport capacity. *Journal of Neuroscience, 23*, 8480–8488.

Moss, D. W., & Bates, T. E. (2001). Activation of murine microglial cell lines by lipopolysaccharide and interferon-gamma causes NO-mediated decreases in mitochondrial and cellular function. *European Journal of Neuroscience, 13*, 529–538.

Müller, N., & Ackenheil, M. (1998). Psychoneuroimmunology and the cytokine action in the CNS: Implications for psychiatric disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 22*, 1–33.

Murmu, M. S., Salomon, S., Biala, Y., Weinstock, M., Braun, K., & Bock, J. (2006). Changes of spine density and dendritic complexity in the prefrontal cortex in offspring of mothers exposed to stress during pregnancy. *European Journal of Neuroscience, 24*, 1477–1487.

Myint, A. M., O'Mahony, S., Kubera, M., Kim, Y. K., Kenny, C., Kaim-Basta, A., Steinbusch, H. W., & Leonard, B. E. (2007). Role of Paroxetine in interferon-alpha-induced immune and behavioural changes in male Wistar rats. *Psychopharmacology, 21*, 843–850.

Nair, A., & Bonneau, R. H. (2006). Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. *Journal of Neuroimmunology, 171*, 72–85.

Nemeroff, C. B., & Owens, M. J. (2002). Treatment of mood disorders. *Nature Neuroscience, 5*, 1068–1070.

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of depression. *Neuron, 34*, 13–25.

Nibuya, M., Morinobu, S., & Duman, R. S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *Journal of Neuroscience, 15*, 7539–7547.

Nibuya, M., Nestler, E. J., & Duman, R. S. (1996). Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *Journal of Neuroscience, 16*, 2365–2372.

Nilsson, L. K., Linderholm, K. R., Engberg, G., Paulson, L., Blennow, K., Lindstrom, L. H., Nordin, C., Karanti, A., Persson, P., & Erhardt, S. (2005). Elevated levels of kynurenic acid in the cerebrospinal fluid of male patients with schizophrenia. *Schizophrenia Research, 80*, 315–322.

Nowak, G., Ordway, G. A., & Paul, I. A. (1995). Alterations in the *N*-methyl-D-aspartate receptor complex in the frontal cortex of suicide victims. *Brain Research, 675*, 157–164.

Nowak, B., Zadrożna, M., Ossowska, G., Sowa-Kućma, M., Gruca, P., Papp, M., Dybała, M., Pilc, A., & Nowak, G. (2010). Alterations in hippocampal calcium binding neurons induced by stress models of depression. Preliminary assessment. *Pharmacological Reports, 62*, 1204–1210.

Nudmamud-Thanoi, S., & Reynolds, G. P. (2004). The NR1 subunit of the glutamate/NMDA receptor in the superior temporal cortex in schizophrenia and affective disorders. *Neuroscience Letters, 372*, 173–177.

O'Sullivan, J. B., Ryan, K. M., Curtin, N. M., Harkin, A., & Connor, T. J. (2009). Noradrenaline reuptake inhibitors limit neuroinflammation in rat cortex following a systemic inflammatory challenge: Implications for depression and neurodegeneration. *The International Journal of Neuropsychopharmacology, 12*, 687–699.

Oehmichen, W., & Gencic, M. (1975). Experimental studies on kinetics and functions of monuclear phagozytes of the central nervous system. *Acta Neuropathologica, 6*, 285–290.

Oliveira, R. M., Guimarães, F. S., & Deakin, J. F. (2008). Expression of neuronal nitric oxide synthase in the hippocampal formation in affective disorders. *Brazilian Journal of Medical and Biological Research, 41*, 333–341.

Ongur, D., Drevets, W. C., & Price, J. L. (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 13290–13295.

Osuch, E. A., Ketter, T. A., Kimbrell, T. A., George, M. S., Benson, B. E., Willis, M. W., Herscovitch, P., & Post, R. M. (2000). Regional cerebral metabolism associated with anxiety symptoms in affective disorder patients. *Biological Psychiatry, 48*, 1020–1023.

Pariante, C. M., Pearce, B. D., Pisell, T. L., Sanchez, C. I., Po, C., Su, C., & Miller, A. H. (1999). The proinflammatory cytokine, interleukin-1alpha, reduces glucocorticoid receptor translocation and function. *Endocrinology, 140*, 4359–4366.

Parker, K. J., Schatzberg, A. F., & Lyons, D. M. (2003). Neuroendocrine aspects of hypercortisolism in major depression. *Hormones and Behavior, 43*, 60–66.

Pei, Z., Pang, H., Qian, L., Yang, S., Wang, T., Zhang, W., Wu, X., Dallas, S., Wilson, B., Reece, J. M., Miller, D. S., Hong, J. S., & Block, M. L. (2007). MAC1 mediates LPS-induced production of superoxide by microglia: The role of pattern recognition receptors in dopaminergic neurotoxicity. *Glia, 55*, 1362–1373.

Pham, K., Nacher, J., Hof, P. R., & McEwen, B. S. (2003). Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. *European Journal of Neuroscience, 17*, 879–886.

Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional neuroanatomy of emotion: A meta-analysis of emotion activation studies in PET and fMRI. *NeuroImage, 16*, 331–348.

Pittenger, C., & Duman, R. S. (2008). Stress, depression, and neuroplasticity: A convergence of mechanisms. *Neuropsychopharmacology, 33*, 88–109.

Post, A., Crochemore, C., Uhr, M., Holsboer, F., & Behl, C. (2000). Differential induction of NF-κ B activity and neural cell death by antidepressants in vitro. *European Journal of Neuroscience, 12*, 4331–4337.

Price, J. L., & Drevets, W. C. (2010). Neurocircuitry of mood disorders. *Neuropsychopharmacology, 35*, 192–216.

Qin, L., Li, G., Qian, X., Liu, Y., Wu, X., Liu, B., Hong, J. S., & Block, M. L. (2005a). Interactive role of the toll-like receptor 4 and reactive oxygen species in LPS-induced microglia activation. *Glia, 52*, 78–84.

Qin, L., Liu, Y., Qian, X., Hong, J. S., & Block, M. L. (2005b). Microglial NADPH oxidase mediates leucine enkephalin dopaminergic neuroprotection. *Annals of the New York Academy of Sciences, 1053*, 107–120.

Raison, C. L., Dantzer, R., Kelley, K. W., Lawson, M. A., Woolwine, B. J., Vogt, G., Spivey, J. R., Saito, K., & Miller, A. H. (2010). CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: Relationship to CNS immune responses and depression. *Molecular Psychiatry, 15*, 393–403.

Rajkowska, G. (2000). Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. *Biological Psychiatry, 48*, 766–777.

Rajkowska, G., & Miguel-Hidalgo, J. J. (2007). Gliogenesis and glial pathology in depression. *CNS and Neurological Disorders – Drug Targets, 6*, 219–233.

Rajkowska, G., Miguel-Hidalgo, J. J., & Wei, J. (1999). Morphometric evidence for neuronal and glial prefrontal pathology in major depression. *Biological Psychiatry, 45*, 1085–1098.

Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A., & Pollmächer, T. (2001). Cytokine-associated emotional and cognitive disturbances in humans. *Archives of General Psychiatry, 58*, 445–452.

Ruhé, H. G., Mason, N. S., & Schene, A. H. (2007). Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: A meta-analysis of monoamine depletion studies. *Molecular Psychiatry, 12*, 331–359.

Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., MacDonald, E., Agerman, K., Haapasalo, A., Nawa, H., Aloyz, R., Ernfors, P., & Castrén, E. (2003). Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *Journal of Neuroscience, 23*, 349–357.

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1986). The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. *Endocrine Reviews, 7*, 284–301.

Sawada, M., Kondo, N., Suzumura, A., & Marunouchi, T. (1989). Production of tumor necrosis factor-alpha by microglia and astrocytes in culture. *Brain Research, 491*, 394–397.

Schwarcz, R., & Pellicciari, R. (2002). Manipulation of brain kynurenines: Glial targets, neuronal effects, and clinical opportunities. *Journal of Pharmacology and Experimental Therapeutics, 303*, 1–10.

Serra-Millàs, M., López-Vílchez, I., Navarro, V., Galán, A.-M., Escolar, G., Penadés, R., Catalán, R., Fañanás, L., Arias, B., & Gastó, C. (2011). Changes in plasma and platelet BDNF levels induced by *S*-Citalopram in major depression. *Psychopharmacology, 216*, 1–8.

Shah, P. J., Glabus, M. F., Goodwin, G. M., & Ebmeier, K. P. (2002). Chronic, treatment resistant depression and right fronto-striatal atrophy. *British Journal of Psychiatry, 180*, 434–440.

Sheline, Y. I., Gado, M. H., & Price, J. L. (1998). Amygdala core nuclei volumes are decreased in recurrent major depression. *Neuroreport, 9*, 2023–2028.

Shimizu, E., Hashimoto, K., Okamura, N., Koike, K., Komatsu, N., Kumakiri, C., Nakazato, M., Watanabe, H., Shinoda, N., Okada, S., & Iyo, M. (2003). Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biological Psychiatry, 54*, 70–75.

Shirayama, Y., Chen, A. C.-H., Nakagawa, S., Russell, D. S., & Duman, R. S. (2002). Brain-derived Neurotrophic factor produces antidepressant effects in behavioral models of depression. *Journal of Neuroscience, 22*, 3251–3261.

Skolnick, P., Layer, R. T., Popik, P., Nowak, G., Paul, I. A., & Trullas, R. (1996). Adaptation of *N*-methyl-D-aspartate receptors following antidepressant treatment: Implications for the pharmacotherapy of depression. *Pharmacopsychiatry, 29*, 23–26.

Sonino, N., Fava, G. A., Belluardo, P., Girelli, M. E., & Boscaro, M. (1993). Course of depression in Cushing's syndrome: Response to treatment and comparison with Graves' disease. *Hormone Research, 39*, 202–206.

Sorra, K. E., & Harris, K. M. (2000). Overview on the structure, composition, function, development, and plasticity of hippocampal dendritic spines. *Hippocampus, 10*, 501–511.

Sousa, N., Lukoyanov, N. V., Madeira, M. D., Almeida, O. F., & Paula-Barbosa, M. M. (2000). Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience, 97*, 253–266.

Southam, E., & Garthwaite, J. (1993). The nitric oxide-cyclic GMP signalling pathway in rat brain. *Neuropharmacology, 32*, 1267–1277.

Španová, A., Kovářů, H., Lisá, V., Lukášová, E., & Rittich, B. (1997). Estimation of apoptosis in C6 glioma cells treated with antidepressants. *Physiological Research, 46*, 161–164.

Steiner, J., Bogerts, B., Sarnyai, Z., Walter, M., Gos, T., Bernstein, H. G., & Myint, A. M. (2011). Bridging the gap between the immune and glutamate hypotheses of schizophrenia and major depression: Potential role of glial NMDA receptor modulators and impaired blood–brain barrier integrity. *World Journal of Biological Psychiatry, 13*(7), 482–492.

Stockmeier, C. A., Mahajan, G. J., Konick, L. C., Overholser, J. C., Jurjus, G. J., Meltzer, H. Y., Uylings, H. B. M., Friedman, L., & Rajkowska, G. (2004). Cellular changes in the postmortem hippocampus in major depression. *Biological Psychiatry, 56*, 640–650.

Streit, W. J., Graeber, M. B., & Kreutzberg, G. W. (1988). Functional plasticity of microglia: A review. *Glia, 1*, 301–307.

Streit, W. J., Walter, S. A., & Pennell, N. A. (1999). Reactive microgliosis. *Progress in Neurobiology, 57*, 563–581.

Szewczyk, B., Poleszak, E., Pilc, A., & Nowak, G. (2010). Ionic glutamate modulators in depression (zinc, magnesium). In P. Skolnick (Ed.), *Glutamate-based therapies for psychiatric disorders* (M. J Parnham & J. Bruinvels (Series Eds.), Milestones in drug therapy; pp. 21–38). Basel AG: Springer.

Szewczyk, B., Kubera, M., & Nowak, G. (2011a). The role of zinc in neurodegenerative inflammatory pathways in depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 35*, 693–701.

Szewczyk, B., Pilc, A., & Nowak, G. (2011b). Zinc and mental health. In L. Rink (Ed.), *Zinc in human health* (Biomedical and health research, pp. 403–416). Amsterdam: IOS Press BV.

Trullas, R., & Skolnick, P. (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *European Journal of Pharmacology, 185*, 1–10.

Tsopelas, C., Stewart, R., Savva, G. M., Brayne, C., Ince, P., Thomas, A., & Matthews, F. E. (2011). Neuropathological correlates of late-life depression in older people. *The British Journal of Psychiatry, 198*, 109–114.

Turchan-Cholewo, J., Dimayuga, F. O., Gupta, S., Keller, J. N., Knapp, P. E., Hauser, K. F., & Bruce-Keller, A. J. (2009). Morphine and HIV-Tat increase microglial-free radical production and oxidative stress: Possible role in cytokine regulation. *Journal of Neurochemistry, 108*, 202–215.

Tynan, R. J., Weidenhofer, J., Hinwood, M., Cairns, M. J., Day, T. A., & Walker, F. R. (2012). A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. *Brain, Behavior, and Immunity, 26*, 469–479.

Valentine, A. D., Meyers, C. A., Kling, M. A., Richelson, E., & Hauser, P. (1998). Mood and cognitive side-effects of interferon-a therapy. *Seminars in Oncology, 25*, 39–47.

Van Craenenbroeck, K., De Bosscher, K., Vanden Berghe, W., Vanhoenacker, P., & Haegeman, G. (2005). Role of glucocorticoids in dopamine-related neuropsychiatric disorders. *Molecular and Cellular Endocrinology, 245*, 10–22.

Vollmayr, B., Simonis, C., Weber, S., Gass, P., & Henn, F. (2003). Reduced cell proliferation in the dentate gyrus is not correlated with the development of learned helplessness. *Biological Psychiatry, 54*, 1035–1040.

Wang, T., Pei, Z., Zhang, W., Liu, B., Langenbach, R., Lee, C., Wilson, B., Reece, J. M., Miller, D. S., & Hong, J. S. (2005). MPP^{+}−induced COX-2 activation and subsequent dopaminergic neurodegeneration. *FASEB Journal, 19*, 1134–1136.

Watanabe, Y., Gould, E., & McEwen, B. S. (1992). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Research, 588*, 341–345.

Wellman, C. L. (2001). Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *Journal of Neurobiology, 49*, 245–253.

Weniger, G., Lange, C., & Irle, E. (2006). Abnormal size of the amygdala predicts impaired emotional memory in major depressive disorder. *Journal of Affective Disorders, 94*, 219–229.

Wolkowitz, O. M., & Reus, V. I. (1999). Treatment of depression with antiglucocorticoid drugs. *Psychosomatic Medicine, 61*, 698–711.

Wu, C. W., Chen, Y. C., Yu, L., Chen, H. I., Jen, C. J., Huang, A. M., Tsai, H. J., Chang, Y. T., & Kuo, Y. M. (2007). Treadmill exercise counteracts the suppressive effects of peripheral lipopolysaccharide on hippocampal neurogenesis and learning and memory. *Journal of Neurochemistry, 103*, 2471–2481.

Zhang, Y., Gu, F., Chen, J., & Dong, W. (2010). Chronic antidepressant administration alleviates frontal and hippocampal BDNF deficits in CUMS rat. *Brain Research, 1366*, 141–148.

Zunszain, P. A., Anacker, C., Cattaneo, A., Choudhury, S., Musaelyan, K., Myint, A. M., Thuret, S., Price, J., & Pariante, C. M. (2012). Interleukin-1β: A new regulator of the kynurenine pathway affecting human hippocampal neurogenesis. *Neuropsychopharmacology, 37*, 939–949.

Part IV

Neurotrophins

Amyloid-Beta, BDNF, and the Mechanism of Neurodegeneration in Alzheimer's Disease

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Contents

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Abstract

The accumulation of amyloid-β is widely considered the primary neurotoxic insult leading to Alzheimer's disease. Although the precise mechanism of this toxicity is not well understood, amyloid-β-induced downregulation of brain-derived neurotrophic factor may be one of the most important contributors to amyloid-β toxicity. Brain-derived neurotrophic factor has diverse neurotrophic effects on the nervous system, including promoting synaptic plasticity and neurogenesis, and it is essential for cognition and memory. Early in the progression of Alzheimer's disease, prior to significant plaque and tangle deposition, declining brain-derived neurotrophic factor expression induced by amyloid-β is associated with mounting impairments in cognition and memory. A variety of approaches have demonstrated that increasing brain-derived neurotrophic factor levels improves learning and memory, highlighting the possibility that therapeutically restoring brain-derived neurotrophic factor levels may prevent or reverse the memory impairments and cognitive decline seen in Alzheimer's disease.

Keywords

Alzheimer's disease • Amyloid-β • BDNF • CREB • Learning and memory • LTP

1 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is the most common cause of dementia in the elderly. AD presents as global cognitive decline with associated memory loss and altered personality. The neuropathological hallmarks of AD include extracellular amyloid-β (Aβ)-containing plaques, intracellular neurofibrillary tangles formed by hyperphosphorylated tau protein, synaptic loss, and resulting neurodegeneration (Hyman et al. 2012; Coleman & Yao 2003; Coleman and Flood 1987; Hyman 1997; Scheff and Price 2003; McKhann et al. 1984). AD primarily affects several regions of the brain that are known for their role in learning and memory, namely, the basal forebrain, hippocampus, cortex, and entorhinal cortex (Hyman et al. 1984; Coyle et al. 1983; Gomez-Isla et al. 1996).

Several theories exist to explain the molecular mechanisms that could lead to this devastating disease. One predominant theory suggests that an imbalance between Aβ production and clearance produces the accumulation of Aβ that is the driving force behind AD neuropathology (Hardy and Selkoe 2002; Iqbal and Grundke-Iqbal 2008). Amyloid-β has many neurotoxic effects; however, the precise mechanism by which Aβ results in the neurodegeneration seen in AD is unclear. The ability of Aβ to downregulate the critical neurotrophic factor brain-derived neurotrophic factor (BDNF) is the main focus of this chapter, as the promotion of synaptic integrity and function by BDNF is of utmost importance throughout life and for the prevention of learning and memory impairments.

2 Characteristics of Alzheimer's Disease

2.1 Pathological Features

Alzheimer's disease (AD) exhibits characteristic pathological features such as senile or amyloid plaques composed of precipitated Aβ and neurofibrillary tangles composed of hyperphosphorylated tau; the presence of these pathologies is necessary for a definitive AD diagnosis (Hyman et al. 2012). The amyloid cascade hypothesis states that the accumulation of toxic Aβ is the upstream driving force behind subsequent pathology and synaptic degeneration (Hardy and Selkoe 2002; Iqbal and Grundke-Iqbal 2008). Nevertheless, amyloid plaques do not correlate well with cognitive decline; neurofibrillary tangles correlate better (Guillozet et al. 2003). However, it is the loss of functional synapses in AD that correlates most strongly with loss of cognitive abilities (Terry et al. 1991).

A significant decline in synaptic connections in AD is followed by the pronounced loss of neurons in the basal forebrain, entorhinal cortex, hippocampus, and cortex. AD has been characterized as a disconnection of the hippocampus, as projections from the basal forebrain, entorhinal cortex, and cortex to the hippocampus are lost in AD (Hyman et al. 1984). Among these brain areas, the entorhinal cortex is one of the earliest areas vulnerable to synaptic degeneration, and because of its connection with the hippocampus and cortex, entorhinal cortex damage in AD significantly impacts learning and memory (Van Hoesen et al. 1991). In addition, studies have indicated that the memory deficits seen in AD are also directly related to the degree of basal forebrain cholinergic atrophy (Coyle et al. 1983). The basal forebrain is the major cholinergic output of the central nervous system, and these neurons as well as their projections to hippocampal and cortical regions are critical for learning, memory, and attention (Baxter and Chiba 1999). The loss of basal forebrain innervation is associated with aging and age-related memory loss (Terry and Katzman 2001; Ypsilanti et al. 2008). In fact, the most common drugs for AD on the market today are cholinergic enhancers.

In addition to loss of connectivity, there may be a loss of functional neurogenesis associated with AD. In many different AD transgenic mouse models, significantly less proliferation of new neurons is present in the hippocampal formation compared to control animals (Mu and Gage 2011). Without the generation of new neurons, the synaptic degeneration resulting from AD is not compensated and learning and memory are impaired. The case is not so clear in human hippocampus, however, where immunostaining with different markers of immature neurons suggests that there is either increased (Jin et al. 2004), no change (Boekhoorn et al. 2006), or decreased (Crews et al. 2010) neurogenesis in AD. More research is clearly needed to determine whether reduced hippocampal neurogenesis contributes significantly to memory loss in AD.

2.2 Clinical Presentation

2.2.1 Impaired Cognition, Learning, and Memory

Perhaps the most clinically apparent symptom of the synaptic loss and neurodegeneration characteristic of AD is the decline of declarative memory, including both episodic and semantic memories (Aronoff et al. 2006). Initially, the loss of synaptic connections in the hippocampus and entorhinal cortex is evident as an early and significant decline in cognitive function and episodic memory (Devanand et al. 2007), which are hallmarks of AD. Episodic memory loss is not only an early event in AD, but in mild cognitive impairment (MCI), it is associated with an increased risk for conversion to AD (Aggarwal et al. 2005; Devanand et al. 2007). MCI is considered a preclinical stage of AD, since as many as 8 % of people with MCI progress to AD per year (Mitchell and Shiri-Feshki 2009). Transgenic mouse models of AD are also significantly impaired on tests of episodic-like memory compared to control animals (Davis et al. 2012). In addition, animal models of AD are impaired in long-term potentiation (LTP), a model for synaptic plasticity and a major cellular constituent underlying learning and memory processes. Aβ, when injected into rat hippocampus or added to hippocampal slices, abolishes LTP without affecting baseline synaptic transmission (Cullen et al. 1997; Chen et al. 2000). Like episodic memory in humans, the LTP deficit in rodent models of AD occurs early; it occurs prior to plaque formation and is accompanied by deficits in spatial learning and memory (Liu et al. 2008; Jacobsen et al. 2006).

2.3 Molecular Pathway for Learning and Memory

2.3.1 CREB

A molecular pathway essential for learning and memory that is disrupted in AD involves the transcription factor cAMP response element binding protein (CREB) (Barco et al. 2003). Aβ interferes with hippocampal LTP via signaling pathways including the Ca(2+)-dependent protein phosphatase calcineurin, Ca(2+)/calmodulin-dependent protein kinase II (CaMKII), and CREB (Yamin 2009). Active, phosphorylated CREB is reduced in the AD brain (Yamamoto-Sasaki et al. 1999) and is also decreased in neurons following treatment with Aβ in vitro (Tong et al. 2001; Garzon and Fahnestock 2007). CREB phosphorylation recruits transcriptional coactivators which are required for the transcription of genes involved in learning and memory. Aβ-induced suppression of transcription induced by the CREB coactivator CRCT1 has been demonstrated in transgenic AD mice, and this suppression is mediated by blockade of L-type voltage-gated calcium channels, reduced calcium influx, and disruption of PP2B/calcineurin-dependent CRTC1 dephosphorylation (España et al. 2010). Restoration of CREB activity in AD transgenic mice by inducing expression of another coactivator, CREB binding protein (CBP), ameliorates learning and memory deficits (Caccamo et al. 2010). Among the genes induced by CREB and its coactivators that are required for learning and memory, the most important for AD may be brain-derived neurotrophic factor (BDNF).

3 BDNF Is a Powerful Neurotrophic Molecule with Pleiotropic Effects on the Nervous System

3.1 Neuronal Survival

BDNF supports the survival of neurons and their connections that are vulnerable in aging and in diseases of the aging brain such as AD, including the hippocampus, entorhinal cortex, neocortex, and basal forebrain (Hyman et al. 1984; Alderson et al. 1990; Knusel et al. 1991; Ghosh et al. 1994; Lindholm et al. 1996; Lowenstein and Arsenault 1996). Early studies demonstrated a more than twofold increase in survival of septal cholinergic neurons following BDNF treatment as measured by acetylcholinesterase (AChE) histochemical staining (Alderson et al. 1990). Further, blocking BDNF signaling either using antibodies or BDNF gene knockdown decreases survival of cortical (Ghosh et al. 1994), hippocampal (Lindholm et al. 1996), and dorsal root ganglion neurons (Korte et al. 1995). Although these results demonstrate that BDNF is a survival-promoting factor for critical neuronal populations during development, conditional knockdown of BDNF in the adult suggests that BDNF is not required for postmitotic neuronal survival, but rather for dendritic maturation and growth of specific neuronal populations such as medium spiny neurons of the striatum (Rauskolb et al. 2010). Furthermore, the high-affinity BDNF receptor, tropomyosin-related kinase B (TrkB), unlike the related receptors TrkA or TrkC, is unable to trigger cell death in the absence of ligand (Nikoletopoulou et al. 2010), suggesting that decreases in BDNF may trigger altered spine morphology, reduced dendritic complexity, synaptic dysfunction, and degeneration but not outright neuronal loss.

3.2 Neurogenesis

BDNF promotes neurogenesis. Direct infusion of BDNF into rat hippocampus increases the number of developing neurons compared to saline injections in both the ipsilateral and contralateral hemispheres, suggesting a widespread effect (Scharfman et al. 2004). This hippocampal neurogenesis is directly related to subsequent cognitive functions, as new neurons are integrated into neural circuitry and play an important role in specific learning and memory tasks such as learning in the Morris water maze, a spatial memory task (Zhao et al. 2008).

Studies investigating the exact molecular mechanisms underlying BDNF-mediated increases in neurogenesis are still underway; however, by signaling through the TrkB receptor, BDNF activates three signaling cascades, namely, the phosphatidyl-inositol-3-kinase (PI3K)/Akt, Ras/extracellular-regulated kinase (ERK), and phospholipase C gamma (PLCγ) pathways, all of which are implicated in BDNF's ability to activate CREB. Specifically, PLCγ promotes the release of intracellular Ca(2+) stores (Berninger et al. 1993) which induce the activation of the CREB kinase, CaMKIV (Finkbeiner et al. 1997; Blanquet and Lamour 1997), and subsequently lead

to the activating phosphorylation of CREB. Further, activation of the Ras/ERK signaling pathway is reportedly sufficient to promote Ca(+2)-/CRE-dependent transcription (Finkbeiner et al. 1997). Lastly, protein kinase A (PKA), which is activated by BDNF signaling predominantly via the PI3K/AKT pathway, can activate CREB by phosphorylating its activation site Ser133 (Gonzalez and Montminy 1989). In support of the latter pathway, the PKA activator rolipram increases the survival of new neurons in a CREB-dependent manner (Fujioka et al. 2004; Merz et al. 2012). The activation of CREB is associated with hippocampal neurogenesis, as phosphorylated CREB is consistently found in newborn hippocampal neurons (Nakagawa et al. 2002a, b; Jagasia et al. 2009). These findings support the possibility that BDNF promotes neurogenesis via mechanisms that rely on its ability to activate the neurogenesis-promoting transcription factor CREB.

3.3 Dendritic Modulation

BDNF regulates dendritic growth, branching and spine maturation through a balance between BDNF and its precursor form, proBDNF. Both mature and proBDNF signal through the TrkB receptor and the pan-neurotrophin receptor $p75^{NTR}$; however, BDNF has a greater affinity for TrkB while proBDNF preferentially binds to $p75^{NTR}$ (Fayard et al. 2005; Teng et al. 2005). Signaling through TrkB promotes dendritic growth and spine maturation via activation of the PI3K/Akt/mTOR signaling pathway, which has been long associated with axonal growth and dendritic complexity (Atwal et al. 2000; Kuruvilla et al. 2000; Markus et al. 2002; Kumar et al. 2005). Therefore, the preferential binding of BDNF to TrkB promotes synaptic development through increased dendritic growth and spine maturation. Conversely, proBDNF inhibits neurite outgrowth through its preferential binding to $p75^{NTR}$, which negatively alters dendrite and spine morphology in addition to spine density (Yamashita et al. 1999; Zagrebelsky et al. 2005; Singh et al. 2008; Koshimizu et al. 2009). $p75^{NTR}$ reduces dendritic growth and branching through activation of a Rho-GTPase, RhoA (Sun et al. 2012), by releasing RhoA from its inactivating Rho-GDI (Yamashita and Tohyama 2003). Thus, the balance between proBDNF and BDNF plays a critical role in the regulation of spine maturation, dendritic growth/retraction, and synaptic density, which underlie learning and memory processes.

3.4 BDNF Plays a Critical Role in LTP

The cleavage of proBDNF to mature BDNF can take place extracellularly by plasmin or matrix metalloproteinases (MMPs) or intracellularly by furin or proprotein convertase 1 (PC1) (Mowla et al. 2001; Lu et al. 2005). The extracellular conversion by plasmin is specifically controlled by the extracellular serine protease tissue plasminogen activator (tPA) (Pang et al. 2004). tPA proteolytically cleaves inactive plasminogen to plasmin which can then cleave proBDNF into

mature BDNF (Lee et al. 2001). This conversion of plasminogen to plasmin by tPA takes place at hippocampal synapses, where subsequent conversion of proBDNF to mature BDNF occurs (Pang et al. 2004). Plasminogen−/− mice are impaired in late-phase long-term potentiation (L-LTP) (Pang et al. 2004). Addition of mature BDNF to these mice was able to rescue L-LTP, while addition of proBDNF was not, implicating proBDNF cleavage to mature BDNF in the mechanisms of LTP.

This ability of BDNF to rescue LTP is related to its preferential signaling through TrkB. Signaling through TrkB activates molecular cascades vital for synaptic integrity and for the production of LTP (Figurov et al. 1996). LTP is induced by the activation of protein kinases such as MAPK, PKA, PI3K, PKC, and CaMKII, which converge at the activation of ERK and modulate the activation of proteins involved in gene transcription and protein synthesis such as CREB (Lynch 2004). BDNF effectively activates protein kinases upstream of ERK through the PI3K/AKT, PKA, and Ras signaling pathways, which play a central role in the activation of CREB and subsequently contribute to neuronal survival and synaptic plasticity. CREB in turn plays a substantial role in upregulating the effector genes necessary for L-LTP (Pittenger and Kandel 1998) such as Arc, a member of the immediate-early gene (IEG) family that has been critically implicated in the maintenance of LTP (Ying et al. 2002; Guzowski et al. 2000), and BDNF itself. Additionally, BDNF is essential for maintaining the phenotype of VP16-CREB mice, which contain a constitutively active form of CREB and prime cells for L-LTP by activating CRE-mediated transcription (Barco et al. 2005). Conversely, proBDNF signaling through $p75^{NTR}$ favors long-term depression (LTD) (Lu 2003; Pang et al. 2004). Although the precise mechanism by which proBDNF signaling via $p75^{NTR}$ promotes LTD is still unknown, it may involve NMDA receptor subtype NR2B. NR2B activation is reportedly required for LTD (Massey et al. 2004), and proBDNF signaling through $p75^{NTR}$ directly potentiates the NR2B component of NMDA synaptic currents in hippocampal slices (Woo et al. 2005). Taken together, current research supports the model that regulation of BDNF/proBDNF processing modulates the balance of LTP/LTD, which underlies learning and memory.

3.5 BDNF Is Essential for Cognition and Memory

Multiple studies have shown that decreases in BDNF such as in heterozygous BDNF knockout mice or BDNF knockdown result in learning and memory deficits (Linnarsson et al. 1997; Gorski et al. 2003; Heldt et al. 2007). A Val66Met polymorphism in the pro-domain of proBDNF which interferes with BDNF secretion results in episodic memory impairments and reduced hippocampal volume, key endophenotypes of AD (Egan et al. 2003; Hariri et al. 2003). This finding shows that an alteration in BDNF levels is sufficient to affect hippocampal activity and that BDNF is critically involved in hippocampal learning and memory processes.

4 BDNF Is Significantly Downregulated in Alzheimer's Disease

4.1 BDNF Loss Is an Early Event in the Progression of AD

Amyloid plaques correlate poorly with cognition, and evidence suggests that cognitive decline precedes Aβ pathology (Van Dam et al. 2003; Westerman et al. 2002; Hanna et al. 2009). This is supported by transgenic mouse models of AD, where deficits in novel object recognition and spontaneous alternation performance in a "Y" maze occur prior to the deposition of Aβ plaques (Holcomb et al. 1998; Francis et al. 2012). Loss of BDNF also occurs prior to plaque deposition in these transgenic mice and coincides with memory deficits (Francis et al. 2012). Further, BDNF is lost early in the progression of AD, as demonstrated by a significant decline in BDNF in individuals with MCI. More than a 30 % reduction in cortical BDNF expression was found in MCI subjects compared to age- and gender-matched, noncognitively impaired controls (Peng et al. 2005), with expression levels nearly as low as in AD subjects. Lastly, like humans, canines develop cognitive impairment and amyloid deposition as they age and are an excellent model of MCI (Cotman & Berchtold 2002; Cotman & Head 2008). BDNF mRNA levels were significantly decreased and correlated with cognitive status in aged, cognitively impaired canines as well, supporting human findings that BDNF loss coincides with memory impairments (Fahnestock et al. 2012). Thus, in both animal models and humans, BDNF is lost early, coinciding with memory loss and prior to the appearance of significant plaque deposition typical of AD.

4.2 BDNF Is Indicative of Cognitive Status

The expression of BDNF is strongly correlated with cognitive status. BDNF mRNA and proBDNF and mature BDNF protein are decreased in the entorhinal, frontal, temporal, and parietal cortices as well as the hippocampus of AD subjects compared to controls (Ferrer et al. 1999; Phillips et al. 1991; Murray et al. 1994; Narisawa-Saito et al. 1996; Connor et al. 1997; Holsinger et al. 2000; Hock et al. 2000; Fahnestock et al. 2002; Michalski and Fahnestock 2003; Peng et al. 2005). Lower levels of both mature BDNF and proBDNF protein correlate with decreased cognitive test scores as measured by the Mini Mental State Examination (MMSE) and Global Cognitive Score (GCS) (Peng et al. 2005).

4.3 Increasing BDNF Levels Can Counteract Memory Impairments

The strong correlation between lowered BDNF and diminished cognition, learning, and memory makes the possibility of rescuing these behavioral symptoms of AD with BDNF an enticing undertaking. Knocking down BDNF in mice results in loss

of synapses, learning and memory deficits, and diminished LTP, whereas BDNF administration rescues or increases LTP and synapses and restores learning and memory (Blurton-Jones et al. 2009; Nagahara et al. 2009; Patterson et al. 1996; Korte et al. 1995). Early studies substantiated findings that BDNF is necessary for LTP: mice with a deletion in the BDNF gene exhibited significantly weakened hippocampal LTP expression (Korte et al. 1995), whereas treating hippocampal BDNF knockout tissue with recombinant BDNF completely restored LTP (Patterson et al. 1996). Similarly, knockdown of the BDNF receptor TrkB can significantly reduce hippocampal LTP (Minichiello et al. 1999). These early studies led to efforts in recent years to deliver exogenous BDNF as a method of restoring cognitive abilities in animal models of AD. However, expression of truncated TrkB receptors in the ventricular ependyma effectively prevents diffusion of BDNF to target tissues when it is administered by intracerebroventricular infusion (Anderson et al. 1995). Furthermore, BDNF does not readily cross the blood–brain barrier. Fusions of BDNF with factors that allow it to be transferred across the blood–brain barrier such as an antibody to the transferrin receptor (Zhang and Pardridge 2001a, b; 2006) or to the insulin receptor (Boado et al. 2007) have facilitated entry of intravenously administered BDNF into brain tissue.

The brain is exquisitely sensitive to BDNF levels, which presents another significant barrier to the successful use of this trophic factor in vivo. Microinjections of microgram levels of BDNF into the hippocampus increase neuronal excitability and can even cause seizures, whereas chronic infusions of similar amounts decrease the TrkB receptor and TrkB activation, resulting in loss of responsiveness to BDNF (Scharfman 1997; Xu et al. 2004). Furthermore, delivery of BDNF can result in impairment of spatial learning rather than improvement if BDNF expression is too high (Pietropaolo et al. 2007). Therefore, more physiological delivery of low doses of BDNF via viral vectors has been extensively investigated.

In the entorhinal cortex-lesioned aged rat, BDNF gene delivery enhanced LTP and partially restored cognitive function (Ando et al. 2002). BDNF gene delivery via lentiviral injection to the entorhinal cortex in a transgenic mouse model of AD not only increased synaptic density (synaptophysin-IR) and synaptic function (p-Erk signaling) but, importantly, improved spatial memory performance in the Morris water maze task (Nagahara et al. 2009). BDNF treatment did not affect neuronal number in this model, although it did rescue neurons from cell loss in a perforant path transection model. BDNF administration also exerted similar effects and rescued learning and memory in the aged, cognitively impaired nonhuman primate. Importantly, this lentiviral expression system has been used to maintain delivery of NGF for up to 1 year in primate basal forebrain, eliminating transient expression as a disadvantage of the system. These experiments were carried out after the onset of disease symptoms and pathology in transgenic mice and after cognitive impairment appeared in aged primates, suggesting that BDNF may be an effective therapy for reversal of cognitive deficits as well as prevention.

However, this delivery method suffers from poor viral diffusion within the parenchyma. This complication is somewhat ameliorated by the implantation of neural stem cells (NSC) which can migrate within brain parenchyma. NSC delivery

into hippocampus of the triple-transgenic (Tg3x-AD) mouse model of AD increased synaptic density and improved hippocampal-dependent learning and memory (Blurton-Jones et al. 2009). BDNF knockdown in these NSCs eliminated the beneficial effects of the cells, demonstrating that BDNF secreted by the NSCs was the active factor (Blurton-Jones et al. 2009).

Alternative approaches to gene delivery include methods of increasing endogenous BDNF levels or signaling. For instance, viral delivery of CREB activators (Caccamo et al. 2010; Espana et al. 2010), successfully reverses synaptic atrophy and learning and memory impairments in transgenic mice. Rolipram, a phosphodiesterase inhibitor and CREB activator, increases BDNF expression (DeMarch et al. 2008) and therefore may have therapeutic effects in AD. Additionally, small molecule BDNF mimetics or TrkB agonists which promote signaling through the TrkB receptor can restore synaptic plasticity and cognitive function (Massa et al. 2010). Moreover, natural products and their derivatives such as certain flavonoids are under active investigation as potential neuroprotective agents, LTP enhancers and activators of CREB, TrkB, and its downstream signaling pathways PI3K/Akt and MAPK/Erk (Maher et al. 2006; Jang et al. 2010). Finally, lifestyle adjustments such as exercise, environmental enrichment, and dietary restriction increase BDNF expression in both animal models and human studies (Mattson et al. 2004b). An enriched environment has been shown to increase BDNF mRNA in rats (Falkenberg et al. 1992), while an exercise routine can regulate transcript-specific BDNF mRNA and protein (Berchtold et al. 2002; Cotman and Berchtold 2002; Zajac et al. 2010). Recently, the combination of an antioxidant-rich diet and environmental enrichment was shown to significantly increase BDNF expression and was correlated with an improvement in memory scores in old, cognitively impaired canines (Fahnestock et al. 2012). Although a variety of molecular, pharmacological, and lifestyle interventions are under investigation and may pave the way for future clinical trials in subjects with AD, increasing brain BDNF levels safely and effectively is not yet a reality. A greater understanding of BDNF regulation and expression may provide additional targets or modalities.

4.4 BDNF Transcriptional and Posttranslational Regulation

The human BDNF gene is 70 kb long and consists of 11 exons and 9 functional promoters, which by alternative splicing result in at least 17 different BDNF transcripts (Pruunsild et al. 2007). This variation in transcript expression allows for tissue-specific regulation of BDNF in response to a variety of developmental and environmental cues (Timmusk et al. 1993; Pruunsild et al. 2007). BDNF transcripts II, III, IV, V, and VII are exclusively localized to the brain, while other BDNF transcripts are expressed in neuronal as well as nonneuronal tissues (Pruunsild et al. 2007). Within the brain, BDNF transcript IV accounts for approximately half of the total BDNF mRNA found in the cortex (Garzon and Fahnestock 2007). BDNF transcript IV is regulated at least in part by CREB (Shieh et al. 1998; Pruunsild et al. 2007; Timmusk et al. 1995).

BDNF protein is synthesized as 36 kDa precursor proBDNF, which can be cleaved by enzymes including plasmin, furin, and matrix metalloproteases to produce 14 kDa mature BDNF (Mowla et al. 2001; Lee et al. 2001; Lu et al. 2005). ProBDNF is expressed widely in the human brain, where it is only partially cleaved to its mature form. Both BDNF and proBDNF are found in relatively high amounts in the hippocampus, cortex, and basal forebrain (Michalski and Fahnestock 2003), areas that are particularly vulnerable in AD.

4.5 BDNF mRNA and Protein Decrease in AD

BDNF levels are dramatically altered in individuals with AD and MCI. BDNF mRNA expression in the hippocampus is decreased nearly twofold in individuals with AD (Phillips et al. 1991), and BDNF mRNA is reduced approximately threefold in the AD parietal cortex (Garzon et al. 2002; Holsinger et al. 2000). This decrease in BDNF mRNA is due to a decrease in four BDNF transcripts: I, II, IV, and VI (Garzon et al. 2002). Of particular interest is transcript IV, since it is the most prevalent transcript in human cortex (Garzon and Fahnestock 2007) and is specifically decreased in human and mouse models of AD (Garzon et al. 2002; Peng et al. 2009).

The decreased BDNF mRNA in AD corresponds to a similar decrease in BDNF protein. Mature BDNF protein is significantly reduced in AD hippocampus (Narisawa-Saito et al. 1996; Connor et al. 1997; Hock et al. 2000), frontal cortex (Ferrer et al. 1999), temporal cortex (Connor et al. 1997), and parietal cortex (Hock et al. 1998; Peng et al. 2005) compared to controls, and proBDNF protein is reduced in AD parietal cortex (Michalski and Fahnestock 2003; Peng et al. 2005). The molecular mechanisms in AD that could lead to this substantial decrease in BDNF expression are beginning to be understood.

5 Amyloid-β Toxicity in Alzheimer's Disease

Although Aβ has been recognized as having non-disease associations, such as acting as an antimicrobial agent (Soscia et al. 2010), it is most well studied as the precipitating insult in AD. Early studies suggested that it was the formation of Aβ plaques that was the primary neurotoxic insult in Alzheimer's disease. However, plaques do not correlate well with decreased cognition, and more recent evidence suggests that soluble forms of Aβ may be more toxic (Ferreira et al. 2007; Walsh and Selkoe 2007; Lacor et al. 2007; Hardy and Selkoe 2002; Garzon and Fahnestock 2007).

5.1 Formation of Toxic, Soluble Aβ

Amyloid-β is a peptide formed by the proteolytic processing of the amyloid precursor protein (APP) (Hardy and Selkoe 2002; Haass et al. 1992; Citron et al.

1992; Shoji et al. 1992). APP is a single-pass, transmembrane protein with a small intracellular C-terminus and a large extracellular segment which contains part of the Aβ sequence (Mattson 2004). APP is initially proteolytically cleaved by α- or β-secretase (also known as beta-site APP cleaving enzyme 1; BACE-1), which releases a soluble fragment of APP (DeStrooper and Annaert 2000). Following primary cleavage by β- or α-secretase, a secondary cleavage of the transmembrane domain, or C-terminal fragment, of APP by γ-secretase results in the formation of either the Aβ peptide or the p3 fragment of APP, respectively (DeStrooper and Annaert 2000; Haass et al. 1992). Gamma-secretase is a complex of integral membrane proteins that includes presenilin-1 (PS-1) or presenilin-2 (PS-2), which when mutated can dramatically alter the production of Aβ. Gamma-secretase cleavage following cleavage by β-secretase can result in Aβ peptides of various lengths (Haass et al. 1992; DeStrooper and Annaert 2000). $A\beta_{1–40}$ is most commonly produced following the successive cleavage of β- and γ-secretases; however, it is $A\beta_{1–42}$ that is of particular interest in the progression of AD because of its greater propensity to aggregate (Tabaton et al. 2010; Glabe 2001). The aggregation of Aβ involves several conformational states from dimers and trimers to high-molecular-weight oligomers and protofibrils and finally insoluble Aβ fibrils (Glabe 2004) and requires a structural change from an α-helical conformation to a more organized β-sheet configuration characteristic of Aβ aggregation (Xu et al. 2005).

Formation of soluble Aβ is increased by mutations in APP, PS-1, and PS-2, hallmarks of familial forms of AD (Citron et al. 1992; Haass et al. 1994; Hutton and Hardy 1997; Scheuner et al. 1996). Such mutations result either in Aβ overproduction or in an increase in the aggregation-prone $A\beta_{1–42}$ (Burdick et al. 1992; Jarrett et al. 1993; Hardy and Selkoe 2002; Selkoe 1994). Increased levels of Aβ increase aggregation and toxicity prior to plaque deposition. Levels of soluble Aβ oligomers in the frontal cortex of individuals with AD are up to 70-fold higher than in control brains (Gong et al. 2003) and correlate with the degree of dementia and of synaptic loss (Lue et al. 1999).

5.2 Mechanisms of Aβ Toxicity

Aβ has many proposed toxic effects that could result in the neuronal dysfunction and neurodegeneration characteristic of AD. Aβ enhances tau hyperphosphorylation and the subsequent formation of neurofibrillary tangles in animal models (Gotz et al. 2001; Lewis et al. 2001; Masliah et al. 2001; Hardy and Selkoe 2002) and in human neuroblastoma cells in vitro (Pennanen and Gotz 2005). This suggests that Aβ may exert its neurotoxic effects via tau hyperphosphorylation and subsequent neurofibrillary tangle formation (Iqbal and Grundke-Iqbal 2008). Another well-studied toxic effect of Aβ accumulation is altered calcium homeostasis: soluble Aβ oligomers have been shown to alter calcium regulation by modulating ion channels, including voltage-gated calcium and potassium channels and nicotinic and NMDA receptors, and by forming its own calcium-conducting

pores, thereby increasing levels of cytosolic calcium (Green et al. 2007; Mattson et al. 1993; Ferreiro et al. 2006; Demuro et al. 2005; Resende et al. 2007). Oligomeric Aβ has also been shown to promote increased reactive oxygen species (ROS) production in primary cortical and hippocampal neurons (Sponne et al. 2003; DeFelice et al. 2007), which has been associated with inflammation and neurodegeneration. Amyloid-β may also indirectly induce neurotoxicity through the activation of proinflammatory responses (Akiyama et al. 2000; Giovannini et al. 2002; Lukiw and Bazan 2000), which may have a role in inhibiting hippocampal memory formation (Tancredi et al. 1992, 2000; Murray and Lynch 1998; Heneka and O'Banion 2007). However, Aβ has its most direct effect on synaptic degeneration through its action on specific signaling cascades.

6 BDNF May Mediate Aβ Toxicity in Alzheimer's Disease

Aβ toxicity results in neurodegeneration by altering signaling pathways upstream and downstream of CREB. CREB phosphorylation and signaling are reduced by exposure to soluble, oligomeric Aβ in vitro (Tong et al. 2001; Garzon and Fahnestock 2007). Aβ treatment inhibits the Ras/ERK and PI3K/AKT signaling pathways (Tong et al. 2004), which are downstream of BDNF/TrkB but are also essential for the phosphorylation of CREB and subsequent expression of BDNF. It has recently been shown that inhibiting glycogen synthase-3β (GSK3β), which can be activated by Aβ, is an effective way to decrease inhibitory phosphorylation of CREB and increase BDNF protein (DaRocha-Souto et al. 2012). Additionally, Aβ has been shown to inactivate PKA in vitro, and increasing PKA activity reverses decreased CREB activation induced by Aβ treatment (Vitolo et al. 2002). Therefore, Aβ disrupts several kinase cascades, leading to a reduction in activity of CREB and in BDNF expression.

Soluble, oligomeric Aβ, but not the fibrillar Aβ found in plaques, decreases CREB activation and downregulates BDNF in vitro, largely via transcript IV (Garzon and Fahnestock 2007). In transgenic mouse models of AD, which also exhibit downregulation of transcript IV, BDNF expression is inversely proportional to the amount of soluble, high-molecular-weight Aβ oligomers (Peng et al. 2009). Thus, inactivation of CREB by soluble, aggregated Aβ may downregulate BDNF transcript IV, resulting in the diminished synaptic connections and memory loss characteristic of AD.

A major consequence of Aβ-induced CREB reduction that is mediated by BDNF is diminished LTP. Soluble Aβ oligomers have been shown to effectively inhibit hippocampal LTP (Lambert et al. 1998; Walsh et al. 2002). Aβ inhibits LTP by specifically interfering with CaMKII and CREB signaling pathways (Yamin 2009), and Aβ-induced reductions in LTP are rescued by BDNF (Zeng et al. 2010). These effects on LTP provide a mechanism for Aβ's interference with learning and memory. Therefore, although Aβ has many proposed mechanisms of toxicity, its modulation of kinase pathways affecting transcription of genes involved in learning and memory, particularly BDNF and CREB, is perhaps the most significant for AD.

7 Conclusion

Alzheimer's disease, which increasingly affects our aging population, is primarily a disease of synaptic dysfunction. Soluble, aggregated amyloid-β is thought to be the primary neurotoxic insult leading to synaptic loss. However, the exact mechanisms that lead from Aβ aggregation to the pathological and physical symptoms of AD are not clear. Aβ accumulation, among other toxic effects, alters kinase cascades, leading to reduced CREB activation and to BDNF downregulation, primarily through transcript IV. This downregulation of BDNF is an early damaging event in the progression of AD and occurs in MCI and prior to plaque deposition in transgenic mice. BDNF promotes the integrity and function of synaptic connections and the function of neuronal circuits required for cognition, learning, and memory, and it is one of the most powerful neural repair molecules known. BDNF administration in animal models of AD can reverse morphological and behavioral symptoms even when administered late in the disease process. This suggests that the therapeutic use of BDNF might arrest or even correct the cognitive decline seen so early in the progression of AD. Methods to deliver or enhance BDNF and its signaling cascade are under investigation. A deeper understanding of the mechanisms that regulate BDNF in AD may provide additional therapeutic options.

References

Aggarwal, N. T., Wilson, R. S., Beck, T. L., Bienias, J. L., & Bennett, D. A. (2005). Mild cognitive impairment in different functional domains and incident Alzheimer's disease. *Journal of Neurology, Neurosurgery, and Psychiatry, 76*(11), 1479–84.

Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G. M., Wyss-Coray, T. (2000). Inflammation and Alzheimer's disease. *Neurobiology of Aging*, *21*, 383–421.

Alderson, R. F., Alterman, A. L., Barde, Y.-A., & Lindsay, R. M. (1990). Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. *Neuron, 5*, 297–306.

Anderson, K. D., Alderson, R. F., Altar, C. A., DiStefano, P. S., Corcoran, T. L., Lindsay, R. M., & Wiegand, S. J. (1995). Differential distribution of exogenous BDNF, NGF, and NT-3 in the brain corresponds to the relative abundance and distribution of high-affinity and low-affinity neurotrophin receptors. *The Journal of comparative neurology, 357*, 296–317.

Ando, S., Kobayashi, S., Waki, H., Kon, K., Fukui, F., Tadenuma, T., Iwamoto, M., Takeda, Y., Izumiyama, N., Watanabe, K., & Nakamura, H. (2002). Animal model of dementia induced by entorhinal synaptic damage and partial restoration of cognitive deficits by BDNF and carnitine. *Journal of neuroscience research, 70*, 519–527.

Aronoff, J. M., Gonnerman, L. M., Almor, A., Arunchalam, S., Kempler, D., & Andersen, E. S. (2006). Information content versus relational knowledge: Semantic deficits in patients with Alzheimer's disease. *Neuropsychologia, 44*(1), 21–35.

Atwal, J. K., Massie, B., Miller, F. D., & Kaplan, D. R. (2000). The TrkB-Shc site signals neuronal survival and local axon growth via MEK and P13-kinase. *Neuron*, *27*(2), 265–277.

Barco, A., Patterson, S. L., Alarcon, J. M., Gromova, P., Mata-Roig, M., Morozov, A., & Kandel, E. R. (2005). Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. *Neuron, 48*(1), 123–137.

Barco, A., Pittenger, C., & Kandel, E. R. (2003). CREB, memory enhancement and the treatment of memory disorders: Promises, pitfalls and prospects. *Expert Opinion on Therapeutic Targets, 7*(1), 101–114.

Baxter, M. G., & Chiba, A. A. (1999). Cognitive functions of the basal forebrain. *Current Opinion in Neurobiology, 9*(2), 178–183.

Berchtold, N. C., Kessiak, J. P., & Cotman, C. W. (2002). Hippocampal brain-derived neurotrophic factor gene regulation by exercise and the medial septum. *Journal of neuroscience research, 68*(5), 511–521.

Berninger, B., García, D. E., Inagaki, N., Hahnel, C., & Lindholm, D. (1993). BDNF and NT-3 induce intracellular Ca^{2+} elevation in hippocampal neurones. *Neuroreport, 4*(12), 1303–1306.

Blanquet, P. R., & Lamour, Y. (1997). Brain-derived neurotrophic factor increases Ca^{2+}/calmodulin-dependent protein kinase 2 activity in hippocampus. *The Journal of biological chemistry, 272*(39), 24133–24136.

Blurton-Jones, M., Kitazawa, M., Martinez-Coria, H., Castello, N. A., Muller, F.-J., Loring, J. F., Yamasaki, T. R., Poon, W. W., Green, K. N., & LaFerla, F. M. (2009). Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease. *Proceedings of the National Academy of Sciences U S A., 106*(32), 13594–13599.

Boado, R. J., Zhang, Y., Zhang, Y., & Pardridge, W. M. (2007). Genetic engineering, expression, and activity of a fusion protein of a human neurotrophin and a molecular trojan horse for delivery across the human blood–brain barrier. *Biotechnology and Bioengineering, 97*(6), 1376–1386.

Boekhoorn, K., Joels, M., & Lucassen, P. J. (2006). Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. *Neurobiology of Disease, 24*(1), 1–14.

Burdick, D., Soreghan, B., Kwon, M., Kosmoski, J., Knauer, M., Henschen, A., Yates, J., Cotman, C., & Glabe, C. (1992). Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. *The Journal of biological chemistry, 265*(1), 546–554.

Caccamo, A., Maldonado, M. A., Bokov, A. F., Majumder, S., & Oddo, S. (2010). CBP gene transfer increases BDNF levels and ameliorates learning and memory deficits in a mouse model of Alzheimer's disease. *PNAS, 107*(52), 22687–22692.

Chen, Q.-S., Kagan, B., Hirakura, Y., & Xie, C.-W. (2000). Impairment of hippocampal long-term potentiation by Alzheimer amyloid β-peptides. *Journal of Neuroscience Research*, *60*, 65–72.

Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A. Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I., & Selkoe, D. J. (1992). Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature, 360*(6405), 673–674.

Coleman, P. D., & Flood, D. G. (1987). Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. *Neurobiology of Aging, 8*, 521–545.

Coleman, P. D., & Yao, P. J. (2003). Synaptic slaughter in Alzheimer's disease. *Neurobiology of Aging, 24*, 1023–1027.

Connor, B., Young, D., Yan, Q., Faull, R. L., Synek, B., & Dragunow, M. (1997). Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain research. Molecular brain research, 49*, 71–81.

Cotman, C. W., & Berchtold, N. C. (2002). Exercise: A behavioral intervention to enhance brain health and plasticity. *Trends in neurosciences, 25*(6), 295–301.

Cotman, C., & Head, E. (2008). The canine (dog) model of human aging and disease: Dietary, environmental and immunotherapy approaches. *Journal of Alzheimer's disease, 15*(4), 685–707.

Coyle, J., Price, D., & DeLong, M. (1983). Alzheimer's disease: A disorder of cortical cholinergic innervation. *Science, 219*(4589), 1184–90.

Crews, L., Adame, A., Patrick, C., Delaney, A., Pham, E., Rockenstein, E., Hansen, L., & Masliah, E. (2010). Increased BMP6 levels in the brains of Alzheimer's disease patients and APP transgenic mice are accompanied by impaired neurogenesis. *The Journal of neuroscience, 30*(37), 12252–62.

Cullen, W., Suh, Y., Anwyl, R., & Rowan, M. (1997). Block of LTP in rat hippocampus in vivo by beta-amyloid precursor protein fragments. *Neuroreport*, *8*(15), 3213–3217.

DaRocha-Souto, B., Coma, M., Pérez-Nievas, B. G., Scotton, T. C., Siao, M., Sánchez-Ferrer, P., Hashimoto, T., Fan, Z., Hudry, E., Barroeta, I., Serenó, L., Rodríguez, M., Sánchez, M. B., Hyman, B. T., & Gómez-Isla, T. (2012). Activation of glycogen synthase kinase-3 beta mediates β-amyloid induced neuritic damage in Alzheimer's disease. *Neurobiology of disease, 45*(1), 425–437.

Davis, K. E., Easton, A., Eacott, M. J., Gigg, J. (2012) Episodic-like memory for what-where-which occasion is selectively impaired in the 3xTgAD mouse model of Alzheimer's disease. *Journal of Alzheimer's disease*. In press.

DeFelice, F., Velasco, P., Lambert, M., Viola, K., Fernandez, S., Ferreira, S., & Klein, W. (2007). Aβ oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *Journal of Biological. Chemistry, 282*(15), 11590–11601.

DeMarch, Z., Giampà, C., Patassini, S., Bernardi, G., & Fusco, F. R. (2008). Beneficial effects of rolipram in the R6/2 mouse model of huntington's disease. *Neurobiology of Disease, 30*(3), 375–387.

Demuro, A., Mina, E., Kayed, R., Milton, S., Parker, I., & Glabe, C. (2005). Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *The Journal of biological chemistry, 280*(17), 17294–17300.

DeStrooper, B., & Annaert, W. (2000). Proteolytic processing and cell biological functions of the amyloid precursor protein. *Journal of cell science, 113*, 1857–1870.

Devanand, D. P., Pradhaban, G., Liu, X., Khandji, A., De Santi, S., Segal, S., & de Leon, M. J. (2007). Hippocampal and entorhinal atrophy in mild cognitive impairment: prediction of Alzheimer disease. *Neurology*, *68*(11), 828–836.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Weinberger, D. R. (2003). The BDNF Val66Met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257–269.

España, J., Valero, J., Miñano-Molina, A. J., Masgrau, R., Martín, E., Guardia-Laguarta, C., Lleó, A., Giménez-Llort, L., Rodríguez-Alvarez, J., & Saura, C. A. (2010). beta-Amyloid disrupts activity-dependent gene transcription required for memory through the CREB coactivator CRTC1. *The Journal of neuroscience, 30*(28), 9402–9410.

Fahnestock, M., Garzon, D., Holsinger, R. M. D., & Michalski, B. (2002). Neurotrophic factors and Alzheimer's disease: Are we focusing on the wrong molecule? *Journal of Neural Transmission. Supplementa*, *62*, 241–252.

Fahnestock, M., Marchese, M., Head, E., Pop, V., Michalski, B., Milgram, W., & Cotman, C. (2012). BDNF increases with behavioral enrichment and an antioxidant diet in the aged dog. *Neurobiology of Aging, 33*, 546–554.

Falkenberg, T., Mohammed, A. K., Henriksson, B., Persson, H., Winblad, B., & Lindefors, N. (1992). Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment. *Neuroscience Letters, 138*(1), 153–156.

Fayard, B., Loeffler, S., Weis, J., Vögelin, E., & Krüttgen, A. (2005). The secreted brain-derived neurotrophic factor precursor pro-BDNF binds to TrkB and p75NTR but not to TrkA or TrkC. *Journal of neuroscience research, 80*(1), 18–28.

Ferreira, S. T., Vieira, M. N., & De Felice, F. G. (2007). Soluble protein oligomers as emerging toxins in Alzheimer's and other amyloid diseases. *IUBMB Life, 59*, 332–345.

Ferreiro, E., Resende, R., Costa, R., Oliveira, C., & Pereira, C. (2006). An endoplasmic-reticulum-specific apoptotic pathway is involved in prion and amyloid-beta peptides neurotoxicity. *Neurobiology of Disease, 23*, 669–678.

Ferrer, I., Marín, C., Rey, M. J., Ribalta, T., Goutan, E., Blanco, R., Tolosa, E., & Martí, E. (1999). BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *Journal of neuropathology and experimental neurology, 58*, 729–739.

Figurov, A., Pozzo-Miller, L. D., Olafsoon, P., Wang, T., & Lu, B. (1996). Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature, 381*(6584), 706–709.

Finkbeiner, S., Tavazoie, S. F., Maloratsky, A., Jacobs, K. M., Harris, K. M., & Greenberg, M. E. (1997). CREB: A major mediator of neuronal neurotrophin responses. *Neuron, 19*(5), 1031–1047.

Francis, B. M., Kim, J., Barakat, M. E., Fraenkl, S., Yücel, Y. H., Peng, S., Michalski, B., Fahnestock, M., McLaurin, J., & Mount, H. T. (2012). Object recognition memory and BDNF expression are reduced in young TgCRND8 mice. *Neurobiology of Aging, 33*(3), 555–563.

Fujioka, T., Fujioka, A., & Duman, R. S. (2004). Activation of cAMP signaling facilitates the morphological maturation of newborn neurons in adult hippocampus. *The Journal of neuroscience, 24*, 319–328.

Garzon, D. J., & Fahnestock, M. (2007). Oligomeric amyloid decreases basal levels of brain-derived neurotrophic factor (BDNF) mRNA via specific downregulation of BDNF transcripts IV and V in differentiated human neuroblastoma cells. *The Journal of neuroscience, 27*, 2628–2635.

Garzon, D. J., Yu, G., & Fahnestock, M. (2002). A new BDNF transcript and decrease in BDNF transcripts 1, 2 and 3 in Alzheimer's disease parietal cortex. *Journal of Neurochemistry, 82*, 1058–1064.

Ghosh, A., Carnahan, J., & Greenberg, M. E. (1994). Requirement for BDNF in activity-dependent survival of cortical neurons. *Science, 263*, 1618–1623.

Giovannini, M., Scali, C., Prosperi, C., Bellucci, A., Vannucchi, M., Rosi, S., & Casamenti, F. (2002). β-Amyloid-induced inflammation and cholinergic hypofunction in the rat brain *in vivo*: Involvement of the p38MAPK pathway. *Neurobiology of Disease*, *11*, 257–274.

Glabe, C. (2001). Intracellular mechanisms of amyloid accumulation and pathogenesis in Alzheimer's disease. *Journal of Molecular Neuroscience, 17*, 137–145.

Glabe, C. (2004). Conformation-dependent antibodies target diseases of protein misfolding. *Trends in biochemical sciences, 29*(10), 542–547.

Gomez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growdon, J. H., & Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *The journal of neuroscience, 16*, 4491–4500.

Gong, Y., Chang, L., Viola, K. L., Lacor, P. N., Lambert, M., Finch, C., Krafft, G., & Klein, W. L. (2003). Alzheimer's disease-affected brain: Presence of oligomeric Aβ ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proceedings National Academy of Sciences USA, 100*, 10417–10422.

Gonzalez, G. A., & Montminy, M. R. (1989). Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell, 59*, 675–680.

Gorski, J. A., Balogh, S. A., Wehner, J. M., & Jones, K. R. (2003). Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. *Neuroscience, 121*(2), 341–354.

Gotz, J., Chen, F., van Dorpe, J., & Nitsch, R. M. (2001). Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. *Science, 293*, 1491–1495.

Green, K. N., Smith, I. F., & Laferla, F. M. (2007). Role of calcium in the pathogenesis of Alzheimer's disease and transgenic models. *Sub-cellular biochemistry, 45*, 507–21.

Guillozet, A. L., Weintraub, S., Mash, D. C., & Mesulam, M. M. (2003). Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Archives of neurology, 60*, 729–736.

Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., McGaugh, J. L., Worley, P. F., & Barnes, C. A. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *The Journal of neuroscience, 20*(11), 3993–4001.

Haass, C., Koo, E., Mellon, A., Hung, A., & Selkoe, D. (1992). Targeting of cell-surface beta-amyloid precursor protein to lysosomes: Alternative processing into amyloid-bearing fragments. *Nature, 357*(6378), 500–503.

Haass, C., Hung, A., Selkoe, D., & Teplow, D. (1994). Mutations associated with a locus for familial Alzheimer's disease result in alternative processing of amyloid beta-protein precursor. *The Journal of biological chemistry, 269*(26), 17741–17748.

Hanna, A., Horne, P., Yager, D., Eckman, C., Eckman, E., & Janus, C. (2009). Amyloid beta and impairment in multiple memory systems in older transgenic APP TgCRND8 mice. *Genes, brain, and behavior, 8*(7), 676–684.

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science, 297*, 353–356.

Hariri, A. R., Goldberg, T. E., Mattay, V. S., Kolachana, B. S., Callicott, J. H., Egan, M. F., & Weinberger, D. R. (2003). Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *The Journal of neuroscience, 23*(17), 6690–6694.

Heldt, S. A., Stanek, L., Chhatwal, J. P., & Ressler, K. J. (2007). Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Molecular psychiatry, 12*(7), 656–670.

Heneka, M., & O'Banion, M. (2007). Inflammatory processes in Alzheimer's disease. *Journal of Neuroimmunology, 184*, 69–91.

Hock, C., Heese, K., Hulette, C., Rosenberg, C., & Otten, U. (2000). Region-specific neurotrophin imbalances in Alzheimer disease: Decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Archives of neurology, 57*, 846–851.

Holcomb, L., Gordon, M. N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., Wright, K., Saad, I., Mueller, R., Morgan, D., Sanders, S., Zehr, C., O'Campo, K., Hardy, J., Prada, C. M., Eckman, C., Younkin, S., Hsiao, K., & Duff, K. (1998). Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature medicine, 4*(1), 97–100.

Holsinger, R. M. D., Schnarr, J., Henry, P., Castelo, V. T., & Fahnestock, M. (2000). Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: Decreased levels in Alzheimer's disease. *Molecular Brain Research, 76*, 347–354.

Hutton, M., & Hardy, J. (1997). The presenilins and Alzheimer's disease. *Human molecular genetics, 6*(10), 1639–1646.

Hyman, B. T. (1997). The neuropathological diagnosis of Alzheimer's disease: Clinical-pathological studies. *Neurobiology of Aging, 18*(4 Suppl), S27–32.

Hyman, B. T., Van Horsen, G. W., Damasio, A. R., & Barnes, C. L. (1984). Alzheimer's disease: Cell-specific pathology isolates the hippocampal formation. *Science, 225*, 1168–1170.

Hyman, B. T., Phelps, C. H., Beach, T. G., Bigio, E. H., Cairns, N. J., Carrillo, M. C., Dickson, D. W., Duyckaerts, C., Frosch, M. P., Masliah, E., Mirra, S. S., Nelson, P. T., Schneider, J. A., Thal, D. R., Thies, B., Trojanowski, J. Q., Vinters, H. V., & Montine, T. J. (2012). National Institute on Aging-Alzheimer's Association guidelines for the neuropathological assessment of Alzheimer's disease. *Alzheimers Dement, 8*(1), 1–13.

Iqbal, K., & Grundke-Iqbal, I. (2008). Alzheimer neurofibrillary degeneration: Significance, etiopathogenesis, therapeutics and prevention. *Journal of Cellular and Molecular Medicine, 12*(1), 38–55.

Jacobsen, J. S., Wu, C.-C., Redwine, J. M., Comery, T. A., Arias, R., Bowlby, M., Martone, R., Morrison, J. H., Pangalos, M. N., Reinhart, P. H., & Bloom, F. E. (2006). Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. *Proceedings of National Academy of Sciences USA, 103*(13), 5161–6.

Jagasia, R., Steib, K., Englberger, E., Herold, S., Faus-Kessler, T., Saxe, M., Gage, F. H., Song, H., & Lie, D. C. (2009). GABA-cAMP response element-binding protein signaling regulates maturation and survival of newly generated neurons in the adult hippocampus. *The Journal of Neuroscience, 29*(25), 7966–7977.

Jang, S. W., Liu, X., Yepes, M., Shepherd, K. R., Miller, G. W., Liu, Y., Wilson, W. D., Xiao, G., Blanchi, B., Sun, Y. E., & Ye, K. (2010). A selective TrkB agonist with potent neurotrophic

activities by 7,8-dihydroxyflavone. *Proceedings of the National Academy of Sciences of the USA, 107*(6), 2687–2692.

Jarrett, J., Berger, E., & Lansbury, P., Jr. (1993). The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: Implications for the pathogenesis of Alzheimer's disease. *Biochemistry, 32*(18), 4693–4697.

Jin, K., Peel, A. L., Mao, X. O., Xie, L., Cottrell, B. A., Henshall, D. C., & Greenberg, D. A. (2004). Increased hippocampal neurogenesis in Alzheimer's disease. *Proceedings of the National Academy of Sciences of the USA, 101*(1), 343–7.

Knusel, B., Winslow, J. W., Rosenthal, A., Burton, L. E., Seid, D. P., Nikolics, K., & Hefti, F. (1991). Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin-3. *Proceedings of the National Academy of Sciences of the USA, 88*, 961–965.

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H., & Bonhoeffer, T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proceedings of the National Academy of Sciences of the USA, 92*, 8856–8860.

Koshimizu, H., Kiyosue, K., Hara, T., Hazama, S., Suzuki, S., Uegaki, K., Nagappan, G., Zaitsev, E., Hirokawa, T., Tatsu, Y., Ogura, A., Lu, B., & Kojima, M. (2009). Multiple functions of precursor BDNF to CNS neurons: Negative regulation of neurite outgrowth, spine formation and cell survival. *Molecular Brain, 2*, 27. doi:10.1186/1756-6606-2-27.

Kumar, V., Zhang, M.-X., Swank, M. W., Kunz, J., & Gang-Yi, W. (2005). Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. *Journal of Neuroscience, 25*(49), 11288–11299.

Kuruvilla, R., Ye, H., & Ginty, D. D. (2000). Spatially and functionally distinct roles of the PI3-K effector pathway during NGF signaling in sympathetic neurons. *Neuron*, *27*(3), 499–512.

Lacor, P. N., Buniel, M. C., Furlow, P. W., Clemente, A. S., Velasco, P. T., Wood, M., Viola, K. L., & Klein, W. L. (2007). Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *Journal of Neuroscience, 27*(4), 796–807.

Lambert, M. P., Barlow, A. K., Chromy, B. A., Edwards, C., Freed, R., Liosatos, M., Morgan, T. E., Rozovsky, I., Trommer, B., Viola, K. L., Wals, P., Zhang, C., Finch, C. E., Krafft, G. A., & Klein, W. L. (1998). Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proceedings of the National Academy of Sciences USA, 95*, 6448–6453.

Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*(5548), 1945–1948.

Lewis, J., Dickson, D. W., Wen-Lang, L., Chisholm, L., Corral, A., Jones, G., Shu-Hui, Y., Sahara, N., Skipper, L., Yager, D., Eckman, C., Hardy, J., Hutton, M., & McGowan, E. (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science, 293*, 1487–1491.

Li, L., Orozco, I. J., Planel, E., Yi, W., Bretteville, A., Krishnamurthy, P., Wang, L., Herman, M., Figueroa, H., Haung Yu, W., Arancio, O., & Duff, K. (2008). A transgenic rat that develops Alzheimer's disease-like amyloid pathology, deficits in synaptic plasticity and cognitive impairment. *Neurobiology of Disease, 31*(1), 46–57.

Lindholm, D., Carroll, P., Tzimagiorgis, G., & Thoenen, H. (1996). Autocrine-paracrine regulation of hippocampal neuron survival by IGF-1 and the neurotrophins BDNF, NT-3 and NT-4. *The European Journal of Neuroscience, 8*(7), 1452–1460.

Linnarsson, S., Björklund, A., & Ernfors, P. (1997). Learning deficit in BDNF mutant mice. *The European Journal of Neuroscience, 9*(12), 2581–2587.

Lowenstein, D. H., & Arsenault, L. (1996). The effects of growth factors on the survival and differentiation of cultured dentate gyrus neurons. *Journal of Neuroscience, 16*(5), 1759–1769.

Lu, B. (2003). BDNF and activity-dependent synaptic modulation. *Learning and memory, 10*, 86–98.

Lu, B., Pang, P. T., & Woo, N. H. (2005). The yin and yang of neurotrophin action. *Nature Reviews Neuroscience, 6*(8), 603–614.

Lue, L. F., Kuo, Y. M., Roher, A. E., Brachova, L., Shen, Y., Sue, L., Beach, T., Kurth, J. H., Rydel, R. E., & Rogers, J. (1999). Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *The American Journal of Pathology, 155*(3), 853–862.

Lukiw, W., & Bazan, N. (2000). Neuroinflammatory signaling upregulation in Alzheimer's disease. *Neurochemical Research, 25*(9/10), 1173–1184.

Lynch, M. A. (2004). Long-term potentiation and memory. *Physiological Reviews, 84*, 87–136.

Maher, P., Akaishi, T., & Abe, K. (2006). Flavonoid fisetin promotes ERK-dependent long-term potentiation and enhances memory. *Proceedings of the National Academy of Sciences USA, 103*, 16568–16573.

Markus, A., Zhong, J., & Snider, W. D. (2002). Raf and akt mediate distinct aspects of sensory axon growth. *Neuron, 35*, 65–76.

Masliah, E., Sisk, A., Mallory, M., & Games, D. (2001). Neurofibrillary pathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Journal of Neuropathology and Experimental Neurology, 60*, 357–368.

Massa, S. M., Yang, T., Xie, Y., Shi, J., Bilgen, M., Joyce, J. N., Nehama, D., Rajadas, J., & Longo, F. M. (2010). Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. *The Journal of Clinical Investigation, 120*(5), 1774–1785.

Massey, P. V., Johnson, B. E., Moult, P. R., Auberson, Y. P., Brown, M. W., Molnar, E., Collingridge, G. L., & Bashir, Z. I. (2004). Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *The Journal of Neuroscience, 24*(36), 7821–7828.

Mattson, M. (2004). Pathways towards and away from Alzheimer's disease. *Nature, 430*, 631–640.

Mattson, M., Barger, S., Cheng, B., Lieberburg, I., Smith-Swintosky, V., & Rydel, R. (1993). β-amyloid precursor protein metabolites and loss of neuronal Ca^{2+} homeostasis in Alzheimer's disease. *TINS, 16*(10), 409–414.

Mattson, M. P., Duan, W., Wan, R., & Guo, Z. (2004). Prophylactic activation of neuroprotective stress response pathways by dietary and behavioral manipulations. *NeuroRx, 1*(1), 111–116.

McKhann, G., Drachman, D. A., Folstein, M., Katzman, R., Price, D. L., Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease – report of the NINCDS–ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. *Neurology 34*, 939–944.

Merz, K., Herold, S., & Lie, C. (2012). CREB in adult neurogenesis – master and partner in development of adult-born neurons? *The European Journal of Neuroscience, 33*, 1078–1086.

Michalski, B., & Fahnestock, M. (2003). Pro-brain-derived neurotrophic factor is decreased in parietal cortex in Alzheimer's disease. *Molecular Brain Research, 111*, 148–154.

Minichiello, L., Korte, M., Wolfer, D., Kühn, R., Unsicker, K., Cestari, V., Rossi-Arnaud, C., Lipp, H. P., Bonhoeffer, T., & Klein, R. (1999). Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron, 24*(2), 401–414.

Mitchell, A. J., & Shiri-Feshki, M. (2009). Rate of progression of mild cognitive impairment to dementia–meta-analysis of 41 robust inception cohort studies. *Acta Psychiatrica Scandinavica, 119*(4), 252–265.

Mowla, S., Farhadi, H., Pareek, S., Atwal, J., Morris, S., Seidah, N., & Murphy, R. (2001). Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *The Journal of Biological Chemistry, 276*(16), 12660–12666.

Mu, Y., & Gage, F. H. (2011). Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Molecular Neurodegeneration, 6*, 85.

Murray, K. D., Gall, C. M., Jones, E. G., & Isackson P. J. (1994). Differential regulation of brain-derived neurotrophic factor and type II calcium/calmodulin-dependent protein kinase messenger RNA expression in Alzheimer's disease. *Neuroscience*, *60*(1), 37–48.

Murray, C. A., & Lynch, M. A. (1998). Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in long-term potentiation. *The Journal of Neuroscience, 18*, 2974–2981.

Nagahara, A. H., Merrill, D. A., Coppola, G., Tsukada, S., Schroeder, B. E., Shaked, G. M., Wang, L., Blesch, A., Kim, A., Conner, J. M., Rockenstein, E., Chao, M. V., Koo, E. H., Geschwind, D., Masliah, E., Chiba, A. A., & Tuszynski, M. H. (2009). Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nature Medicine, 15*(3), 331–337.

Nakagawa, S., Kim, J. E., Chen, J., Fujioka, T., Malberg, J., Tsuji, S., & Duman, R. S. (2002a). Localization of phosphorylated cAMP response element-binding protein in immature neurons of adult hippocampus. *Journal of the Neuroscience, 22*(22), 9868–9876.

Nakagawa, S., Kim, J. E., Lee, R., Malberg, J. E., Chen, J., Steffen, C., Zhang, Y. J., Nestler, E. J., & Duman, R. S. (2002b). Regulation of neurogenesis in adult mouse hippocampus by cAMP and cAMP response element-binding protein. *Journal of Neuroscience, 22*(9), 3673–3682.

Narisawa-Saito, M., Wakabayashi, K., Tsuji, S., Takahashi, H., & Nawa, H. (1996). Regional specificity of alterations in NGF, BDNF and NT-3 levels in Alzheimer's disease. *Neuroreport, 7*(18), 2925–2928.

Nikoletopoulou, V., Lickert, H., Frade, J. M., Rencurel, C., Giallonardo, P., Zhang, L., Bibel, M., & Barde, Y. A. (2010). Neurotrophin receptors TrkA and TrkC cause neuronal death whereas TrkB does not. *Nature, 467*(7311), 59–63.

Pang, P. T., Teng, H. K., Zaitsev, E., Woo, N. T., Sakata, K., Zhen, S., Teng, K. K., Yung, W. H., Hempstead, B. L., & Lu, B. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science*, *306*, 487–491.

Patterson, S. L., Abel, T., Deuel, T. A., Martin, K. C., Rose, J. C., & Kandel, E. R. (1996). Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron, 16*, 1137–1145.

Peng, S., Wuu, J., Mufson, E. J., & Fahnestock, M. (2005). Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *Journal of Neurochemistry, 93*, 1412–1421.

Peng, S., Garzon, D. J., Marchese, M., Klein, W., Ginsberg, S. D., Francis, B. M., Mount, H. T., Mufson, E. J., Salehi, A., & Fahnestock, M. (2009). Decreased brain-derived neurotrophic factor depends on amyloid aggregation state in transgenic mouse models of Alzheimer's disease. *The Journal of Neuroscience, 29*(29), 9321–9329.

Pennanen, L., & Gotz, J. (2005). Different tau epitopes define Abeta(42)-mediated tau insolubility. *Biochemical and Biophysical Research Communications, 337*(4), 1097–1101.

Phillips, H. S., Hains, J. M., Armanini, M., Laramee, G. R., Johnson, S. A., & Winslow, J. W. (1991). BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron, 7*, 695–702.

Pietropaolo, S., Paterna, J. C., Büeler, H., Feldon, H., & Yee, B. K. (2007). Bidirectional changes in water-maze learning following recombinant adenovirus-associated viral vector (rAAV)-mediated brain-derived neurotrophic factor expression in the rat hippocampus. *Behavioural Pharmacology*, *18*(5–6), 533–547.

Pittenger, C., & Kandel, E. (1998). A genetic switch for long-term memory. *C. R. Acad. Sci. III, 321*(2–3), 91–96.

Pruunsild, P., Kazantseva, A., Aid, T., Palm, K., & Timmusk, T. (2007). Dissecting the human BDNF locus: Bidirectional transcription, complex splicing, and multiple promoters. *Genomics, 90*(3), 397–406.

Rauskolb, S., Zagrebelsky, M., Dreznjak, A., Deogracias, R., Matsumoto, T., Wiese, S., Erne, B., Sendtner, M., Schaeren-Wiemers, N., Korte, M., & Barde, Y. A. (2010). Global deprivation of brain-derived neurotrophic factor in CNS reveals an area-specific requirement for dendritic growth. *Journal of Neuroscience, 30*(5), 1739–1749.

Resende, R., Pereira, C., Agostinho, P., Vieira, A., Malva, J., & Oliveira, C. (2007). Susceptibility of hippocampal neurons to Aβ peptide toxicity is associate with perturbation of Ca^{2+} homeostasis. *Brain Research, 1143*, 11–21.

Scharfman, H. E. (1997). Hyperexcitability in combined entorhinal/hippocampal slices of adult rat after exposure to brain-derived neurotrophic factor. *Journal of Neurophysiology, 78*, 1082–1095.

Scharfman, H., Goodman, J., Macleod, A., Phani, S., Antonelli, C., & Croll, S. (2004). Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Experimental Neurology, 192*(2), 348–356.

Scheff, S. W., & Price, D. A. (2003). Synaptic pathology in Alzheimer's disease: A review of ultrastructural studies. *Neurobiology of Aging, 24*, 1029–1046.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T. D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., & Younkin, S. (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nature Medicine, 2*(8), 864–870.

Selkoe, D. J. (1994). Amyloid beta-protein precursor: New clues to the genesis of Alzheimer's disease. *Current Opinion in Neurobiology, 4*(5), 708–716.

Shieh, P. B., Hu, S. C., Bobb, K., Timmusk, T., & Ghosh, A. (1998). Identification of a signaling pathway involved in calcium regulation of BDNF expression. *Neuron, 20*(4), 727–740.

Shoji, M., Golde, T. E., Ghiso, J., Cheung, T. T., Estus, S., Shaffer, L. M., Cai, X. D., McKay, D. M., Tintner, R., Frangione, B., et al. (1992). Production of the Alzheimer amyloid beta protein by normal proteolytic processing. *Science, 258*(5079), 126–129.

Singh, K. K., Park, K. J., Hong, E. J., Kramer, B. M., Greenberg, M. E., Kaplan, D. R., & Miller, F. D. (2008). Developmental axon pruning mediated by BDNF-p75NTR-dependent axon degeneration. *Nature Neuroscience, 11*(6), 64–658.

Soscia, S., Kirby, J., Washicosky, K., Tucker, S., Ingelsson, M., Hyman, B., Burton, M. A., Goldstein, L. E., Duong, S., Tanzi, R. E., Moir R. D. (2010). The Alzheimer's disease-associated amyloid β-protein is an antimicrobial peptide. *PLoS ONE, 5*(3) e9505. doi: 10.1371/journal.pone.0009505.

Sponne, I., Fifre, A., Drouet, B., Klein, C., Koziel, V., Pinçon-Raymond, M., Olivier, J. L., Chambaz, J., & Pillot, T. (2003). Apoptotic neuronal cell death induced by the non-fibrillar amyloid-β peptide proceeds through an early reactive oxygen species-dependent cytoskeleton perturbation. *The Journal of Biological Chemistry, 278*(5), 3437–3445.

Sun, Y., Lim, Y., Li, F., Liu, S., Lu, J.-J., Haberberger, R., Zhong, J.-H., & Zhou, X. F. (2012). ProBDNF collapses neurite outgrowth of primary neurons by activating RhoA. *PLOS ONE, 7*(4), e35883. doi:10.1371/journal.pone.0035883.

Tabaton, M., Zhu, X., Perry, G., Smith, M., & Giliberto, L. (2010). Signaling effect of amyloid-β_{42} on the processing of AβPP. *Experimental Neurology, 221*(1), 18–25.

Tancredi, V., D'Arcangelo, G., Grassi, F., Tarroni, P., Palmieri, G., Santoni, A., & Eusebi, F. (1992). Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. *Neuroscience Letters, 146*(2), 176–178.

Tancredi, V., D'Antuono, M., Cafè, C., Giovedì, S., Buè, M. C., D'Arcangelo, G., Onofri, F., & Benfenati, F. (2000). The inhibitory effects of interleukin-6 on synaptic plasticity in the rat hippocampus are associated with an inhibition of mitogen-activated protein kinase ERK. *Journal of Neurochemistry, 75*(2), 634–643.

Teng, H. K., Teng, K. K., Lee, R., Wright, S., Tevar, S., Almeida, R. D., Kermani, P., Torkin, R., Chen, Z. Y., Lee, F. S., Kraemer, R. T., Nykjaer, A., & Hempstead, B. L. (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *The Journal of Neuroscience, 25*(22), 5455–5463.

Terry, R. D., & Katzman, R. (2001). Life span and synapses: Will there be a primary senile dementia? *Neurobiology of Aging, 22*, 347–348.

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A., & Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Annals of Neurology, 30*(4), 572–580.

Timmusk, T., Palm, K., Metsis, M., Reintam, T., Paalme, V., Saarma, M., & Persson, H. (1993). Multiple promoters direct tissue-specific expression of the rate BDNF gene. *Neuron, 10*, 475–489.

Timmusk, T., Lendahl, U., Funakoshi, H., Arenas, E., Persson, H., & Metsis, M. (1995). Identification of brain-derived neurotrophic factor promoter regions mediating tissue-specific, axotomy-, and neuronal activity-induced expression in transgenic mice. *The Journal of Cell Biology, 128*, 185–199.

Tong, L., Thornton, P. L., Balazs, R., & Cotman, C. W. (2001). Beta-amyloid-(1–42) impairs activity-dependent cAMP-response element-binding protein signaling in neurons at concentrations in which cell survival is not compromised. *Journal of Biological Chemistry, 276*, 17301–17306.

Tong, L., Balazs, R., Thornton, P. L., & Cotman, C. W. (2004). Beta-amyloid peptide at sublethal concentrations downregulates brain-derived neurotrophic factor functions in cultured cortical neurons. *The Journal of Neuroscience, 24*, 6799–6809.

Van Dam, D., D'Hooge, R., Staufenbiel, M., Van Ginneken, C., Van Meir, F., & De Deyn, P. P. (2003). Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. *European Journal of Neuroscience, 17*(2), 388–396.

Van Hoesen, G. W., Hyman, B. T., & Damasio, A. R. (1991). Entorhinal cortex pathology in Alzheimer's disease. *Hippocampus, 1*(1), 1–8.

Vitolo, O. V., Sant'Angelo, A., Costanzo, V., Battaglia, F., Arancio, O., & Shelanski, M. (2002). Amyloid beta peptide inhibition of the PKA/CREB pathway and long-term potentiation: Reversibility by drugs that enhance cAMP signaling. *Proceedings of the National Academy of Sciences of the USA, 99*(20), 13217–13221.

Walsh, D. M., & Selkoe, D. J. (2007). A beta oligomers – a decade of discovery. *Journal of Neurochemistry, 101*, 1172–1184.

Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J., & Selkoe, D. J. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature, 416*, 535–539.

Westerman, M. A., Cooper-Blacketer, D., Mariash, A., Kotilinek, L., Kawarabayashi, T., Younkin, L. H., Carlson, G. A., Younkin, S. G., & Ashe, K. H. (2002). The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease. *J. Neurosci, 22*(5), 1858–1867.

Woo, N. H., Teng, H. K., Siao, C. J., Chiaruttini, C., Pang, P. T., Milner, T. A., Hempstead, B. L., & Lu, B. (2005). Activation of p75 NTR by proBDNF facilitates hippocampal long-term depression. *Nature Neuroscience, 8*(8), 1069–1077.

Xu, B., Michalski, B., Racine, R. J., & Fahnestock, M. (2004). The effects of brain-derived neurotrophic factor (BDNF) administration on kindling induction, Trk expression and seizure-related morphological changes. *Neuroscience, 126*(3), 521–531.

Xu, Y., Shen, J., Lou, X., Zhu, W., Chen, K., Ma, J., & Jiang, H. (2005). Conformational transition of amyloid beta-peptide. *Proceedings of the National Academy of Sciences of the USA, 102*(15), 5403–5407.

Yamamoto-Sasaki, M., Ozawa, H., Saito, T., Rosler, M., & Riederer, P. (1999). Impaired phosphorylation of cyclic AMP response element binding protein in the hippocampus of dementia of the Alzheimer's type. *Brain Research, 842*(2), 300–303.

Yamashita, T., & Tohyama, M. (2003). The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. *Nature Neuroscience, 6*(5), 461–467.

Yamashita, T., Tucker, K. L., & Barde, Y.-A. (1999). Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. *Neuron, 24*, 585–593.

Yamin, G. (2009). NMDA receptor-dependent signaling pathways that underlie amyloid beta-protein disruption of LTP in the hippocampus. *Journal of Neuroscience Research, 87*(8), 1729–36.

Ying, S. W., Futter, M., Rosenblum, K., Webber, M. J., Hunt, S. P., Bliss, T. V., & Bramham, C. R. (2002). Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: Requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. *Journal of Neuroscience, 22*(5), 1532–1540.

Ypsilanti, A. R., Girão da Cruz, M. T., Burgess, A., & Aubert, I. (2008). The length of hippocampal cholinergic fibers is reduced in the aging brain. *Neurobiology Aging, 29*(11), 1666–1679.

Zagrebelsky, M., Holz, A., Dechant, G., Barde, Y.-A., Bonhoeffer, T., & Korte, M. (2005). The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons. *Journal of Neuroscience, 25*(43), 9989–9999.

Zajac, M. S., Pang, T. Y., Wong, N., Weinrich, B., Leang, L. S., Craig, J. M., Saffery, R., & Hannan, A. J. (2010). Wheel running and environmental enrichment differentially modify exon-specific BDNF expression in the hippocampus of wild-type and pre-motor symptomatic male and female Huntington's disease mice. *Hippocampus, 20*(5), 621–636.

Ze-F, W., Li, H.-L., Li, X.-C., Zhang, Q., Tian, Q., Wang, Q., Xu, H., & Wang, J.-Z. (2006). Effects of endogenous beta-amyloid overproduction on tau phosphorylation in cell culture. *Journal of Neurochemistry, 98*, 1167–1175.

Zeng, Y., Zhao, D., & Xie, C. W. (2010). Neurotrophins enhance CaMKII activity and rescue amyloid-β-induced deficits in hippocampal synaptic plasticity. *Journal of Alzheimer's Disease, 21*, 823–831.

Zhang, Y., & Pardridge, W. M. (2001a). Conjugation of brain-derived neurotrophic factor to a blood–brain barrier drug targeting system enables neuroprotection in regional brain ischemia following intravenous injection of the neurotrophin. *Brain Research, 889*(1–2), 49–56.

Zhang, Y., & Pardridge, W. M. (2001b). Neuroprotection in transient focal brain ischemia after delayed intravenous administration of brain-derived neurotrophic factor conjugated to a blood–brain barrier drug targeting system. *Stroke, 32*(6), 1378–1384.

Zhang, Y., & Pardridge, W. M. (2006). Blood–brain barrier targeting of BDNF improves motor function in rats with middle cerebral artery occlusion. *Brain Research, 1111*(1), 227–229.

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell, 132*, 645–660.

BDNF V66M Polymorphism and Brain Functions

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Contents

Abstract

Brain derived neurotrophic factor (BDNF) plays important roles in neuronal survival, axonal and dendritic growth, synaptic plasticity, learning and memory and emotional behavior. Recently, a single nucleotide polymorphism (SNP) in the BDNF gene leading to a valine to methionine substitution at position 66 in BDNF prodomain (Val66Met) has been found to decrease activity-dependent BDNF secretion and to be associated with increased susceptibility to neuropsychiatric disorders. BDNF Val66Met polymorphism only exists in human and represents the first genetic alteration in a neurotrophin that has been linked to a human disease. In this chapter, we will focus on the biological consequences of BDNFVal66Met polymorphism in the context of central nervous system structure and function with combined molecular and cellular, human behavioral and imaging, and transgenic mouse studies.

Keywords

$BDNF_{Met}$ • Behavior • Brain structure • Neuropsychiatric disorders • Secretion

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1 Introduction

Brain-derived neurotrophic factor (BDNF), a molecule known to regulate neuronal survival and differentiation, plays an important role in activity-dependent plasticity processes, such as long-term potentiation (LTP), learning, and memory (Huang and Reichardt 2001; Chao 2003). BDNF is widely expressed in the development and adult mammalian brain and has been implicated in the actions of both antidepressants and mood stabilizers, as well as the pathophysiology of affective disorders (Schuman 1999; Castren and Rantamaki 2010; Krishnan and Nestler 2010). Therefore, BDNF plays a significant role in regulating specific synaptic functioning in learning, memory, and neuropsychiatric disorders. Recently, a single nucleotide polymorphism (SNP) in the BDNF gene leading to a valine-to-methionine substitution at position 66 in BDNF prodomain (Val66Met) has been identified and shown to influence human hippocampal volume and memory (Egan et al. 2003). This BDNF polymorphism is found to be associated with altered susceptibility to neuropsychiatric disorders including bipolar disorder, Alzheimer's disease, and depression (Neves-Pereira et al. 2002; Sklar et al. 2002; Ventriglia et al. 2002; Nederhof et al. 2010). BDNF Val66Met polymorphism only exists in human and represents the first genetic alteration in a neurotrophin that has been linked to a human disease. This chapter will be focused on the biological consequences of BDNF Val66Met polymorphism in the context of central nervous system structure and function.

2 $BDNF_{Met}$ SNP and Mental Disorders

The human BDNF gene is located on the short arm of chromosome 11 (11p14.1). One frequent, nonconservative polymorphism in the human BDNF gene (dbSNP number rs6265) has been identified, a single nucleotide polymorphism (SNP) at nucleotide 196 (G/A) producing an amino acid substitution (valine to methionine) at codon 66 (Val66Met). The frequencies for the alleles of the Val66Met polymorphism were A = 20–30 % and G = 70–80 % in Caucasians (Neves-Pereira et al. 2002; Egan et al. 2003). However, the frequency of $BDNF_{Met}$ SNP varies depending upon ethnicity. The frequency of healthy individuals who carried G/G (Val/Val) was significantly decreased in Japanese (33.8 %) than that in Italians (48.7 %) or in Americans (68.4 %) (Shimizu et al. 2004), which suggests that BDNF might act to produce ethnic traits.

Accumulating evidence suggests that BDNF plays a role in the pathophysiology of psychiatric diseases. A recent series of studies have linked BDNF Val66Met polymorphism with memory impairments as well as altered susceptibility to neuropsychiatric disorders, such as bipolar disorders, Alzheimer's disease, Parkinson's disease, depression, and eating disorders (Neves-Pereira et al. 2002; Ventriglia et al. 2002; Masaki et al. 2003; Ribases et al. 2003; Schumacher et al. 2005). This BDNF polymorphism represents the first alternation in a neurotrophin gene that has been linked to clinical pathology. Anxiety is a

common symptom among most psychiatric disorders. Several recent studies have looked at the relationship between the $BDNF_{Met}$ variant and the anxiety traits. The results have been conflicting, with the Val allele associated with vulnerability in one study and the Met allele designated as the "risk" allele in another study (Sen et al. 2003; Jiang et al. 2005; Lang et al. 2005). Inconsistency across genetic studies may be attributable to sampling and measurement issues, genetic heterogeneity due to differential sampling of populations, or low frequency of homozygous Met carriers, which may lessen the effect size of any particular association. It may also relate to a failure to take into account relevant gene-by-gene and gene-by-environment interactions. A recent investigation found the association between incident stroke and depression with the strongest association for Met/Met genotype participants (Kim et al. 2008), which provides evidence for a gene-environment interaction with respect to the impact of stroke on depression. Other studies revealed that BDNF interacted with childhood adversity or stressful life events that predicted greater likelihood of depression among Met carriers and did not influence Val/Val carriers, which further confirmed that the association of $BDNF_{Met}$ SNP with depression was modified by the environment factor such as stress (Bukh et al. 2009; Carver et al. 2011). A significant three-way interaction between BDNF genotype, 5-HTTLPR, and maltreatment history in predicting depression was also reported (Kaufman et al. 2006). Children with the Met allele of the BDNF gene and two short alleles of 5-HTTLPR had the highest depression scores, but the vulnerability associated with these two genotypes was only evident in the maltreated children. To further elucidate the role of $BDNF_{Met}$ variant in psychiatric disease, the $BDNF_{Met}$ knock-in mouse would be an ideal tool since the transgenic mouse would provide a more homogeneous gene background and the gene-by-gene and gene-by-environment effects will be more easily controlled in mouse behavior study.

3 Molecular Mechanism Underlying $BDNF_{Met}$ Variant

Brain-derived neurotrophic factor (BDNF) has been shown to play significant roles in neuronal survival, axonal and dendritic growth and guidance, synaptic structure and connections, neurotransmitter release, LTP, and synaptic plasticity (Chao 2003). Thus, the influence of BDNF spans from developmental neurobiology to neurodegenerative and psychiatric disorders. The actions of BDNF are dictated by two classes of cell surface receptors, the TrkB receptor tyrosine kinase and the p75 neurotrophin receptor ($p75^{NTR}$), a member of the TNF receptor superfamily (Chao et al. 2006). BDNF binding to TrkB receptor triggers receptor dimerization, transphosphorylation of intracellular tyrosine residues, and subsequent activation of the PI-3 kinase, Ras/MAPK, and PLC-γ pathways, thus influencing transcriptional events that have a multitude of effects on cell cycle, neurite outgrowth, and synaptic plasticity (Huang and Reichardt 2003; Teng and Hempstead 2004; Chao et al. 2006). Signal transduction through p75 independently gives rise to increase

in JNK (c-Jun N-terminal kinase), NF-κB (nuclear factor κB), and ceramide, thus triggering apoptosis (Roux and Barker 2002). Consistent with the critical role of BDNF in synaptic plasticity, BDNF is synthesized and released in a manner that is dependent on neuronal activity (Lessmann et al. 2003; Lu 2003; Bramham and Messaoudi 2005). In the mammalian brain, BDNF is synthesized as a precursor called proBDNF, which is proteolytically cleaved to generate mature BDNF. ProBDNF may preferentially bind $p75^{NTR}$, whereas mature BDNF preferentially binds TrkB receptor (Teng et al. 2005; Nagappan et al. 2009; Yang et al. 2009).

The molecular mechanisms underlying altered $BDNF_{Met}$ function have begun to be studied primarily in vitro cell culture systems. The Met substitution in BDNF prodomain was shown in neurosecretory cells and primary cultured neurons to lead to four trafficking defects: (1) decreased variant $BDNF_{Met}$ distribution into neuronal dendrites, (2) decreased variant $BDNF_{Met}$ targeting to secretory granules, (3) subsequent impairment in BDNF-regulated secretion, and (4) impaired dendritic targeting of BDNF mRNA (Egan et al. 2003; Chen et al. 2004; Chen et al. 2005; Chiaruttini et al. 2009). The molecular mechanisms underlying the trafficking defects associated with $BDNF_{Met}$ variant are described in detail below.

When expressed together in the same cell, $BDNF_{Met}$ alters the trafficking of wild-type BDNF ($BDNF_{Val}$) through the formation of heterodimers that are less efficiently sorted into the regulated secretory pathway (Chen et al. 2004). These findings are consistent with previous studies indicating that the prodomain of neurotrophins plays important role in regulating their intracellular trafficking to secretory pathways (Suter et al. 1991; Mowla et al. 1999). These in vitro studies with $BDNF_{Met}$ point to the presence of a specific trafficking signal in the BDNF prodomain region encompassing the Met substitution that is required for efficient BDNF sorting. Following this hypothesis, a trafficking protein, sortilin, was identified to be necessary for the efficient sorting of BDNF to the regulated secretory pathway. Sortilin interacts specifically with BDNF in a region encompassing the Met substitution (Chen et al. 2005). Replacement of Met at this position led to decreased interaction of BDNF with sortilin and suggests that decreased protein-protein interaction between BDNF and the trafficking machinery is one plausible molecular model for the secretion defect observed with the variant BDNF. Targeting of BDNF mRNA to dendrites also plays a key role in mediating synaptic plasticity (An et al. 2008; Tongiorgi and Baj 2008). Constitutive dendritic targeting of BDNF mRNA is mediated by translin, an RNA-binding protein implicated in RNA trafficking (Chiaruttini et al. 2009). $BDNF_{Met}$ mutation blocks dendritic targeting of BDNF mRNA by disrupting its interaction with translin (Chiaruttini et al. 2009). A $BDNF_{Met}$ knock-in transgenic mouse was recently generated and provided a unique tool to study the in vivo consequences of $BDNF_{Met}$ SNP (Chen et al. 2006). The transcription of $BDNF_{Met}$ is regulated by endogenous BDNF promoters in the $BDNF_{Met}$ knock-in mouse, which fully mimics the human $BDNF_{Met}$ polymorphism. When hippocampal neurons were cultured from $BDNF_{Met}$ mice, a significant decrease in the BDNF-regulated secretion was detected, which suggested an insufficiency in available BDNF in $BDNF_{Met}$ mice (Chen et al. 2006). In $BDNF_{Met/Met}$ mice, pilocarpine-induced dendritic trafficking

of BDNF mRNA was also blocked, which further confirmed the in vitro study (Chiaruttini et al. 2009). Thus, the in vitro findings of the trafficking defects associated with $BDNF_{Met}$ variant were verified in $BDNF_{Met}$ knock-in mouse.

It also remains possible that there are additional defects in $BDNF_{Met}$ processing, although in vitro studies in neurons suggest no defect in $BDNF_{Met}$ processing (Egan et al. 2003; Chen et al. 2004). It has been reported that tissue plasminogen activator, by activating the extracellular protease plasmin, converts proBDNF to the mature BDNF and that such conversion is critical for late-phase long-term potentiation expression in the mouse hippocampus (Pang et al. 2004). Given that proBDNF preferentially activates $p75^{NTR}$ over TrkB receptor, it is likely that proteolytic conversation of proBDNF may be implicated in the BDNF function. Whether $BDNF_{Met}$ affects BDNF processing should be further examined in vivo in the $BDNF_{Met}$ knock-in mice.

4 Brain Structure Alterations Associated with $BDNF_{Met}$ Polymorphism

The fundamental question is what the in vivo consequences of $BDNF_{Met}$ SNP are. One of the most reliable effects observed in carriers of the Met allele is a reduction in hippocampal volume. In studies of human brain morphometry using structural MRI scans, Val/Met individuals have repeatedly been shown to have a smaller hippocampal volume relative to controls who are homozygous for Val allele (Val/Val) (Pezawas et al. 2004; Szeszko et al. 2005; Bueller et al. 2006). This difference may be related to the role that BDNF and its receptors play in the development as well as continued plasticity of the brain (Huang and Reichardt 2001; Lu et al. 2005). In addition to hippocampus, studies have shown decreased volume in the dorsolateral prefrontal cortex, an area associated with planning and higher-order cognitive functioning, in carriers of the Met allele (Pezawas et al. 2004). It was also found that Met allele carriers have smaller temporal and occipital lobar gray matter volumes (Ho et al. 2006). Interestingly, the effect of $BDNF_{Met}$ SNP on brain structure is modified by neuropsychiatric disorders. The bipolar disorder patients who carried the $BDNF_{Met}$ allele had the smallest hippocampus volumes compared to individuals without bipolar disorder and Val/Val homozygotes, and these decreases are most prominent in left anterior hippocampus (Chepenik et al. 2009; Matsuo et al. 2009). Within the bipolar cohort, individuals with one or more BDNF Met alleles showed greater losses in prefrontal gyrification index (GI) over time (Mirakhur et al. 2009). These studies suggest that the $BDNF_{Met}$ SNP may play a more prominent role in brain structure differences in subjects affected with bipolar disorder. Within schizophrenia patients, Met allele carriers had significantly greater reductions in frontal gray matter volume than Val-homozygous patients (Ho et al. 2007). Major depression patients had significantly smaller hippocampal volumes compared with controls and significantly smaller hippocampal volumes were observed for patients carrying the Met-BDNF allele compared with subjects homozygous for

the Val-BDNF allele, which suggest that Met-BDNF allele carriers might be at risk to develop smaller hippocampal volumes and may be susceptible to major depression (Frodl et al. 2007).

Brain structure alterations associated with $BDNF_{Met}$ SNP were further analyzed in $BDNF_{Met}$ knock-in mouse. Using Cavalieri volume estimation, a significant decrease (about 14 %) in hippocampal volume in $BDNF_{Met}$ mice was observed, which was comparable to the decrease in the humans carriers of Met allele (Pezawas et al. 2004; Chen et al. 2006). Ventromedial prefrontal cortex (vmPFC) volume was also found to be significantly decreased in $BDNF_{Met/Met}$ mice compared with wild-type mice (Yu et al. 2009). Golgi staining and fractal dimension analyses further revealed a significant decrease in dendritic complexity in the dentate gyrus and vmPFC neurons from $BDNF_{Met}$ mice (Chen et al. 2006; Yu et al. 2009), which might in part account for the decreased volume in $BDNF_{Met}$ carriers.

5 Behavior Phenotypes Associated with $BDNF_{Met}$ Polymorphism

One of the common clinical symptoms among the psychiatric disease affected by the $BDNF_{Met}$ variant is the impairment of higher cognitive abilities. Individuals with the Val/Met genotype have been shown to be associated with poorer episodic memory in comparison with Val homozygotes, which rely heavily on the hippocampus (Egan et al. 2003; Hariri et al. 2003). Moreover, functional magnetic resonance imaging (fMRI) studies showed Met carriers exhibited relatively diminished hippocampal activation compared with Val/Val individuals during both the encoding and retrieval processes of a declarative memory task (Egan et al. 2003; Hariri et al. 2003). These findings taken together suggest that carriers of the Met allele have a selective impairment in hippocampal-dependent memory. In bipolar patients, the performance in Wisconsin card sort task (WCST) was significantly better in subjects with Val/Val-BDNF genotype compared with Val/Met genotype, which suggests a role of $BDNF_{Met}$ SNP in prefrontal cognitive function in bipolar illness (Rybakowski et al. 2003). In both patients with schizophrenia and healthy volunteers, Met allele carriers had poorer verbal memory performance than their Val-homozygous counterparts. In visuospatial abilities, Met allele-associated visuospatial impairment was specific to patients with schizophrenia but not healthy volunteers (Ho et al. 2006). These studies suggest that the association between the $BDNF_{Met}$ variant and poor medial temporal lobe-related memory performance and that the $BDNF_{Met}$ variant may have a specific role in conferring visuospatial dysfunction in schizophrenia (Ho et al. 2006). However, $BDNF_{Met}$ variant did not appear to impact general intellectual abilities or affect attention, problem solving, and language skills (Ho et al. 2006). A recent study also showed that $BDNF_{Met}$ polymorphism is associated with differences in brain motor system function, altered short-term plasticity, and greater error in short-term motor learning (McHughen et al. 2010).

The $BDNF_{Met}$ knock-in mouse provided a unique tool to assess the behavior phenotypes associated with $BDNF_{Met}$ polymorphism in a controlled genetic and environmental background which was not feasible in humans. In the fear conditioning test, $BDNF_{Met}$ mice showed significant less context-dependent memory than wild-type mice (Chen et al. 2006), which was consistent with the hippocampal-dependent learning defect in human carriers of the $BDNF_{Met}$ allele. When placed in conflict settings, $BDNF_{Met/Met}$ mice display increased anxiety-related behaviors in three separate tests (elevated plus maze, open field, and novelty-induced hypophagia) and thus provide a link between $BDNF_{Met}$ SNP and anxiety (Chen et al. 2006). Interestingly, the elevated anxiety in $BDNF_{Met/Met}$ mice could not be rescued by a common serotonin reuptake inhibitor (SSRI) fluoxetine, which suggests that humans with the $BDNF_{Met}$ allele may not have optimal responses to this class of antidepressants. The $BDNF_{Met/Met}$ mouse may serve as a valuable model to identify novel pharmacologic approaches to treat anxiety symptoms that underlie many neuropsychiatric disorders. $BDNF_{Met/Met}$ mouse was further found to exhibit abnormalities in aversive memory extinction (Yu et al. 2009). This abnormality in extinction learning may be explained by alterations in neuronal morphology, as well as decreased neural activity in the vmPFC. Recently, Soliman et al. reported the impaired extinction learning in human $BDNF_{Met}$ allele carriers relative to non-Met allele carriers, which was paralleled by atypical frontoamygdala activity in humans (Soliman et al. 2010). Understanding the effect of $BDNF_{Met}$ allele on impaired learning of cues that signal safety versus threat provided insight into the role of $BDNF_{Met}$ SNP in anxiety disorders. In $BDNF_{Met/Met}$ mouse, D-cycloserine (DCS), a partial agonist of NMDA receptor, was effective in rescuing the extinction defect (Yu et al. 2009), suggesting that when coupled with behavior therapy, DCS may be an effective treatment option for anxiety disorders in humans with this genetic variant BDNF.

6 Conclusion

Brain-derived neurotrophic factor (BDNF) plays important roles in activity-dependent plasticity processes and has been implicated in the pathophysiology of affective disorders. The human genetic variant BDNF (Val66Met) represents the first example of neurotrophin family member and has been linked to psychiatric disorders. Combined molecular and cellular, human behavioral and imaging, and transgenic mouse approaches were employed to assess the impact of $BDNF_{Met}$ SNP on brain structure and function. The mouse model provides the opportunity to test dose-dependent effects of the $BDNF_{Met}$ allele in a controlled genetic and environmental background not feasible in humans. The human behavioral and imaging findings provide confidence that cross-species translation is biologically valid. $BDNF_{Met}$ variant has been associated with cognitive impairment and anxiety; drug discovery strategies to increase BDNF release from synapses, prolong the half-life of secreted BDNF, or directly activate TrkB receptors may provide greater therapeutic responses for human populations with this common BDNF polymorphism. In addition, the $BDNF_{Met/Met}$ mouse may serve as a valuable model to evaluate novel pharmacologic strategy in humans with this polymorphic allele and may ultimately guide personalized medicine for related clinical disorders.

References

An, J. J., Gharami, K., Liao, G. Y., Woo, N. H., Lau, A. G., Vanevski, F., Torre, E. R., Jones, K. R., Feng, Y., Lu, B., & Xu, B. (2008). Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell, 134*, 175–187.

Bramham, C. R., & Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. *Progress in Neurobiology, 76*, 99–125.

Bueller, J. A., Aftab, M., Sen, S., Gomez-Hassan, D., Burmeister, M., & Zubieta, J. K. (2006). BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. *Biological Psychiatry, 59*, 812–815.

Bukh, J. D., Bock, C., Vinberg, M., Werge, T., Gether, U., & Vedel Kessing, L. (2009). Interaction between genetic polymorphisms and stressful life events in first episode depression. *Journal of Affective Disorders, 119*, 107–115.

Carver, C. S., Johnson, S. L., Joormann, J., Lemoult, J., & Cuccaro, M. L. (2011). Childhood adversity interacts separately with 5-HTTLPR and BDNF to predict lifetime depression diagnosis. *Journal of Affective Disorders, 132*, 89–93.

Castren, E., & Rantamaki, T. (2010). The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. *Developmental Neurobiology, 70*, 289–297.

Chao, M. V. (2003). Neurotrophins and their receptors: A convergence point for many signalling pathways. *Nature Reviews Neuroscience, 4*, 299–309.

Chao, M. V., Rajagopal, R., & Lee, F. S. (2006). Neurotrophin signalling in health and disease. *Clinical Science (London), 110*, 167–173.

Chen, Z. Y., Patel, P. D., Sant, G., Meng, C. X., Teng, K. K., Hempstead, B. L., & Lee, F. S. (2004). Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *Journal of Neuroscience, 24*, 4401–4411.

Chen, Z. Y., Ieraci, A., Teng, H., Dall, H., Meng, C. X., Herrera, D. G., Nykjaer, A., Hempstead, B. L., & Lee, F. S. (2005). Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. *Journal of Neuroscience, 25*, 6156–6166.

Chen, Z. Y., Jing, D., Bath, K. G., Ieraci, A., Khan, T., Siao, C. J., Herrera, D. G., Toth, M., Yang, C., McEwen, B. S., Hempstead, B. L., & Lee, F. S. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science, 314*, 140–143.

Chepenik, L. G., Fredericks, C., Papademetris, X., Spencer, L., Lacadie, C., Wang, F., Pittman, B., Duncan, J. S., Staib, L. H., Duman, R. S., Gelernter, J., & Blumberg, H. P. (2009). Effects of the brain-derived neurotrophic growth factor val66met variation on hippocampus morphology in bipolar disorder. *Neuropsychopharmacology, 34*, 944–951.

Chiaruttini, C., Vicario, A., Li, Z., Baj, G., Braiuca, P., Wu, Y., Lee, F. S., Gardossi, L., Baraban, J. M., & Tongiorgi, E. (2009). Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. *Proceedings of the National Academy of Sciences U S A, 106*, 16481–16486.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Weinberger, D. R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257–269.

Frodl, T., Schule, C., Schmitt, G., Born, C., Baghai, T., Zill, P., Bottlender, R., Rupprecht, R., Bondy, B., Reiser, M., Moller, H. J., & Meisenzahl, E. M. (2007). Association of the brain-derived neurotrophic factor Val66Met polymorphism with reduced hippocampal volumes in major depression. *Archives of General Psychiatry, 64*, 410–416.

Hariri, A. R., Goldberg, T. E., Mattay, V. S., Kolachana, B. S., Callicott, J. H., Egan, M. F., & Weinberger, D. R. (2003). Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *Journal of Neuroscience, 23*, 6690–6694.

Ho, B. C., Milev, P., O'Leary, D. S., Librant, A., Andreasen, N. C., & Wassink, T. H. (2006). Cognitive and magnetic resonance imaging brain morphometric correlates of brain-derived neurotrophic factor Val66Met gene polymorphism in patients with schizophrenia and healthy volunteers. *Archives of General Psychiatry, 63*, 731–740.

Ho, B. C., Andreasen, N. C., Dawson, J. D., & Wassink, T. H. (2007). Association between brain-derived neurotrophic factor Val66Met gene polymorphism and progressive brain volume changes in schizophrenia. *The American Journal of Psychiatry, 164*, 1890–1899.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: Roles in neuronal development and function. *Annual Review of Neuroscience, 24*, 677–736.

Huang, E. J., & Reichardt, L. F. (2003). Trk receptors: Roles in neuronal signal transduction. *Annual Review of Biochemistry, 72*, 609–642.

Jiang, X., Xu, K., Hoberman, J., Tian, F., Marko, A. J., Waheed, J. F., Harris, C. R., Marini, A. M., Enoch, M. A., & Lipsky, R. H. (2005). BDNF variation and mood disorders: A novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. *Neuropsychopharmacology, 30*, 1353–1361.

Kaufman, J., Yang, B. Z., Douglas-Palumberi, H., Grasso, D., Lipschitz, D., Houshyar, S., Krystal, J. H., & Gelernter, J. (2006). Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. *Biological Psychiatry, 59*, 673–680.

Kim, J. M., Stewart, R., Kim, S. W., Yang, S. J., Shin, I. S., Kim, Y. H., & Yoon, J. S. (2008). BDNF genotype potentially modifying the association between incident stroke and depression. *Neurobiology of Aging, 29*, 789–792.

Krishnan, V., & Nestler, E. J. (2010). Linking molecules to mood: New insight into the biology of depression. *The American Journal of Psychiatry, 167*, 1305–1320.

Lang, U. E., Hellweg, R., Kalus, P., Bajbouj, M., Lenzen, K. P., Sander, T., Kunz, D., & Gallinat, J. (2005). Association of a functional BDNF polymorphism and anxiety-related personality traits. *Psychopharmacology (Berl), 180*, 95–99.

Lessmann, V., Gottmann, K., & Malcangio, M. (2003). Neurotrophin secretion: Current facts and future prospects. *Progress in Neurobiology, 69*, 341–374.

Lu, B. (2003). BDNF and activity-dependent synaptic modulation. *Learning & Memory, 10*, 86–98.

Lu, B., Pang, P. T., & Woo, N. H. (2005). The yin and yang of neurotrophin action. *Nature Reviews Neuroscience, 6*, 603–614.

Masaki, T., Matsushita, S., Arai, H., Takeda, A., Itoyama, Y., Mochizuki, H., Kamakura, K., Ohara, S., & Higuchi, S. (2003). Association between a polymorphism of brain-derived neurotrophic factor gene and sporadic Parkinson's disease. *Annals of Neurology, 54*, 276–277.

Matsuo, K., Walss-Bass, C., Nery, F. G., Nicoletti, M. A., Hatch, J. P., Frey, B. N., Monkul, E. S., Zunta-Soares, G. B., Bowden, C. L., Escamilla, M. A., & Soares, J. C. (2009). Neuronal correlates of brain-derived neurotrophic factor Val66Met polymorphism and morphometric abnormalities in bipolar disorder. *Neuropsychopharmacology, 34*, 1904–1913.

McHughen, S. A., Rodriguez, P. F., Kleim, J. A., Kleim, E. D., Marchal Crespo, L., Procaccio, V., & Cramer, S. C. (2010). BDNF val66met polymorphism influences motor system function in the human brain. *Cerebral Cortex, 20*, 1254–1262.

Mirakhur, A., Moorhead, T. W., Stanfield, A. C., McKirdy, J., Sussmann, J. E., Hall, J., Lawrie, S. M., Johnstone, E. C., & McIntosh, A. M. (2009). Changes in gyrification over 4 years in bipolar disorder and their association with the brain-derived neurotrophic factor valine(66) methionine variant. *Biological Psychiatry, 66*, 293–297.

Mowla, S. J., Pareek, S., Farhadi, H. F., Petrecca, K., Fawcett, J. P., Seidah, N. G., Morris, S. J., Sossin, W. S., & Murphy, R. A. (1999). Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. *The Journal of Neuroscience, 19*, 2069–2080.

Nagappan, G., Zaitsev, E., Senatorov, V. V., Jr., Yang, J., Hempstead, B. L., & Lu, B. (2009). Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. *Proceedings of the National Academy of Sciences U S A, 106*, 1267–1272.

Nederhof, E., Bouma, E. M., Oldehinkel, A. J., & Ormel, J. (2010). Interaction between childhood adversity, brain-derived neurotrophic factor val/met and serotonin transporter promoter polymorphism on depression: The TRAILS study. *Biological psychiatry, 68*, 209–212.

Neves-Pereira, M., Mundo, E., Muglia, P., King, N., Macciardi, F., & Kennedy, J. L. (2002). The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: Evidence from a family-based association study. *American Journal of Human Genetics, 71*, 651–655.

Pang, P. T., Teng, H. K., Zaitsev, E., Woo, N. T., Sakata, K., Zhen, S., Teng, K. K., Yung, W. H., Hempstead, B. L., & Lu, B. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science, 306*, 487–491.

Pezawas, L., Verchinski, B. A., Mattay, V. S., Callicott, J. H., Kolachana, B. S., Straub, R. E., Egan, M. F., Meyer-Lindenberg, A., & Weinberger, D. R. (2004). The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *The Journal of Neuroscience, 24*, 10099–10102.

Ribases, M., Gratacos, M., Armengol, L., de Cid, R., Badia, A., Jimenez, L., Solano, R., Vallejo, J., Fernandez, F., & Estivill, X. (2003). Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Molecular Psychiatry, 8*, 745–751.

Roux, P. P., & Barker, P. A. (2002). Neurotrophin signaling through the p75 neurotrophin receptor. *Progress in Neurobiology, 67*, 203–233.

Rybakowski, J. K., Borkowska, A., Czerski, P. M., Skibinska, M., & Hauser, J. (2003). Polymorphism of the brain-derived neurotrophic factor gene and performance on a cognitive prefrontal test in bipolar patients. *Bipolar Disorders, 5*, 468–472.

Schumacher, J., Jamra, R. A., Becker, T., Ohlraun, S., Klopp, N., Binder, E. B., Schulze, T. G., Deschner, M., Schmal, C., Hofels, S., Zobel, A., Illig, T., Propping, P., Holsboer, F., Rietschel, M., Nothen, M. M., & Cichon, S. (2005). Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and major depression. *Biological Psychiatry, 58*, 307–314.

Schuman, E. M. (1999). Neurotrophin regulation of synaptic transmission. *Current Opinion in Neurobiology, 9*, 105–109.

Sen, S., Nesse, R. M., Stoltenberg, S. F., Li, S., Gleiberman, L., Chakravarti, A., Weder, A. B., & Burmeister, M. (2003). A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology, 28*, 397–401.

Shimizu, E., Hashimoto, K., & Iyo, M. (2004). Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: The possibility to explain ethnic mental traits. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 126B*, 122–123.

Sklar, P., Gabriel, S. B., McInnis, M. G., Bennett, P., Lim, Y. M., Tsan, G., Schaffner, S., Kirov, G., Jones, I., Owen, M., Craddock, N., DePaulo, J. R., & Lander, E. S. (2002). Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. *Molecular Psychiatry, 7*, 579–593.

Soliman, F., Glatt, C. E., Bath, K. G., Levita, L., Jones, R. M., Pattwell, S. S., Jing, D., Tottenham, N., Amso, D., Somerville, L. H., Voss, H. U., Glover, G., Ballon, D. J., Liston, C., Teslovich, T., Van Kempen, T., Lee, F. S., & Casey, B. J. (2010). A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science, 327*, 863–866.

Suter, U., Heymach, J. V., Jr., & Shooter, E. M. (1991). Two conserved domains in the NGF propeptide are necessary and sufficient for the biosynthesis of correctly processed and biologically active NGF. *The EMBO Journal, 10*, 2395–2400.

Szeszko, P. R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., Ashtari, M., Napolitano, B., Bilder, R. M., Kane, J. M., Goldman, D., & Malhotra, A. K. (2005). Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. *Molecular Psychiatry, 10*, 631–636.

Teng, K. K., & Hempstead, B. L. (2004). Neurotrophins and their receptors: Signaling trios in complex biological systems. *Cellular and Molecular Life Sciences, 61*, 35–48.

Teng, H. K., Teng, K. K., Lee, R., Wright, S., Tevar, S., Almeida, R. D., Kermani, P., Torkin, R., Chen, Z. Y., Lee, F. S., Kraemer, R. T., Nykjaer, A., & Hempstead, B. L. (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *The Journal of Neuroscience, 25*, 5455–5463.

Tongiorgi, E., & Baj, G (2008) Functions and mechanisms of BDNF mRNA trafficking. Novartis found Symposium 289:136–147; discussion 147–151, 193–135.

Ventriglia, M., Bocchio Chiavetto, L., Benussi, L., Binetti, G., Zanetti, O., Riva, M. A., & Gennarelli, M. (2002). Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. *Molecular Psychiatry, 7*, 136–137.

Yang, J., Siao, C. J., Nagappan, G., Marinic, T., Jing, D., McGrath, K., Chen, Z. Y., Mark, W., Tessarollo, L., Lee, F. S., Lu, B., & Hempstead, B. L. (2009). Neuronal release of proBDNF. *Nature Neuroscience, 12*, 113–115.

Yu, H., Wang, Y., Pattwell, S., Jing, D., Liu, T., Zhang, Y., Bath, K. G., Lee, F. S., & Chen, Z. Y. (2009). Variant BDNF Val66Met polymorphism affects extinction of conditioned aversive memory. *Journal of Neuroscience, 29*, 4056–4064.

Blood Brain-Derived Neurotrophic Factor Levels and Mood Disorders

Reiji Yoshimura and Jun Nakumura

Contents

Abstract

Blood levels of brain-derived neurotrophic factor (BDNF) plays an important role in mood disorders. It is considered that blood (serum or plasma) BDNF could be a candidate biological state marker for depressive state. In addition, blood BDNF levels might also reflect psychological job stress in healthy workers.

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1 Introduction

Depression is heterogeneous and is diagnosed on the basis of a patient's symptoms, not on the basis of a laboratory test. Therefore, it is important to search for biological markers for depression due to the need of finding diagnostic adjuncts. Brain-derived neurotrophic factor (BDNF), a major neurotrophic factor, has been found to play a critical role in long-term potentiation, a cellular mechanism of learning and memory, suggesting that it can influence neuroplasticity (Fignov et al. 1996). BDNF is also needed for the survival and guidance of neurons during development as well as the survival and function of neurons during adulthood (Duman et al. 2000). The atrophy and loss of hippocampal or cerebral cortical neurons or of glia could result from a stress-induced loss of neurotrophic factors, from other processes that compromise neuronal function and activity, or from other insults depending on the patient's genetic background (Shelton 2000). There is growing evidence that BDNF might have a crucial role in depression (Duman et al. 2000; Mowla et al. 2001). The source of circulating BDNF remains unknown. Platelets, brain neurons, and vascular endothelial cells are currently considered to be putative sources. It was demonstrated that BDNF crosses the blood–brain barrier (Pan et al. 1998) and that BDNF levels in the brain and serum have been shown to undergo similar changes during the maturation and aging process in rats (Karege et al. 2002). Furthermore, Lang et al. reported that serum BDNF concentrations reflect some aspects of neuronal plasticity, as indicated by the association of BDNF levels with those of *N*-acetyl-aspartate, which reflects neuronal damage in the cerebral cortex (Lang et al. 2007). These results indicated that blood BDNF levels might in part reflect the BDNF levels in the brain. Moreover, Klein et al. (2011) recently reported that measures of blood and plasma BDNF levels reflect brain-tissue BDNF levels. We reported that serum BDNF levels are approximately 20 times greater than those in platelet-poor plasma, and a close correlation was found between plasma and serum levels (Yoshimura et al. 2010a). Platelets are the major source of serum BDNF, as they sequester large quantities of it for release during clotting. Sources of circulating plasma BDNF include vascular endothelial cells as well as brain cells (Lommatzsch et al. 2005). Platelets have a life span of approximately 10 days, whereas plasma BDNF is minimally affected by the amount stored in platelets, and therefore is likely to be a more suitable index of brain BDNF levels (Marano et al. 2007) (Fig. 1).

In this chapter, the role of serum BDNF and its association with mood disorders predominantly will be discussed mainly based on our previous findings.

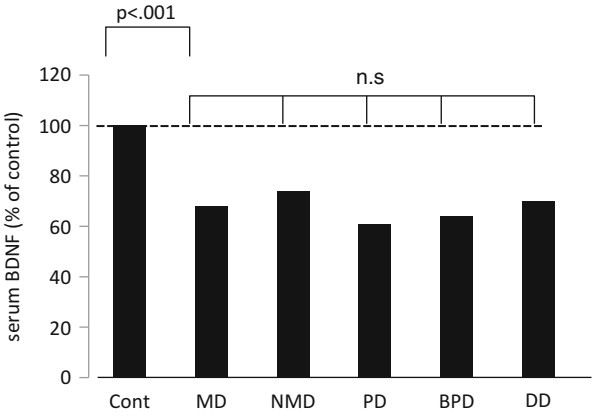

Fig. 1 Serum BDNF levels in various depressed types. Abbreviations: Cont, control; MD, major depression with melancholia; NMD, major depression without melancholia; PD, major depression with psychotic features; BPD, depressive episode with bipolar disorder; DD, double depression

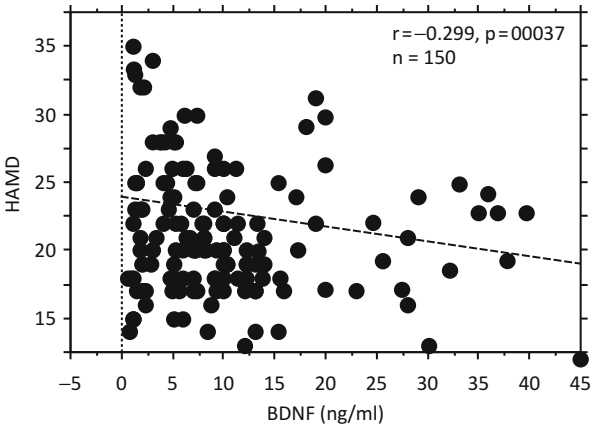

Fig. 2 HAMD scores and serum BDNF levels

2 Blood BDNF Levels in Patients with Major Depressive Disorder

The serum BDNF levels were significantly lower in those patients than in the healthy subjects: No difference in serum BDNF levels between with melancholia and without melancholia. In addition, no difference was observed in serum BDNF levels between the patients with psychotic features (psychotic depression) and without ones. There have been at least three meta-analyses of studies of blood BDNF levels in patients with major depressive disorder (Bocchio-Chiavetto et al. 2010; Brunori et al. 2010; Sen et al. 2008). The results of those investigations have consistently revealed that blood BDNF levels in patients with major depressive disorder are significantly lower than those of healthy subjects. A negative correlation was also found between the scores of Hamilton Rating Scale for Depression (HAMD) and serum BDNF levels, which means serum BDNF levels reflect the severity of depressive state (Yoshimura et al. 2011; Shimizu et al. 2003). From these findings, it is deduced blood BDNF level is a candidate biomarker for depression (Fig. 2).

3 Blood BDNF Levels in Patients with Bipolar I Disorder

Plasma BDNF levels in depressive episodes were significantly lower than those in manic episodes in patients with bipolar I disorder or healthy subjects (Yoshimura et al. 2006). Fernandes et al. recently performed meta-analysis including our study, and they concluded that blood BDNF levels are consistently reduced during maniac and depressive episodes, but not during euthymic state in patients with bipolar I disorder (Fernandes et al. 2011).

4 Blood BDNF Levels in Patients with Dysthymic Disorder

Dysthymic disorder has a number of typical characteristics such as low drive, low self-esteem, and a low capacity for pleasure. They will usually find little pleasure in usual activities and pastimes (Griffiths et al. 2000). In addition, BDNF levels among patients with dysthymic disorder, major depressive disorder, and healthy controls were measured. Serum BDNF levels in the dysthymic disorder group were significantly lower than those in the healthy controls. Moreover, no difference was found between the dysthymic disorder group and the major depressive disorder group (Yoshimura et al. 2011). On the other hand, Aydemir et al. (2007) reported that serum BDNF concentrations of the dysthymic group were significantly higher than that of the major depressive disorder group, and it was not different from the level of the control group. One of the reasons of the discrepancy of the results might be due to the difficulty of the diagnosis of dysthymic disorder. Actually, it is difficult to diagnose patients with dysthymic disorder because of the subtle nature of the symptoms and the patients often can hide them in social situations, making it challenging for others to detect symptoms. Additionally, dysthymic disorder often occurs at the same time as other psychological disorders which adds a level of complexity to determine the presence of dysthymic disorder, particularly because there is often an overlap in the symptoms of other psychiatric disorders (Sansone et al. 2009).

5 Blood BDNF Levels in Patients with Major Depressive Disorder with Alcohol Dependence

Alcohol dependence is often comorbid with major depressive disorder (Regiet et al. 1990). Alcohol use in depression patients increases their severity of depressive state and suicidal risk. Zanardini et al. (2011) reported that serum BDNF levels were decreased in patients with alcohol dependence However, no reports have been found comparing blood BDNF levels with and without alcohol dependence in depressed patients. Serum BDNF levels in the patients with or without alcohol dependence were significantly lower than those in the healthy controls. There was however no difference in serum BDNF levels between the depressed patients with and without alcohol dependence (Umene-Nakano et al. 2009).

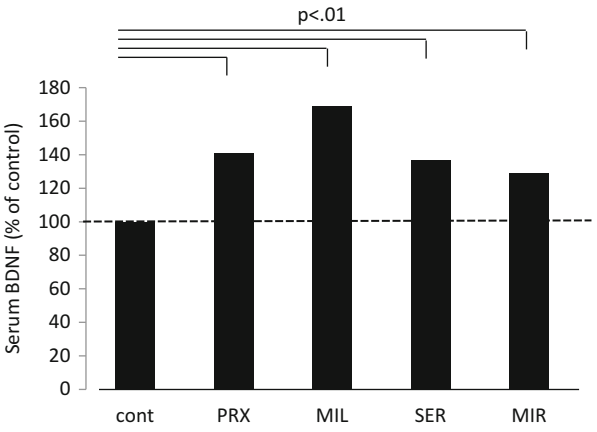

Fig. 3 Effects of various antidepressants on serum BDNF levels. Abbreviations: Cont, control; MIL, milnacipran; MIR, mirtazapine; PRX, paroxetine; SER, sertraline

6 Effects of Antidepressants on Serum BDNF Levels in Depressive Patients

Recent review by Molendijk et al. demonstrated that St John's wart and selective serotonin reuptake inhibitor (SSRI), but not serotonin noradrenaline reuptake inhibitor (SNRI), tricyclic antidepressant (TCA), or noradrenergic and specific serotonergic antidepressants (NaSSAs) increased serum BDNF levels (Molendijk et al. 2010). Effects of paroxetine, a SSRI, and milnacipran, a SNRI, on serum BDNF levels in patients with major depressive disorder were investigated. Treatment with paroxetine and milnacipran for at least 8 weeks, but not 4 weeks, both increased serum BDNF levels in responders, but not in nonresponders to the same degree (Yoshimura et al. 2007). Taken together, serotonin rather than noradrenaline might play an essential role for increasing BDNF (Molendijk et al. 2010) (Fig. 3).

7 Effects of Repetitive Transcranial Magnetic Stimulation (rTMS) or Electrical Convulsive Therapy (ECT) on Blood BDNF Levels

rTMS or ECT is effective for treatment-resistant depression. A systematic review by Lam et al. (2008) reported that the pooled response and remission rate rates were 25 % and 17 %, respectively. ECT is widely used for treatment in major depressive disorder, bipolar disorder, and schizophrenia. The mechanisms of rTMS and ECT have not been fully elucidated. To the best of our knowledge, this is the first report that rTMS increased plasma BDNF levels at least 5 weeks in responders with treatment-resistant depressed patients (Yukimasa et al. 2006). Furthermore, ECT also increased serum BDNF levels in responders with refractory patients within 4 weeks (Okamoto et al. 2008). Taking these findings into account, it can be deduced that rTMS or ECT rapidly and robustly might increase blood BDNF levels

in depressed patients. Bocchio-Chiavetto et al. have demonstrated that ECT significantly increased serum BDNF levels in paralleled with clinical improvement of depressive symptoms (Bocchio-Chiavetto et al. 2006). On the other hand, Fernandes et al. (2009) reported that no changes were observed in the serum BDNF before and after the ECT treatment. Zanardini et al. (2006) reported that a significant increase of serum BDNF was found after rTMS treatment. It still remains controversial: How rTMS or ECT increased blood BDNF levels in depressed patients. Farther replications in larger sample will be performed.

8 Polymorphism of BDNF Gene Val66Met and Response to SSRIs

The relationship between the BDNF gene polymorphism (Val66Met) and the clinical response of patients with major depressive disorder to selective serotonin reuptake inhibitors (SSRIs; paroxetine and sertraline) was investigated. In addition, serum BDNF levels in these patients were considered together with the clinical response. A negative association was found between the serum BDNF levels and the Hamilton Rating Scale for Depression (HAMD) scores. No correlation was observed between BDNF gene Val66Met polymorphism and response to SSRIs or between BDNF Val66Met polymorphism and serum BDNF levels. These results suggest that serum BDNF level is a state biomarker for depression, but that in depressed patients, presence of the BDNF Val66Met polymorphism is independent of both the response to SSRI treatment and serum BDNF levels (Yoshimura et al. 2011). In contrast, recent meta-analysis by Zou et al. (2010) demonstrated that the association between BDNF Val66Met polymorphism and treatment response in patients with major depressive disorder, and Val66Met heterozygous patients has a better response rate in comparison to Val66Val homozygote patients, especially in the Asian population.

9 Adding an Atypical Antipsychotic Drug to an Antidepressant Increased Rapidly Plasma BDNF Levels in Treatment-Resistant Depressed Patients: Preliminary Findings

Only two-thirds of depressive patients respond to antidepressant treatment. Addition of an atypical antipsychotic drug to ongoing treatment with an antidepressant has been considered effective and well-tolerated, and that the improvements of depressive symptoms were related with the increase of plasma BDNF levels after adding the atypical antipsychotic drug to the antidepressant (Yoshimura et al. 2010b). No relationship was however observed between BDNF gene Val66Met polymorphism and the response to the atypical antipsychotic drug to the antidepressant. To the best of our knowledge, this is the first report demonstrating an atypical antipsychotic drug to an antidepressant enhances plasma BDNF levels in refractory depressive patients.

10 Longitudinal Follow-Up Study of Serum BDNF Levels and the Relapse of Depressive Episodes

Long-term study of serum BDNF levels in patients with major depressive disorder. A longitudinal follow-up study measuring serum BDNF levels after remission of depressive episodes. Serum BDNF levels at relapse were significantly lower than those at remission or after 6 months after remission. No difference was observed between levels at remission and at 6 months after remission. These results indicate that serum BDNF levels at 6 months after remission might not be associated with subsequent depressive episodes.

11 No Association Between Responses to the Addition of an Atypical Antipsychotic Drug to a SSRI or SNRI and BDNF (Val66Met) Polymorphism in Refractory Major Depressive Disorder: Preliminary Study

The association between the BDNF (Val66Met) polymorphism and the response to the addition of an atypical antipsychotic drug to a SSRI or SNRI in treatment-refractory depression was observed. Forty-two patients meeting the DSM-IV criteria for major depressive disorder and who were treated with at least two courses of a single antidepressant, but whose HAMD-17 scores were 15 points or more and were reduced less than 50 % over at least a 4-week treatment periods, were enrolled in the study. No correlation was found between the BDNF (Val66Met) polymorphism and a positive response to the atypical antipsychotic addition. In addition, patients who experienced remission were followed up 1 year after discontinuation of the atypical antipsychotic drug at least within 3 months. Eleven of 19 patients (47 %) relapsed within a year. These results suggest that the BDNF (Val66Met) polymorphism is not associated with the response to the augmentation of a SSRI or SNRI with an atypical antipsychotic drug, and the combination regimen of an atypical antipsychotic drug and a SSRI or SNRI should be continued for 3 months or more in refractory depressed patients in the Japanese population (Fig. 4).

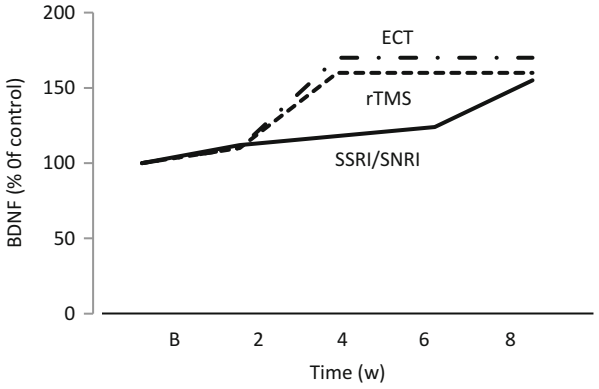

Fig. 4 Time courses of blood BDNF levels in different treatments for depression

12 Mental Stress, Personality Trait, and Blood BDNF Levels

Lang et al. first reported a negative correlation between the BDNF serum concentration and the depression-related factor neuroticism (Yoshimura et al. 2011). These results indicate that low BDNF levels in healthy humans with depressive personality traits might constitute a risk marker, reflecting a personality profile that is linked to vulnerability to mood disorders. Recently, Terracciano et al. also reported that serum BDNF might represent a biological correlate of neuroticism and not just of transient depressive state (Lang et al. 2004). Associations between plasma BDNF levels and personality trait evaluated by using NEO-FFI (neuroticism, extroversion, openness, agreeableness, and conscientiousness) also were examined. A positive correlation was observed between plasma BDNF levels and extroversion, but not neuroticism (Terracciano et al. 2011). The discrepancy of our results and those in other two studies remain controversial. One of the reasons might be the difference in the ethnicity. An association between psychological job stress and serum BDNF levels in Japanese hospital employee also was examined. Psychological job stress was evaluated. A negative correlation was observed between the scores of psychological stress and serum BDNF levels (Mitoma et al. 2008; Okuno et al. 2011). In other words, subjects with high psychological job stress had low BDNF levels. These results suggest that serum BDNF levels reflect psychological job stress in healthy workers.

13 Conclusion

Blood (serum and plasma) BDNF levels are useful state biomarker for depressive stateon. Blood BDNF levels reflect the severity of depressive state. SSRIs and SNRIs increased serum BDNF levels in the responders with major depressive disorder. Adding an atypical antipsychotic drug to a SSRI or a SNRI improved clinical symptoms in patients with refractory depression, which was accompanied with the increase plasma BDNF levels. The polymorphism of BDNF gene Val66Met was neither associated with the response to SSRIs nor an atypical augmentation to SSRIs or SNRIs. Further research should be done to reconfirm our preliminary findings.

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References

Aydemir, O., Deveci, A., Taskin, O. E., et al. (2007). Serum brain-derived neurotrophic factor level in dysthymia: a comparative study with major depressive disorder. *Progress in Neuropsychopharmacology Biological Psychiatry, 31*(5), 1023–1026.

Bocchio-Chiavetto, L., Zanardi, R., Bortolomasi, M., et al. (2006). Electroconvulsive therapy (ECT) increases serum brain derived neurotrophic factor (BDNF) in drug resistant depressed patients. *European Neuropsychopharmacology, 16*, 620–624.

Bocchio-Chiavetto, L., Bagnardi, V., Zanardini, R., et al. (2010). Serum and plasma BDNF levels in major depression: A replication study and meta-analysis. *World Journal of Biological Psychiatry, 11*, 763–773.

Brunori, A. R., Lopes, M., & Fregni, F. (2010). A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: Implications for the role of neuroplasticity in depression. *International Journal of Neuropsychopharmacology, 11*, 1169–1180.

Duman, R. S., Malberg, J., Nakagawa, S., et al. (2000). Neuronal plasticity and survival in mood disorders. *Biological Psychiatry, 48*, 732–739.

Fernandes, B. S., Gama, C. S., Cereser, K. M., et al. (2011). Brain-derived neurotrophic factor as a state-marker of mood episode in bipolar disorders: A systematic review and meta-analysis. *Journal of Psychiatry Research, 45*, 995–1004.

Fernandes, B., Gama, C. S., Massuda, R., et al. (2009). Serum brain-derived neurotrophic factor (BDNF) is not associated with response to electro convulsive therapy (ECT): a pilot study in drug resistant depressed patients. *Neuroscience Letters, 453*(3), 195–198.

Fignov, A., Pozzo-Miller, L. D., Olafsson, P., et al. (1996). Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature, 381*, 706–709.

Griffiths, J., Ravindran, A. V., Merali, S., et al. (2000). Dysthymia: a review of pharmacological and behavioral factors. *Molecular Psychiatry, 5*(3), 242–261.

Karege, F., Schward, M., & Cisse, M. (2002). Postnatal development profile of brain-derived neurotrophic factor in rat brain and platelets. *Neuroscience Letters, 328*, 261–264.

Klein, A. B., Williamson, R., Santini, M. A., et al. (2011). Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *International Journal of Neuropsychopharmacology, 14*, 347–353.

Lam, R. W., Chan, P., Wilkins-Ho, M., et al. (2008). Repetitive transcranial magnetic stimulation for treatment-resistant depression: a systematic review and meta analysis. *Canadian Journal of Psychiatry, 53*(9), 621–631.

Lang, U. E., Hellweg, R., & Gallinat, J. (2004). BDNF serum concentrations in healthy volunteers are associated with depression-related personality traits. *Neuropsychopharmacol, 29*, 795–798.

Lang, U. E., Hellweg, R., Seifert, F., et al. (2007). Correlation between serum brain-derived neurotrophic factor level and in vivo marker of clinical integrity. *Biological Psychiatry, 62*, 530–535.

Lommatzsch, M., Zinger, D., Schuhbaeck, K., et al. (2005). The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiology of Aging, 26*, 115–123.

Marano, C. M., Phatak, P., Vemulalli, U. R., et al. (2007). Increased plasma concentration of brain-derived neurotrophic factor with electroconvulsive therapy: A pilot study in patients with major depression. *Journal of Clinical Psychiatry, 68*, 512–517.

Mitoma, M., Yoshimura, R., Sugita, A., et al. (2008). Stress at work alters serum brain-derived neurotrophic factor (BDNF) levels and plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in healthy volunteers: BDNF and MHPG as possible biological markers of mental stress? *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 32*, 679–685.

Molendijk, M. L., Bus, B. A. A., Spinhoven, P. H., et al. (2010). Serum levels of brain-derived neurotrophic factor in major depressive disorder: State-trait issues, clinical features and pharmacological treatment. *Molecular Psychiatry, 21*, 1–8.

Mowla, S. J., Farhadi, H. F., Pareek, S., et al. (2001). Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *Journal of Biological Chemistry, 276*, 12660–12666.

Okamoto, T., Yoshimura, R., Ikenouchi-Sugita, A., et al. (2008). Efficacy of electroconvulsive therapy is associated with changing blood levels of homovanillic acid and brain-derived

neurotrophic factor (BDNF) in refractory depressed patients: A pilot study. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 32*, 1185–1190.

Okuno, K., Yoshimura, R., Ueda, N., et al. (2011). Relationship between stress, social adaptation, personality traits, brain-derived neurotrophic factor and 3-methoxy-4-hydroxyphenylglycol plasma concentrations in employees at a publishing company in Japan. *Psychiatry Research, 186*, 326–332.

Pan, W., Banks, W. A., Fasold, M. B., et al. (1998). Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology, 37*, 1553–1561.

Regiet, D. A., Farmer, M. E., Rae, D. S., et al. (1990). Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area(ECA) study. *JAMA, 263*(19), 2511–2518.

Sansone, R. A., & Sansone, L. A. (2009). Early-versus late-onset dysthymia: a meaningful clinical dysfuncton? *Psychiatry, 6*(11), 14–17.

Sen, D., Duman, R., & Sanacora, G. (2008). Serum brain-derived neurotrophic factor, depression, and implications. *Biological Psychiatry, 64*, 527–532.

Shelton, R. C. (2000). Cellular mechanisms in the vulnerability and depression and response to antidepressants. *Psychiatric Clinics of North America, 23*, 713–729.

Shimizu, E., Hashimoto, K., Okamura, N., et al. (2003). Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biological Psychiatry, 54*(1), 70–75.

Terracciano, A., Lobina, M., Prias, M. G., et al. (2011). Neuroticism, depressive symptoms, and serum BDNF. *Psychosomatic Medicine, 73*(8), 638–642.

Umene-Nakano, W., Yoshimura, R., Ikenouchi-Sugita, A., et al. (2009). Serum brain-derived neurotrophic factor in comobidity of depression and alcohol dependence. *Human Psychopharmacology, 25*, 409–413.

Yoshimura, R., Nakano, Y., Hori, H., et al. (2006). Effect of risperidone on plasma catecholamine metabolites and brain-derived neurotrophic factor in patients with bipolar disorders. *Human Psychopharmacology, 21*, 433–438.

Yoshimura, R., Mitoma, M., Sugita, A., et al. (2007). Effects of paroxetine or milnacipran on serum brain-derived neurotrophic factor in depressed patients. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 31*, 1034–1037.

Yoshimura, R., Ikenouchi-Sugita, A., Hori, H., et al. (2010a). A close relation plasma and serum levels of brain-derived neurotrophic factor (BDNF) in healthy volunteers. *International Journal of Psychiatry in Clinical Practice, 14*, 220–222.

Yoshimura, R., Ikenouchi-Sugita, A., Hori, H., et al. (2010b). Adding a low dose atypical antipsychotic drug to an antidepressant induced a rapid increase of plasma brain-derived neurotrophic factor levels in patients with treatment-resistant depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 34*, 308–312.

Yoshimura, R., Kishi, T., Suzuki, A., et al. (2011). The brain-derived neurotrophic factor (BDNF) polymorphism Val66Met is associated with neither serum BDNF level nor selective rerotonin reuptake inhibitors in depressed Japanese patients. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 35*, 1022–1025.

Yukimasa, T., Yoshimura, R., Tamagawa, A., et al. (2006). High-frequency repetitive transcranial stimulation improves refractory depression by influencing catecholamine and brain-derived neurotrophic factors. *Pharmacopsychiatry, 39*, 52–59.

Zanardini, R., Fontana, A., Pagono, R., et al. (2011). Alterations of brain-derived neurotrophic factor serum levels in patients with alcohol dependence. *Alcoholism*: *Clinical and Experimental Research*, *35*(8), 1529–1533.

Zanandini, R., Gazzoli, A., Ventriglin, M., et al. (2006). Effect of repetitive transcranial magnetic stimulation on serum brain derived neurotrophic factor in drug registant depressed patients. *Journal of Affective Disorders, 91*(1), 83–86.

Zou, Y. E., Ye, D. Q., Feng, X. L., et al. (2010). Meta-analysis of BDNF Val 66 Met polymorphism associated with treatment response in patients with major depressive disorder. *European Neuropsychopharmacology, 20*(8), 535–544.

Neuroprotection in Demyelinating Diseases: The Therapeutic Potential of the Neurotrophins

Junhua Xiao, Richard A. Hughes, and Simon S. Murray

Contents

Abstract

The myelin sheath has evolved to exert critical influences upon the central and peripheral nervous systems. Recently, the neurotrophins have been implicated in influencing the dynamic and complex signals that occur between neurons and myelinating glial cells – Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system – that regulate myelination. Somewhat surprisingly, the neurotrophins have been found to influence

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myelination in a complex manner involving both promyelinating and inhibitory signals that can be directed against either neuronal or glial cells that ultimately regulate central and peripheral myelin formation in distinct ways. The neurotrophins and their receptors have also been shown to influence the severity and affect remyelination in distinct in vivo models of demyelinating disease. Together, these data indicate that the selective targeting of neurotrophin receptors to promote remyelination offers an exciting prospect for the treatment of demyelinating diseases.

List of Abbreviations

CGRP	Calcitonin gene-related peptide
CNS	Central nervous system
DPN	Diabetic peripheral neuropathy
DRG	Dorsal root ganglion
EAE	Experimental autoimmune encephalomyelitis
EAN	Experimental autoimmune neuritis
Erk	Extracellular signal-regulated kinase
GPCR	G Protein-coupled receptor
MAPK	Mitogen-activated protein kinase
MS	Multiple sclerosis
NRG	Neuregulin
NTR	Neurotrophin receptor
OPCs	Oligodendrocyte progenitor cells
PNS	Peripheral nervous system
TrkB	Tropomyosin-related kinase B

1 Introduction

The myelin sheath is critical for normal nervous system function. Its evolution has effectively given the nervous system enhanced compactness, reduced its metabolic demands, improved action potential synchrony, and allowed the rapid processing of complex information. This has been achieved via at least two distinct and critical influences on the nervous system. The first is to increase the speed at which the action potential is propagated along the axon, by permitting saltatory conduction. Electrophysiological studies have clearly shown the impact this has on nervous system function, demonstrating that nervous system demyelination results in slowing of the conduction velocity of action potentials and in more severe cases can actually lead to conduction block (McDonald and Sears 1969). While these studies theoretically accounted for the neurological deficits encountered in people suffering from demyelinating diseases, the clinical picture appeared more complex. Brain imaging studies revealed that white matter lesion load did not necessarily correlate with the extent of neurological deficit. Indeed,

histological studies have now revealed that injured and transected axons are present within both demyelinated and actively demyelinating lesions and that progressive axonal loss resulting from demyelination is a key contributor to clinical decline (Trapp et al. 1997; Bitsch et al. 2000). It is now increasingly apparent that the second critical influence that myelinating glial cells exert is to confer protective and trophic support to the axons which they ensheathe (Yin et al. 2006). Collectively, these studies indicate that there is an intimate relationship between damage to either the myelinating glial cell or the myelin sheath itself and axonal degeneration. The evolution of the myelin sheath and saltatory conduction reduced the metabolic demands and energy expenditure required by the nerve cell to relay the action potential down its axon, and perhaps this at least in part contributes to the axonal-protective properties of myelin. However it is also clear that the myelinating cells support axonal integrity and survival independent of the myelin sheath itself (Lappe-Siefke et al. 2003; Lee et al. 2012; Oluich et al. 2012). Thus, the protective influences that myelinating glial cells and the myelin sheath exert on axonal integrity suggest that any successful therapy against demyelinating diseases must consider both survival of myelinating glial cells and myelin regeneration.

In human demyelinating diseases such as multiple sclerosis (MS) and some peripheral demyelinating neuropathies, the myelinating glial cells progressively die, disrupting these myelin sheaths and placing axonal integrity at risk. The body has an innate mechanism for remyelination, whereby myelin sheaths are restored to the damaged axons, protecting them from further degeneration (Irvine and Blakemore 2008; Franklin et al. 2012). In MS, this is affected by regenerating oligodendrocytes through the generation, recruitment, and differentiation of OPCs resident within the CNS. However, remyelination is often incomplete, becomes less efficient with age, and following successive demyelinating events is ultimately insufficient and invariably leads to irreversible axonal damage and progressive and extensive disability (De Stefano et al. 1998).

In these diseases an autoimmune response is commonly observed, which is generally believed to contribute to the extent of demyelination. While immune-targeted therapies are available to limit demyelination, no treatments are currently available to directly promote myelin repair. Intuitively, studies that examine the growth factors and molecular mechanisms that promote myelination during development and remyelination following injury are essential if the therapeutic targets that promote myelin repair and thereby limit axonal injury and ultimately disability are to be identified. Recent reviews have examined the neurotrophin family of growth factors and the roles they play in regulating myelination during development in detail (Rosenberg et al. 2006; Xiao et al. 2009a). We briefly review here the current understanding of the mechanisms that the neurotrophins and their receptors utilize to influence myelination during development and identify the protective roles they exert in demyelinating diseases. Finally we discuss the evolving potential for the selective targeting of neurotrophin receptors for therapeutic benefit in the context of myelin repair following a demyelinating insult.

2 The Neurotrophins, Their Receptors, and Myelination During Development

2.1 The Neurotrophins and Their Receptors

The neurotrophin family of growth factors comprises nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). They are widely expressed and exist in two distinct forms. They are initially synthesized as larger precursor proteins, known as proneurotrophins, which subsequently undergo proteolytic cleavage either intracellularly or extracellularly to yield the mature neurotrophins. It is generally accepted that the proneurotrophins modulate cell death (Teng et al. 2010); however, there is emerging evidence they can also exert distinct influences including modulating synaptic plasticity and axonal competition at developing neuromuscular synapses (Woo et al. 2005; Je et al. 2012). To date, there is no evidence to indicate that the proneurotrophins directly influence myelination, whether central or peripheral.

In contrast, it is well established that the mature neurotrophins exert profound and diverse effects upon neural development as well as in maintaining and modulating the adult mature nervous system. The mature neurotrophins signal through two distinct classes of structurally unrelated transmembrane receptors: the receptor tyrosine kinase tropomyosin-related kinase (Trk) family and p75NTR (Chao 2003). These receptors exhibit a distinct capacity to interact with the neurotrophins. Whereas all the neurotrophins can bind nonselectively and with approximately the same affinity to p75NTR, they display selectivity in their interaction with Trk receptors: NGF binds selectively to TrkA, BDNF and NT4/5 to TrkB, and NT-3 to TrkC (Chao 2003; Huang and Reichardt 2003). Increasing the complexity of these ligand-receptor interactions, when Trk and p75NTR are co-expressed on the same cell, they can also form a receptor complex wherein p75NTR modulates the affinity of Trk receptor binding and selectivity of Trk receptor activation (Esposito et al. 2001). Despite this complexity, genetic analysis of mice deficient in either the neurotrophins or their receptors reveals that, by and large, Trk receptor signalling transduces the key influences of the neurotrophins (Conover and Yancopoulos 1997; Huang and Reichardt 2003).

2.2 The Neurotrophins Regulate Schwann Cell Myelination

It is well established that both peripheral neurons and Schwann cells express a variety of neurotrophin receptors, as well as expressing and secreting neurotrophins. Analyses of either neurotrophin or neurotrophin receptor knockout mice reveal the absolute requirement of the ligands and their receptors for peripheral neuron development, showing that ~80 % of DRG neurons are dependent on NGF for survival (Crowley et al. 1994), ~30 % are dependent on BDNF (Jones et al. 1994), and ~70 % are dependent on NT-3 (Liebl et al. 1997; Tessarollo et al. 1997).

Consequently, while it is clear that the neurotrophins and their receptors exert critical influences on the development of the peripheral nervous system, analysis of the precise effects that these molecules have on peripheral myelination in vivo has been problematic. However, development and manipulation of in vitro myelination assays has provided insight into the roles the neurotrophins and their receptors are playing in this context. Somewhat surprisingly, despite Schwann cells expressing several neurotrophin receptors, the key influences that the neurotrophins exert on peripheral myelination appear to be directed through neuronally expressed receptors that ultimately regulate axonal receptivity to myelination by Schwann cells.

Chan and colleagues were the first to identify that NGF exerts a positive influence upon Schwann cell myelination. Through the use of in vitro myelination assays, they identified that NGF enhanced Schwann cell myelination specifically of TrkA-expressing DRG neurons. This influence of NGF was specific to TrkA, as NGF also promoted myelination of neurons not expressing p75NTR and exerted no influence on TrkB-expressing DRG neurons (where TrkA expression is absent) (Chan et al. 2004). Thus, NGF exerts its promyelinating influence selectively through neuronally expressed TrkA receptors (Fig. 1). Contextually this fits well developmentally, as ~80 % of DRG neurons express TrkA during the early postnatal period when peripheral nerves are actively being myelinated. Thus, TrkA is well placed to direct key influences upon DRG neurons to regulate their myelination in vivo (Wiggins et al. 1975; Silos-Santiago et al. 1995; Molliver and Snider 1997; Xiao et al. 2009a). Interestingly, TrkA expression is strikingly developmentally regulated, in that while ~80 % of DRG neurons express TrkA during embryogenesis and early postnatal life, the expression of TrkA is remarkably downregulated in adult DRG neurons (Molliver and Snider 1997). This suggests that the utility of NGF as a potential remyelination therapy in adults may be limited.

The influence that BDNF exerts upon peripheral myelination is considerably more complex, with evidence demonstrating that BDNF can promote or inhibit the myelination of distinct subsets of DRG neurons. Like NGF, BDNF regulates Schwann cell myelination through activation of neuronally expressed receptors. However, the precise influence that BDNF exerts is dependent on the subset of neurotrophin receptors expressed on the particular DRG neuron. BDNF inhibits Schwann cell myelination of TrkB+ DRG neurons; however, in the absence of neuronal TrkB, BDNF can promote the myelination via activation of neuronal p75NTR (Chan et al. 2001; Cosgaya et al. 2002; Xiao et al. 2009b). The contrasting influence that BDNF exerts upon Schwann cell myelination is therefore context specific, due to BDNF activating distinct neuronal receptors – p75NTR to promote myelination and TrkB to inhibit it (Xiao et al. 2009b) (Fig. 1). For this reason, the utility of BDNF itself as a potential remyelination therapy in adults may also be limited. This also reveals a fundamental difference as to how NGF and BDNF promote Schwann cell myelination: NGF promotes myelination via activation of neuronal TrkA receptors (Chan et al. 2004), whereas BDNF promotes myelination via activation of neuronal p75NTR receptors (Xiao et al. 2009b) (Fig. 1).

In contrast to the situation for NGF and BDNF, there have been relatively few studies that have attempted to identify the precise influence that NT-3 exerts on

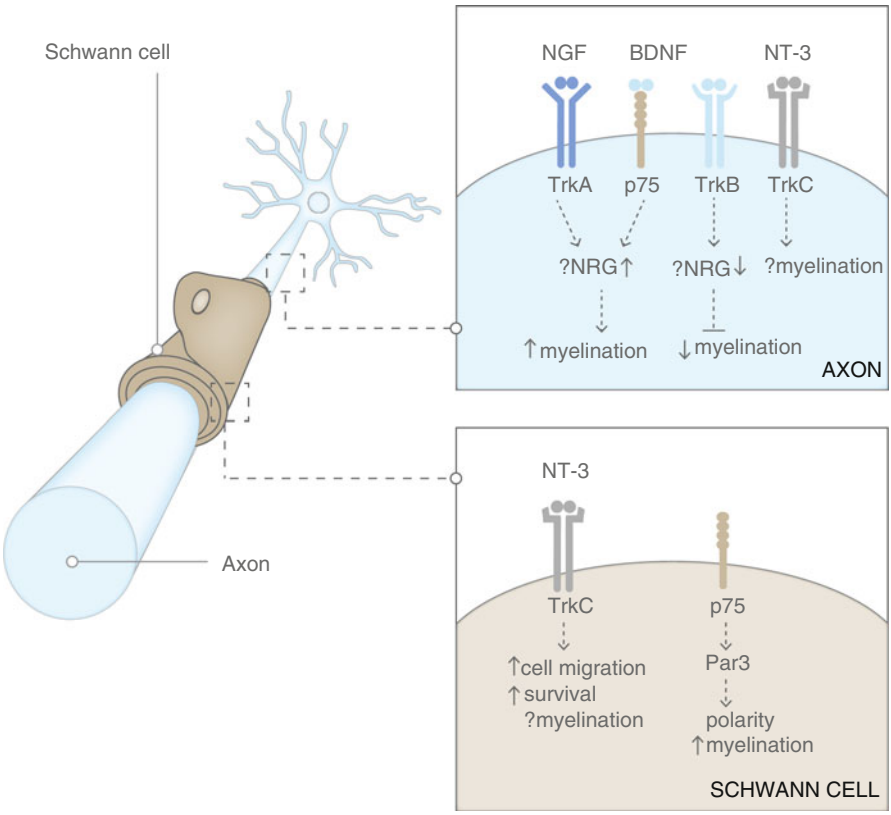

Fig. 1 Neurotrophin influences on Schwann cell myelination. Top panel: Neuronal influences of the neurotrophins. NGF exerts a promyelinating influence via TrkA activation. BDNF promotes myelination via p75NTR activation, but inhibits myelination via TrkB. NGF and BDNF putatively differentially regulate neuregulin (*NRG*) expression to regulate myelination. The precise influence that neuronal TrkC receptors exert is unclear. *Bottom panel*: Neurotrophin influences on Schwann cells. NT-3 effects Schwann cell migration and survival, whereas BDNF influences Schwann cell polarity in a p75NTR-dependent manner. The precise role these influences exert on myelination is unclear

Schwann cell myelination. In vitro, exogenous NT-3 inhibits myelin formation, and injection of NT-3 adjacent to the sciatic nerve of neonatal mouse pups also results in reduced myelin protein formation, supporting an inhibitory role for NT-3 upon peripheral myelination, most likely via activation of the TrkC receptor (Chan et al. 2001; Cosgaya et al. 2002) (Fig. 1). However, as Schwann cells and some DRG neurons express TrkC, the precise mechanism that NT-3 utilizes to influence myelination remains unclear.

Collectively, these data demonstrate that the influences the neurotrophins exert upon the myelination of peripheral axons are highly dependent on the complement of neurotrophin receptors expressed at critical times in development. It is clear that

at least NGF and BDNF direct neuronal events that alter axonal receptivity to Schwann cell myelination. Importantly, the critical neuronal event that determines the ensheathment fate of peripheral axons is the expression of neuregulin-1 type III (Taveggia et al. 2005; Nave and Salzer 2006; Birchmeier and Nave 2008). Thus, it is likely that neurotrophin signalling within the distinct subsets of DRG neurons differentially regulates the expression of neuregulin-1 type III. However, this has yet to be definitively demonstrated.

It is intriguing that Schwann cells also express several neurotrophin receptors, including p75NTR, TrkC, and truncated TrkB receptors. Emerging data identify that the neurotrophins also exert direct influences on Schwann cells. BDNF can regulate Schwann cell migration (Yamauchi et al. 2004) as well as Schwann cell polarity and alignment to axons in a p75NTR-dependent manner (Chan et al. 2006) (Fig. 1). NT-3, acting through Schwann cell-expressed TrkC receptors, also promotes Schwann cell migration (Yamauchi et al. 2003; Yamauchi et al. 2005a, b), and analysis of NT-3 mutant mice suggests it also promotes survival of maturing Schwann cells (Woolley et al. 2008) (Fig. 1). These events all ultimately impact upon the process of myelination and clearly suggest that neurotrophins exert distinct influences directly upon both neurons and Schwann cells that collectively contribute to the orchestration of peripheral myelination.

2.3 The Neurotrophins Regulate Oligodendrocyte Myelination

As in the peripheral nervous system, a variety of neurotrophins and neurotrophin receptors are expressed by both the neurons and oligodendrocytes of the central nervous system. Analyses of central nervous system myelination in either neurotrophin or neurotrophin receptor knockout mice have also proven largely problematic, as many of these mice die in the embryonic or early postnatal period prior to CNS myelination being complete. In any case, analyses of these mice are confounded by the global deletion, whereupon the potential selective influences that neuronal and oligodendroglial receptors exert upon myelination cannot be discriminated. In vitro myelination assays have again proved critical here, providing insight into the influences that the neurotrophins and their receptors exert upon oligodendrocyte myelination. Perhaps surprisingly, the data here clearly indicate that the neurotrophins exert quite distinct influences upon peripheral and central nervous system myelination.

The first indication that the neurotrophins did not exert uniform influences upon peripheral and central myelination came with the study of NGF. Chan and colleagues, adopting the same approach that demonstrated that NGF promoted Schwann cell myelination, found that NGF inhibited oligodendrocyte myelination (Chan et al. 2004). Mechanistically NGF was shown to be acting via the activation of neuronally expressed TrkA receptors in both cases but with starkly contrasting outcomes (Fig. 2, top panel). This reaffirmed what had been known for some time – that axonal control over peripheral and central nervous system myelination is mediated via unique and mutually exclusive mechanisms – but surprisingly

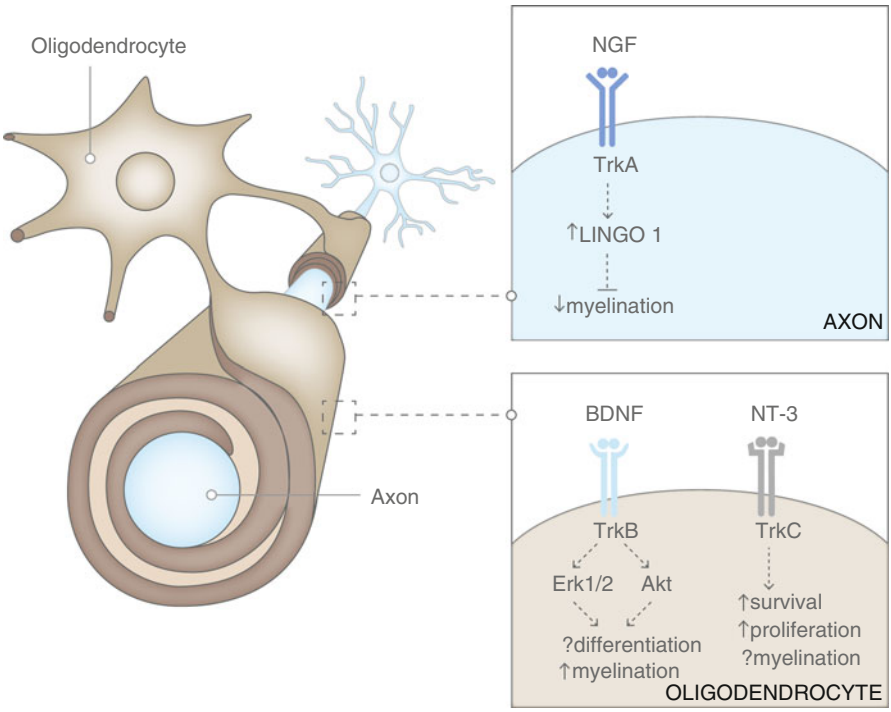

Fig. 2 Neurotrophin influences on oligodendrocyte myelination. Top panel: Neuronal influences of the neurotrophins. NGF inhibits oligodendrocyte myelination via regulating LINGO-1 expression. *Bottom panel*: Neurotrophin influences on oligodendrocytes. BDNF activates TrkB to promote oligodendrocyte myelination, potentially via Erk1/Erk2 or Akt activation. NT-3 effects oligodendrocyte survival and proliferation; however, the precise influence it exerts on myelination is unclear

revealed that a common growth factor could exert opposite effects upon myelination. In this context, NGF regulates axonal expression of LINGO-1, a membrane-bound protein that acts as a negative regulator of oligodendrocyte but not Schwann cell myelination (Mi et al. 2005; Lee et al. 2007) (Fig. 2, top panel). As an aside, LINGO-1 exerts profoundly negative influences on oligodendrocyte myelination (Mi et al. 2005), and LINGO-1 antagonists have been found to promote remyelination in animal models of CNS demyelination (Pepinsky et al. 2011a, b). A phase 1 clinical trial utilizing a LINGO-1 antagonist has recently been completed.

Analysis of the impact that BDNF exerted on oligodendrocyte myelination also revealed marked differences to its influence on Schwann cell myelination. Both BDNF knockout and heterozygous mice display a hypomyelinated phenotype in the CNS (Cellerino et al. 1997; Vondran et al. 2010; Xiao et al. 2010), suggesting a key role for BDNF in promoting oligodendrocyte myelination. This was verified in vitro, with BDNF found to promote myelination via a direct effect upon

oligodendrocyte-expressed TrkB receptors (Cohen et al. 1996; Du et al. 2003; Xiao et al. 2010) (Fig. 2). This was the first demonstration that a neurotrophin exerted its promyelinating influence by directly activating receptors expressed by the myelinating glial cell. This was subsequently verified in vivo through the analysis of mice in which TrkB had been deleted specifically in oligodendrocytes. This deletion significantly disrupted normal myelination in the CNS, resulting in a significant reduction in myelin protein expression and myelination of CNS white matter tracts during development (Wong et al. 2013). Interestingly, analyses revealed significantly reduced myelin thickness without oligodendrocyte number being affected, indicating that TrkB exerts an important influence in selectively promoting myelin ensheathment. These conditional knockout mice also exhibited an increased density of oligodendrocyte progenitor cells (OPCs) in CNS white matter tracts, which in vitro analyses suggested was dependent upon TrkC and p75 expression (Wong et al. 2013). This is another exemplar demonstrating the complexity of the neurotrophin signalling.

As the signalling by the Trk receptors is reasonably well characterized (Huang and Reichardt 2001), this had the potential to provide novel insight into the molecular control of myelination. Indeed, several canonical signalling pathways downstream of TrkB have been implicated in promoting oligodendrocyte myelination. Activation of both the MAPK (Chew et al. 2010; Fyffe-Maricich et al. 2011; Ishii et al. 2012) and Akt (Narayanan et al. 2009; Tyler et al. 2009) signalling pathways promote oligodendrocyte differentiation and myelination. Through screening candidate signalling pathways in in vitro myelination assays, it was found that BDNF-induced activation of extracellular signal-regulated kinase 1 and 2 (Erk1/Erk2) signalling in oligodendrocytes positively correlated with myelination (Xiao et al. 2012). Indeed, Erk1/Erk2 has been identified as a key signalling pathway that regulates the promyelinating effect of BDNF (Xiao et al., 2012; Ishii et al., 2012).

NT-3 is well known as an oligodendrocyte survival factor and was in fact one of the first growth factors identified to support the survival and proliferation of oligodendrocyte progenitor cells in vitro and in vivo (Barres et al. 1993, 1994; Kumar and de Vellis 1996; Kumar et al. 1998; Saini et al. 2004) (Fig. 2). It is important to note though that in addition to these effects upon progenitor cell survival, exogenous NT-3 also promotes oligodendrocyte myelination in vitro (Yan and Wood 2000; Rubio et al. 2004). The phenotype of the NT-3 knockout mouse supports a promyelinating role for NT-3 in vivo (Kahn et al. 1999). However, insufficient attention has been paid to NT-3 in this context, and whether its primary influence in the central nervous system in vivo is the generation and proliferation of OPCs or as a distinct promyelinating factor or both remains to be definitively established.

Similar to that observed for Schwann cell myelination, the influences the neurotrophins exert upon oligodendrocyte myelination are highly dependent on the complement of neurotrophin receptors expressed by the different cell types. While NGF initiates neuronal events that alter axonal receptivity to oligodendrocyte myelination, BDNF and NT-3 directly target oligodendrocyte-expressed

receptors to influence myelination. Somewhat surprisingly, despite p75NTR being expressed by both neurons and oligodendrocytes, it does not appear to exert a significant influence upon central nervous system myelination (Xiao et al. 2010), unlike that observed with Schwann cell myelination (Cosgaya et al. 2002; Chan et al. 2006; Xiao et al. 2009b).

Collectively, identification of the key influences that the neurotrophins exert on both peripheral and central nervous system myelination has revealed that their actions are highly contextual, with no unified response apparent. This is at least in part reflected in the complex nature with which the neurotrophins interact with their respective receptors but also reflects the distinct mechanisms that have evolved to generate central and peripheral myelin. From a therapeutic perspective this is potentially problematic, demanding that any potential neurotrophin-based approach be selective for central or peripheral remyelination, with the caveat that what might promote remyelination in the CNS could inhibit remyelination in the PNS. Nevertheless, neurotrophin-based approaches have been adopted to investigate their efficacy in promoting remyelination following a demyelinating injury to both the CNS and PNS.

3 The Neurotrophins, Their Receptors, and Remyelination Following Injury

Demyelination is the pathological process in which myelin sheaths are lost from around axons (Franklin and Ffrench-Constant 2008), with MS and Guillain-Barre syndrome (GBS) being the most common demyelinating diseases of the central and peripheral nervous systems, respectively (Miller 1996). Demyelination can result from a direct insult targeted at the myelin forming glial cells, which commonly occurs in CNS demyelination (Franklin and Ffrench-Constant 2008). The process of remyelination, in which entire myelin sheaths are restored to demyelinated axons, can reinstate saltatory conduction, resolve functional deficits, and promote axonal integrity and neuronal survival (Jeffery and Blakemore 1997; Liebetanz and Merkler 2006; Zawadzka and Franklin 2007). In recent years, multiple studies have examined the influence that the neurotrophins exert upon models of CNS autoimmune demyelination and remyelination. In contrast, there have been relatively few studies examining the influence that the neurotrophins exert upon models of peripheral nervous demyelination.

3.1 The Neurotrophins Promote Peripheral Nervous System Remyelination

Peripheral neuropathy encompasses complex neurological conditions, covering many disorders of the peripheral nervous system (Hughes 2008). The causes of peripheral neuropathy are protean: they can be either genetic in origin or acquired, with acquired neuropathies having either metabolic, ischemic, inflammatory,

or toxic precipitants (Hughes and Cornblath 2005; Hughes 2008). Peripheral neuropathies are also usefully classified as either axonal or demyelinative in origin. In axonal neuropathies the primary insult is directed to the neuron, and demyelination follows as a secondary consequence, whereas in demyelinative neuropathies the insult is directed to the Schwann cell that ensheathes the axon with myelin. Neuropathies are nevertheless not always pure; for example, there are circumstances in which the pathogenic insult is directed to both the axon and the Schwann cell; furthermore, in severe demyelinative neuropathy there is often secondary axonal degeneration because of the loss of trophic support that the Schwann cells otherwise provide.

Axonal peripheral neuropathy is most commonly caused by diabetes but can also result from a range of metabolic disorders, infectious agents, vasculitis, toxins, drugs, autoimmune and paraproteinemia neuropathies, and inherited polyneuropathies (McHugh and McHugh 2004). In experimental models of peripheral polyneuropathy, a reduction in the availability of the neurotrophins and alterations in the expression of their receptors has been observed, affecting both neuronal survival and maintenance of a myelinated phenotype (Apfel 1999a; Pittenger and Vinik 2003). The most widely utilized model of peripheral neuropathy is diabetic peripheral neuropathy (DPN) (Apfel et al. 1994, 1998; Apfel 1999a, b; Fressinaud et al. 2003; Pittenger and Vinik 2003). While DPN itself represents a heterogeneous variety of disorders affecting the peripheral nervous system, it is modelled by inducing diabetes in rats by administration of streptozocin.

DPN is characteristically described as degeneration of both small- and large-diameter sensory fibers, axonal degeneration, demyelination and atrophy, and ultimately associated with failed axonal regeneration, remyelination, and synaptogenesis (Pittenger and Vinik 2003). There is altered expression of neurotrophins and their receptors, as well as neuronal trafficking of neurotrophins in experimental models of DPN. It has been suggested that such changes could in fact contribute to the pathogenesis of DPN, but the data are far from definitive (Fressinaud et al. 2003; Pittenger and Vinik 2003). While large myelinated fibers can be affected in this model, it is the neuropathy associated with the small thinly myelinated fibers and sympathetic neurons that causes the majority of symptoms that adversely affect quality of life (Apfel 1999b). In this context, experimental depletion of NGF or its receptors duplicates many of the neuronal abnormalities observed in this model (Pittenger and Vinik 2003). So perhaps unsurprisingly NGF, which selectively supports the survival of the subset of smaller DRG and sympathetic neurons, was trialled as a potential therapy for DPN due to the consequences associated with degeneration of these neuronal populations (Apfel 2002). A number of animal studies initially demonstrated benefit following NGF treatment for toxic and diabetic peripheral polyneuropathy, which lead to the first clinical trials using neurotrophin-based strategies (Apfel 1999a, b). However, while significant improvement was seen in early trials, studies were discontinued at the phase III stage due to painful side effects (Apfel 2001, 2002). As NT-3 supports the survival of larger DRG neurons, by adopting a similar rationale, it too was trialled as a therapy in animal models of DPN. Utilizing an adenovirus-based vector approach

for the delivery of NT-3 in drug-induced diabetic rats, the slowing of motor nerve conduction velocity and muscle denervation was prevented, suggesting NT-3 retards the development of DPN (Pradat et al. 2001). Combination studies with both NGF and NT-3 in animal models of peripheral neuropathy including DPN suggested that these factors can ameliorate neuronal degeneration (Apfel 1999a). However, as indicated above, this is not a true demyelinating neuropathy, so the lessons learned here in the context of remyelination are unclear.

The contemporary view is that NGF promotes peripheral nerve myelination via activation of neuronal TrkA receptors (Chan et al. 2004). In the adult, no motor neurons and relatively few DRG neurons express TrkA, which tend to be thin and nonmyelinated anyway, so the rationale for utilizing NGF as a strategy to promote peripheral remyelination has limited basis. In fact the pain associated with this clinical trial may be attributable to NGF producing peripheral sensitization of nociceptive DRG neurons through the activation of TrkA. Indeed recent work has demonstrated that increases in peripheral nerve sensitization and pain are due to TrkA activation, p38 phosphorylation, and TRP channel expression (Bron et al. 2003; Obata et al. 2006).

With NGF being an unsuitable therapeutic candidate, what of BDNF? It is certainly implicated in remyelination, as endogenous BDNF has been shown to promote peripheral nerve regeneration and remyelination after sciatic nerve crush injury (Zhang et al. 2000). Electrical stimulation enhanced the remyelination of injured peripheral nerves by inducing BDNF expression and promoting Schwann cell polarization (Wan et al. 2010). However, recent findings have identified that the influence BDNF exerts upon peripheral myelination is more complex, due to BDNF activating distinct receptors: p75NTR to promote myelination and TrkB to inhibit it (Xiao et al. 2009b). On the simple basis of these contrasting influences, BDNF also presents as a less than ideal therapeutic candidate. Nevertheless the identification that BDNF promotes peripheral myelination via neuronal p75NTR is of some interest (Xiao et al. 2009b), as p75NTR has been identified as a global positive regulator of peripheral myelination during development (Cosgaya et al. 2002). In a sciatic nerve injury model in vivo, remyelination was found to be impaired in p75NTR−/− mice (Song et al. 2006). However, it is important to note that approximately 50 % of the DRG neurons are lost in the p75NTR−/− mouse (Murray et al. 1999), complicating this analysis. In a similar sciatic nerve injury model but in nude mice, nerve grafts derived from p75NTR−/− or wild-type mice were transplanted, supplying a source of Schwann cells for remyelination. Analysis of these mice identified that those receiving p75NTR−/− grafts had poorer motor performance, reduced myelin sheath thickness, decreased axonal diameter, and reduced myelin protein expression (Tomita et al. 2007). Collectively these data suggest that p75NTR also plays an important role in the remyelinating process, albeit in a nerve injury model.

Thus, the potential that the neurotrophins hold in promoting peripheral myelination is far from clear, and two issues stand out as requiring attention. The first is the choice of model. Clearly models that utilize nerve injury as the primary pathology are complex, and identifying the selective influence that any putative

therapy exerts on remyelination is confounded. There is a demonstrated need to assess any potential neurotrophin-based therapeutic strategy in a targeted primary demyelinating model, such as experimental autoimmune neuritis (EAN), an autoimmune disease model induced by inoculation with PNS antigen (Gold et al. 2000). The second standout issue is the complexity in the interactions between neurotrophins and their receptors. The cell biology has identified that the neurotrophins exert differing and at times contrasting influences on peripheral myelination, dependent on the complement of neurotrophin receptors expressed by the peripheral neuron. Thus, the therapeutic potential of the neurotrophins per se maybe functionally limited, yet it remains plausible that the selective molecular targeting of p75NTR may, in principle, exert a unified promyelinating response in the PNS. A couple of research groups have recently identified compounds that act as putative selective p75NTR modulators (Massa et al. 2006; Fletcher et al. 2008; Fletcher and Hughes 2009), so the potential of a selective agonist that uniformly promotes the myelination of distinct subsets of peripheral neurons remains a distinct possibility.

3.2 The Neurotrophins Promote Central Remyelination

There are two major groups of CNS demyelinating diseases. The first is the leukodystrophies, a group of genetic disorders that affect the production or metabolism of one of several of the component molecules of myelin. The second is inflammatory demyelinating diseases, caused by autoimmune mediated damage to myelin and oligodendrocytes, with MS being by far the most significant of these diseases. Experimental models of CNS demyelination, including experimental autoimmune encephalomyelitis (EAE) and toxic models of demyelination such as lysolecithin, ethidium bromide, and cuprizone models, have been widely used to examine the therapeutic potential of candidate molecules and to assess the affect they exert upon oligodendroglial and immune cells, as well as their influence on the extent of demyelination and remyelination (Rodriguez 2007).

While the roles that the neurotrophins exert on CNS myelination have been extensively studied in the context of development (Barres et al. 1993; Cellerino et al. 1997; Chan et al. 2004; Xiao et al. 2010), the influence that they exert upon demyelination and remyelination has received substantially less attention. BDNF is the neurotrophin most widely expressed in the CNS and unsurprisingly has been the focus of most attention.

In the normal CNS, BDNF is primarily derived from neurons (Lewin and Barde 1996). However, in inflamed areas in the CNS, such as occurs in MS and EAE, activated astrocytes express increased levels of endogenous BDNF (Stadelmann et al. 2002; Burbach et al. 2004; Linker et al. 2010). Moreover, BDNF is also produced by several immune cell subtypes in vitro, such as activated T cells, B cells, and monocytes (Kerschensteiner et al. 1999; Stadelmann et al. 2002). Thus, BDNF is well placed to exert a modulatory effect upon myelination in this context (Stadelmann et al. 2002). Interestingly in the EAE model, the MS

immune-modulatory drug glatiramer acetate causes a sustained increase in BDNF expression in the brain, which its protective effects are dependent upon (Aharoni et al. 2005; De Santi et al. 2009; Lee et al. 2011).

Several recent studies have implicated BDNF in exerting a functionally protective role following CNS demyelination. In response to the cuprizone model of demyelination, BDNF heterozygote mice exhibit greater demyelination and reduced remyelination in the absence of any change to the total number of oligodendroglia, microglia, astrocytes, or extent of axonal injury (VonDran et al. 2011). In mice in which BDNF has been selectively deleted under the influence of the GFAP promoter, and thus theoretically deleted in oligodendrocytes, astrocytes, and some neurons, a more severe course of EAE and increased axonal loss was observed (Linker et al. 2010). Interestingly, the protective effect of BDNF in this model is temporally restricted, as an inducible conditional knockout strategy demonstrated that BDNF exerts its protective effects primarily during the initial phases of EAE, reducing clinical symptoms and structural damage, whereas deletion of BDNF in later stages of the disease had no significant effect on either disease course or axonal integrity (Lee et al. 2011). This suggests there may be an early window of therapeutic opportunity for the modulation of BDNF levels, at least in EAE.

It appears that not only central nervous system-derived BDNF is important, as mice deficient for BDNF in T cells exhibited progressive disability and enhanced axonal loss in EAE, while mice overexpressing BDNF in T cells exhibited less severe EAE and axonal protection (Linker et al. 2010). Despite this, however, CNS-derived BDNF appears more critical than immune cell-derived BDNF. Experiments utilizing bone marrow chimeras reveal that following CNS deletion of BDNF, immune cell-derived BDNF cannot influence disease severity (Lee et al. 2011). This suggests that while immune cell-derived BDNF can modulate disease, CNS resident cells are the major source of biologically relevant BDNF in autoimmune demyelination. Whether in this context BDNF exerts its influence directly on the myelinated axon/neuron-oligodendrocyte interface remains an open question. In an alternate strategy, treatment with BDNF-engineered bone marrow stem cells also resulted in a significant delay to EAE onset, reduced clinical severity, reduced expression of pro-inflammatory cytokines TNF-α and IFN-γ, and increased expression of anti-inflammatory cytokines such as IL-4, IL-10, and IL-11 (Makar et al. 2009). In addition, immune cell expression of TrkB has been shown to play an important role in EAE immunopathogenesis by modulating autoreactive T cell survival and behavior (De Santi et al. 2009). This indicates that BDNF may also influence the immune response, suggesting its protective effect at least in part could be indirect. Ultimately the identity of the cells that BDNF targets for its protective effect may not be absolutely identified until a new strategy is taken, whereupon the EAE model is undertaken in mice in which the BDNF receptor is conditionally targeted in distinct candidate cell types.

In the CNS, TrkB expression is present in various cell types including neurons, oligodendroglial cells, astrocytes, as well as infiltrating immune cells (Cahoy et al. 2008; De Santi et al. 2009), so there is no shortage of potential BDNF targets.

Interestingly, the detection of phosphorylated TrkB was increased in the neurons of EAE-diseased mice compared to the intact axons of control mice, suggesting BDNF is at least exerting a neuronal effect in this context (Linker et al. 2010). As both in vitro and in vivo analyses have identified that oligodendroglial TrkB potentiates CNS myelination (Xiao et al. 2010; Wong et al. 2013), it would be interesting to investigate directly whether oligodendrocyte expression and phosphorylation of TrkB is altered in models of demyelination/remyelination. This would provide some insight into whether the selective promyelinating influence that oligodendroglial TrkB signalling exerts during development is also relevant to remyelination. What is beyond dispute however is that regardless of the model or the strategy to manipulate BDNF or TrkB expression, the effect they exert is a uniformly promyelinating one. Future studies aimed at identifying the cellular and molecular basis of the protective and regenerative effects that BDNF exerts in the context of animal models of demyelinating diseases are critical now in order to establish how these responses might ultimately be targeted and modulated for therapeutic benefit.

4 The Therapeutic Potential of Targeting Neurotrophin Receptors for Promoting Remyelination

Despite substantial increases in our understanding of the mechanisms of myelination, demyelination, and remyelination, current therapeutic interventions for demyelinating diseases are suboptimal. These advances in knowledge have yet to be translated into the clinic, meaning that current therapies focus on reducing inflammation rather than directly promoting remyelination. However, as discussed above, there is now a strong body of evidence from preclinical studies pointing to the therapeutic potential of neurotrophins in preventing or slowing demyelination and promoting remyelination.

Human clinical trials assessing the efficacy of human recombinant NGF as a treatment for peripheral neuropathy were undertaken over a decade ago and were discontinued due to side effects including pain (Apfel et al. 1994; Apfel 1999b; Apfel et al. 2000; Apfel 2001, 2002). While the effect on pain sensitization is likely to be an NGF-specific side effect, the complexity of neurotrophin receptor interactions and the more recent identification of the context-dependent influences the neurotrophins exert upon peripheral myelination described above confound the clinical use of recombinant neurotrophins for this purpose. In addition the pharmacokinetic behavior of the neurotrophins is generally not optimal for pharmaceutical use. BDNF, for example, has a half-life in the circulation of less than 1 min in the rat (Poduslo and Curran 1996), and, as one would expect with a protein, there is no strong evidence that it can cross the blood–brain barrier.

Given the difficulties inherent with using recombinant neurotrophins themselves to treat demyelinating and other CNS disorders, increasing attention has turned to the development of alternative strategies to harness neurotrophic action for clinical use. One means of doing this is to use functional mimetics of the neurotrophins.

These compounds would have the advantage of being able to selectively target p75NTR or Trk receptors and could be tailored to have appropriate pharmacokinetic properties. Moreover, exciting recent data describing G protein-coupled receptor (GPCR) transactivation of Trk receptors and their downstream signalling pathways raise the possibility of using an even wider range of small molecules to elicit promyelinating effects.

4.1 Putative p75NTR Agonists

Unlike NGF, BDNF does not induce painful side effects and is safe and well tolerated in both animals and humans (Mitsumoto et al. 1994; Ikeda et al. 1995). Based on published data (Chan et al. 2001; Cosgaya et al. 2002; Xiao et al. 2009b), BDNF itself could be considered as a suitable promyelinating candidate to test in the context of peripheral neuropathy. However, as described above, while BDNF promotes myelination of peripheral axons via activation of neuronally expressed p75NTR, it also inhibits the myelination of TrkB-expressing neurons (Xiao et al. 2009b). Thus, at least in the context of peripheral myelination, compounds that selectively activate p75NTR, without concomitantly activating TrkB, would be expected in principle to produce a unified promyelinating response in the periphery.

Recently, two different classes of selective modulators of p75NTR have been described. Structure-based design approaches have been used to obtain conformationally constrained, low molecular weight peptides based on the solvent-exposed loops of BDNF involved in binding to its receptors (Fig. 3). Using this approach, monomeric mimetics of loop 4, exemplified by the cyclic pentapeptide cyclo-[DPro-Ala-Lys-Lys-Arg] (cyclo-DPAKKR), have been developed (Fletcher et al. 2008; Fletcher and Hughes 2009), consisting of the cationic "Lys-Lys-Arg" motif shown to be required for the binding of BDNF to p75NTR (Ryden et al. 1995), presented in a highly conformationally constrained cyclic template. Like BDNF, cyclo-DPAKKR promotes the survival of embryonic chick sensory neurons in culture (Fletcher et al. 2008). However, its mechanism of action is distinct to BDNF, in that it does not cause TrkB autophosphorylation, nor does it lead to phosphorylation of MAPK, a downstream mediator of TrkB signalling, consistent with an effect through p75NTR (Fletcher et al. 2008). In addition, cyclo-DPAKKR has low molecular weight (580) and is highly resistant to proteolysis. These properties of cyclo-DPAKKR and analogues render them unique pharmacological tools to investigate the influence of selective modulation of p75NTR on PNS myelination.

Indeed, recent data indicate the effects that cyclo-DPAKKR exerts upon peripheral myelination are entirely consistent with it acting as a selective p75NTR activator: *promoting* the myelination of distinct subsets of DRG neurons in vitro and requiring neuronal expression of p75NTR. Importantly, local injection of cyclo-DPAKKR adjacent to the developing sciatic nerve in vivo also significantly enhanced myelin protein expression and increased the number of myelinated axons (Xiao et al. 2013). It was also identified that the promyelinating effect of cyclo-DPAKKR was coincident with an increase in the expression of neuregulin-1

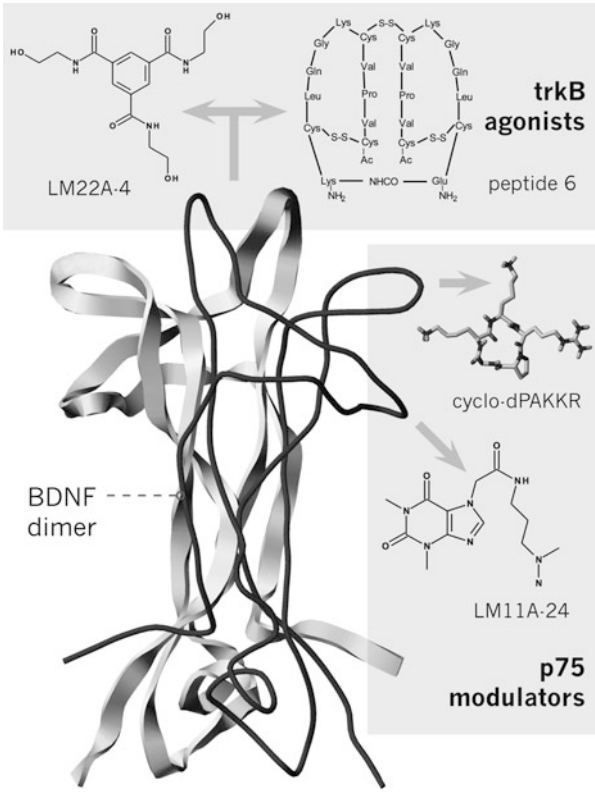

Fig. 3 Molecular structure of BDNF and mimetics. The central image is the three-dimensional structure of the BDNF dimer (*ribbon representation*). The TrkB agonists, mimicking the loop 2 region of BDNF, are shown above: LM22A-4 (Massa et al. 2010) and the tricyclic dimeric peptide 6 (O'Leary and Hughes 2003). The p75NTR modulators are shown to the right: cyclo-DPAKKR (3D depiction of an NMR-derived structure in solution; (Fletcher et al. 2008)) derived from loop 4 of BDNF and LM11A-24 (2D structure; (Massa et al. 2006)) derived from loop 1 of NGF (Images not drawn to scale)

type III which provides the key instructive axonal signal that initiates myelination (Michailov et al. 2004; Taveggia et al. 2005). Collectively, these data demonstrate that cyclo-DPAKKR promotes peripheral myelination in vitro and in vivo, suggesting it is a strategy worthy of further investigation for the treatment of peripheral demyelinating diseases.

Recently, Frank Longo's group described a tandem in silico and in vitro approach that leads to the identification of a novel p75NTR ligand, LM11A-24 (Massa et al. 2006). This small molecule, a derivative of caffeine that was selected as a mimic of loop 1 of NGF, has been found to inhibit Aß-induced neurodegeneration and synaptic impairment, block proNGF-induced cell death, and prevent the death of motor neurons in vitro (Pehar et al. 2006; Yang et al. 2008). As a putative selective p75NTR modulator, it would be interesting to investigate the effect that LM11A-24 exerts on Schwann cell myelination in vitro. In particular, it will be intriguing to see if it is able to uniformly promote myelin formation of distinct populations of DRG neurons in vitro and whether this influence can be replicated on peripheral myelin development in vivo. Ultimately, the logical extension of this approach would be to investigate whether both cyclo-DPAKKR and LM11A-24 have the ability to reduce the severity/promote remyelination in an animal model of peripheral nerve demyelination such as EAN.

4.2 Putative TrkB Agonists

In contrast to its apparent inhibitory influence upon PNS myelination, TrkB signalling has been shown to play a key role in promoting CNS myelination. Thus, the selective targeting of TrkB offers a potential means of promoting CNS remyelination. A version of the structure-based design approach has been used to obtain conformationally constrained, low molecular weight peptides that mimic solvent-exposed loop 2 of BDNF, the region involved in binding TrkB (Fig. 3). Using this approach, monomeric mimetics of loop 2 that act as TrkB antagonists (O'Leary and Hughes 1998) and dimeric mimetics of loop 2 that are highly potent putative TrkB agonists (O'Leary and Hughes 2003) have been successfully developed. Like BDNF itself, the putative TrkB agonists promote neuronal survival in cell culture (O'Leary and Hughes 2003). The best of these dimeric peptides, the conformationally constrained tricyclic dimeric peptide 6, is highly potent with an EC50 for the survival of sensory neurons in culture of 11pM (O'Leary and Hughes 2003), that is, the same order of magnitude as BDNF itself, despite being less than one-tenth the molecular weight of the parent protein.

Similarly, an in silico screen with a BDNF pharmacophore followed by low-throughput in vitro screening in mouse fetal hippocampal neurons led to the discovery of small molecules with nanomolar neurotrophic activity specific to TrkB, typified by LM22A-4 (Massa et al. 2010). LM22A-4 prevents neural cell death with an efficacy equal to BDNF in vitro and causes hippocampal and striatal TrkB activation and improved motor learning in an in vivo model of traumatic brain injury (Massa et al. 2010). It also improves functional recovery and increases neurogenesis following hypoxic-ischemic stroke (Han et al. 2012) and rescues TrkB phosphorylation and improves respiratory function in a model of Rett syndrome (Schmid et al. 2012). Together, these studies demonstrate the potential utility of BDNF – particularly its solvent-exposed loops – as a template for the discovery of novel low molecular compounds that mimic the action of the parent protein.

Adopting a distinct approach, 7,8-dihydroxyflavone was identified in a cell-based screen as a high-affinity TrkB agonist and provokes receptor dimerization, autophosphorylation, and activation of downstream signalling (Jang et al. 2010). Dihydroxyflavone protects neurons from apoptosis in vitro in a TrkB-dependent manner and is neuroprotective in several models of neuronal injury such as kainic acid-induced apoptosis, the transient MCAO occlusion model of stroke, and the MPTP model of Parkinson's disease. It also exerts positive influences in in vivo models of Rett syndrome (Johnson et al. 2012) and depression (Liu et al. 2010, 2012).

The discovery of these small molecules that function as specific TrkB ligands has made modulating selected components of neurotrophin signalling possible for the first time. As the selective modulation of TrkB could achieve more favorable effects than those mediated by BDNF, which also stimulates other neurotrophin receptors, these small molecules provide a potentially powerful therapeutic tool for the treatment of demyelinating disease. The scene is now set to examine the effects

that these compounds exert upon oligodendrocyte myelination in vitro and if successful to extend this work to identify whether they exert a protective influence in animal models of CNS demyelination.

4.3 Transactivation of Trk Neurotrophin Receptors by GPCR Ligands

One final strategy for the targeting of neurotrophin receptors lies in the discovery that Trk receptors can be transactivated by GPCRs (Lee and Chao 2001). Transactivation of receptor tyrosine kinases by GPCRs is now recognized as an important signalling mechanism allowing the cell to respond to a vast array of extracellular stimuli (Daub et al. 1996; Luttrell et al. 1999; Rajagopal et al. 2004). Both TrkA and TrkB receptors can be activated, in the absence of NGF or BDNF, via a GPCR-dependent mechanism. At least two GPCR ligands, adenosine and pituitary adenylate cyclase-activating peptide (PACAP), can activate Trk receptors to promote neuronal survival in vitro, an effect dependent upon Trk-mediated activation of Akt activity (Lee and Chao 2001; Lee et al. 2002; Rajagopal et al. 2004). The effect of adenosine and PACAP upon Trk receptor activation is unusual in that it displays very slow kinetics and selectively activates intracellular receptors. Nevertheless, this effect has been shown to be therapeutically relevant in vivo, as transactivation of the TrkB receptor by adenosine agonists promotes motor neuron survival after facial nerve lesioning (Wiese et al. 2007). While studies to date have focussed selectively on neuronal survival, in principle GPCR transactivation of Trk receptors could also exert a promyelinating influence, as not only is the Akt pathway activated by Trk receptors following transactivation but it is also implicated in promoting oligodendrocyte myelination (Lee and Chao 2001; Narayanan et al. 2009; Tyler et al. 2009). Thus, in the context of oligodendrocyte myelination, further studies are clearly warranted to investigate the influence that GPCR transactivation of Trk receptors exerts. However, for Schwann cell myelination the case is less clear, as in vitro studies have revealed that Trk receptor signalling in peripheral neurons exerts contrasting effects upon Schwann cell myelination and the signalling mechanisms that mediate these distinct responses have yet to be elucidated. In totality, a more complete understanding of the potential influences that GPCR transactivation exerts upon models of both central and peripheral myelination in vitro is required in order to establish an evidence-based rationale for future investigations aimed at determining the therapeutic potential of this mechanism of neurotrophin receptor activation.

5 Conclusion

It has proven useful to identify the precise influence and the cellular and molecular basis of how the neurotrophins regulate myelination in vitro. Based on these findings, it is now important to attempt to verify these conclusions through the specific conditional targeting of the neurotrophins and their receptors in vivo, in order to

develop a clear understanding of the key influences the neurotrophins exert upon myelination. Future studies aimed at identifying whether these mechanisms are relevant not only to development but also in the context animal models of demyelinating diseases will afford specific insight into potential therapeutic approaches that may effect remyelination. The fact that the neurotrophins can exert positive as well as negative influences on myelination in vitro highlights another challenge. However, the evolving development of small molecule neurotrophin mimetics that selectively target distinct neurotrophin receptors affords a degree of hope that ultimately evidence-based rational approaches that enhance these responses will provide therapeutic benefit in the context of human demyelinating disease.

References

Aharoni, R., Eilam, R., Domev, H., Labunskay, G., Sela, M., & Arnon, R. (2005). The immunomodulator glatiramer acetate augments the expression of neurotrophic factors in brains of experimental autoimmune encephalomyelitis mice. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 19045–19050.

Apfel, S. C. (1999a). Neurotrophic factors and diabetic peripheral neuropathy. *European Neurology, 41*(Suppl 1), 27–34.

Apfel, S. C. (1999b). Neurotrophic factors in peripheral neuropathies: Therapeutic implications. *Brain Pathology, 9*, 393–413.

Apfel, S. C. (2001). Neurotrophic factor therapy–prospects and problems. *Clinical Chemistry and Laboratory Medicine, 39*, 351–355.

Apfel, S. C. (2002). Nerve growth factor for the treatment of diabetic neuropathy: What went wrong, what went right, and what does the future hold? *International Review of Neurobiology, 50*, 393–413.

Apfel, S. C., Arezzo, J. C., Brownlee, M., Federoff, H., & Kessler, J. A. (1994). Nerve growth factor administration protects against experimental diabetic sensory neuropathy. *Brain Research, 634*, 7–12.

Apfel, S. C., Kessler, J. A., Adornato, B. T., Litchy, W. J., Sanders, C., & Rask, C. A. (1998). Recombinant human nerve growth factor in the treatment of diabetic polyneuropathy. NGF study group. *Neurology, 51*, 695–702.

Apfel, S. C., Schwartz, S., Adornato, B. T., Freeman, R., Biton, V., Rendell, M., Vinik, A., Giuliani, M., Stevens, J. C., Barbano, R., & Dyck, P. J. (2000). Efficacy and safety of recombinant human nerve growth factor in patients with diabetic polyneuropathy: A randomized controlled trial. RhNGF clinical investigator group. *JAMA : The Journal of the American Medical Association, 284*, 2215–2221.

Barres, B. A., Schmid, R., Sendnter, M., & Raff, M. C. (1993). Multiple extracellular signals are required for long-term oligodendrocyte survival. *Development, 118*, 283–295.

Barres, B. A., Raff, M. C., Gaese, F., Bartke, I., Dechant, G., & Barde, Y. A. (1994). A crucial role for neurotrophin-3 in oligodendrocyte development. *Nature, 367*, 371–375.

Birchmeier, C., & Nave, K. A. (2008). Neuregulin-1, a key axonal signal that drives schwann cell growth and differentiation. *Glia, 56*, 1491–1497.

Bitsch, A., Schuchardt, J., Bunkowski, S., Kuhlmann, T., & Bruck, W. (2000). Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. *Brain, 123*(Pt 6), 1174–1183.

Bron, R., Klesse, L. J., Shah, K., Parada, L. F., & Winter, J. (2003). Activation of Ras is necessary and sufficient for upregulation of vanilloid receptor type 1 in sensory neurons by neurotrophic factors. *Molecular and Cellular Neurosciences, 22*, 118–132.

Burbach, G. J., Hellweg, R., Haas, C. A., Del, T. D., Deicke, U., & Abramowski, D. (2004). Induction of brain-derived neurotrophic factor in plaque-associated glial cells of aged APP23 transgenic mice. *Journal of Neuroscience, 24*, 10.

Cahoy, J. D., Emery, B., Kaushal, A., Foo, L. F., Zamanian, J. L., Christopherson, K. S., Xing, Y., Lubischer, J. L., Krieg, P. A., Krupenko, S. A., Thompson, W. J., & Barres, B. A. (2008). A transcriptome database for astrocytes, neurons, and oligodendrocytes: A New resource for understanding brain development and function. *Journal of Neuroscience, 28*, 14.

Cellerino, A., Carroll, P., Thoenen, H., & Barde, Y. A. (1997). Reduced size of retinal ganglion cell axons and hypomyelination in mice lacking brain-derived neurotrophic factor. *Molecular and Cellular Neuroscience, 9*, 397–408.

Chan, J. R., Cosgaya, J. M., Wu, Y. J., & Shooter, E. M. (2001). Neurotrophins are key mediators of the myelination program in the peripheral nervous system. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 14661–14668.

Chan, J. R., Watkins, T. A., Cosgaya, J. M., Zhang, C., Chen, L., Reichardt, L. F., Shooter, E. M., & Barres, B. A. (2004). NGF controls axonal receptivity to myelination by schwann cells or oligodendrocytes. *Neuron, 43*, 183–191.

Chan, J. R., Jolicoeur, C., Yamauchi, J., Elliott, J., Fawcett, J. P., Ng, B. K., & Cayouette, M. (2006). The polarity protein par-3 directly interacts with p75NTR to regulate myelination. *Science, 314*, 832–836.

Chao, M. V. (2003). Neurotrophins and their receptors: A convergence point for many signalling pathways. *Nature Reviews Neuroscience, 4*, 299–309.

Chew, L. J., Coley, W., Cheng, Y., & Gallo, V. (2010). Mechanisms of regulation of oligodendrocyte development by p38 mitogen-activated protein kinase. *Journal of Neuroscience, 30*, 11011–11027.

Cohen, R. I., Marmur, R., Norton, W. T., Mehler, M. F., & Kessler, J. A. (1996). Nerve growth factor and neurotrophin-3 differentially regulate the proliferation and survival of developing rat brain oligodendrocytes. *Journal of Neuroscience, 16*, 6433–6442.

Conover, J. C., & Yancopoulos, G. D. (1997). Neurotrophin regulation of the developing nervous system: Analyses of knockout mice. *Reviews in the Neurosciences, 8*, 13–27.

Cosgaya, J. M., Chan, J. R., & Shooter, E. M. (2002). The neurotrophin receptor p75NTR as a positive modulator of myelination. *Science, 298*, 1245–1248.

Crowley, C., Spencer, S. D., Nishimura, M. C., Chen, K. S., Pitts-Meek, S., Armanini, M. P., Ling, L. H., McMahon, S. B., Shelton, D. L., Levinson, A. D., et al. (1994). Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. *Cell, 76*, 1001–1011.

Daub, H., Weiss, F. U., Wallasch, C., & Ullrich, A. (1996). Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature, 379*, 557–560.

De Santi, L., Annunziata, P., Sessa, E., & Bramanti, P. (2009). Brain-derived neurotrophic factor and TrkB receptor in experimental autoimmune encephalomyelitis and multiple sclerosis. *Journal of Neurological Sciences, 287*, 17–26.

De Stefano, N., Matthews, P. M., Fu, L., Narayanan, S., Stanley, J., Francis, G. S., Antel, J. P., & Arnold, D. L. (1998). Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. *Brain, 121*(Pt 8), 1469–1477.

Du, Y., Fischer, T. Z., Lee, L. N., Lercher, L. D., & Dreyfus, C. F. (2003). Regionally specific effects of BDNF on oligodendrocytes. *Developmental Neuroscience, 25*, 116–126.

Esposito, D., Patel, P., Stephens, R. M., Perez, P., Chao, M. V., Kaplan, D. R., & Hempstead, B. L. (2001). The cytoplasmic and transmembrane domains of the p75 and Trk a receptors regulate high affinity binding to nerve growth factor. *Journal of Biological Chemistry, 276*, 32687–32695.

Fletcher, J. M., & Hughes, R. A. (2009). Modified low molecular weight cyclic peptides as mimetics of BDNF with improved potency, proteolytic stability and transmembrane passage in vitro. *Bioorganic & Medicinal Chemistry, 17*, 2695–2702.

Fletcher, J. M., Morton, C. J., Zwar, R. A., Murray, S. S., O'Leary, P. D., & Hughes, R. A. (2008). Design of a conformationally defined and proteolytically stable circular mimetic of brain-derived neurotrophic factor. *Journal of Biological Chemistry, 283*, 33375–33383.

Franklin, R. J., & Ffrench-Constant, C. (2008). Remyelination in the CNS: From biology to therapy. *Nature Reviews Neuroscience, 9*, 839–855.

Franklin, R. J., Ffrench-Constant, C., Edgar, J. M., & Smith, K. J. (2012). Neuroprotection and repair in multiple sclerosis. *Nature Reviews. Neurology, 8*, 624–634.

Fressinaud, C., Jean, I., & Dubas, F. (2003). Selective decrease in axonal nerve growth factor and insulin-like growth factor I immunoreactivity in axonopathies of unknown etiology. *Acta Neuropathologica, 105*, 477–483.

Fyffe-Maricich, S. L., Karlo, J. C., Landreth, G. E., & Miller, R. H. (2011). The ERK2 mitogen-activated protein kinase regulates the timing of oligodendrocyte differentiation. *Journal of Neuroscience, 31*, 843–850.

Gold, R., Hartung, H. P., & Toyka, K. V. (2000). Animal models for autoimmune demyelinating disorders of the nervous system. *Molecular Medicine Today, 6*, 88–91.

Han, J., Pollak, J., Yang, T., Siddiqui, M. R., Doyle, K. P., Taravosh-Lahn, K., Cekanaviciute, E., Han, A., Goodman, J. Z., Jones, B., Jing, D., Massa, S. M., Longo, F. M., & Buckwalter, M. S. (2012). Delayed administration of a small molecule tropomyosin-related kinase B ligand promotes recovery after hypoxic-ischemic stroke. *Stroke, 43*, 1918–1924.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: Roles in neuronal development and function. *Annual Review of Neuroscience, 24*, 677–736.

Huang, E. J., & Reichardt, L. F. (2003). Trk receptors: Roles in neuronal signal transduction. *Annual Review of Biochemistry, 72*, 609–642.

Hughes, R. (2008). Peripheral nerve diseases: The bare essentials. *Practical Neurology, 8*, 396–405.

Hughes, R. A., & Cornblath, D. R. (2005). Guillain-Barre syndrome. *Lancet, 366*, 1653–1666.

Ikeda, K., Klinkosz, B., Greene, T., Cedarbaum, J. M., Wong, V., Lindsay, R. M., & Mitsumoto, H. (1995). Effects of brain-derived neurotrophic factor on motor dysfunction in wobbler mouse motor neuron disease. *Annals of Neurology, 37*, 505–511.

Irvine, K. A., & Blakemore, W. F. (2008). Remyelination protects axons from demyelination-associated axon degeneration. *Brain, 131*, 1464–1477.

Ishii, A., Fyffe-Maricich, S. L., Furusho, M., Miller, R. H., & Bansal, R. (2012). ERK1/ERK2 MAPK signaling is required to increase myelin thickness independent of oligodendrocyte differentiation and initiation of myelination. *Journal of Neuroscience, 32*, 8855–8864.

Jang, S. W., Liu, X., Yepes, M., Shepherd, K. R., Miller, G. W., Liu, Y., Wilson, W. D., Xiao, G., Blanchi, B., Sun, Y. E., & Ye, K. (2010). A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 2687–2692.

Je, H. S., Yang, F., Ji, Y., Nagappan, G., Hempstead, B. L., & Lu, B. (2012). Role of pro-brain-derived neurotrophic factor (proBDNF) to mature BDNF conversion in activity-dependent competition at developing neuromuscular synapses. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 15924–15929.

Jeffery, N. D., & Blakemore, W. F. (1997). Locomotor deficits induced by experimental spinal cord demyelination are abolished by spontaneous remyelination. *Brain, 120*(Pt 1), 27–37.

Johnson, R. A., Lam, M., Punzo, A. M., Li, H., Lin, B. R., Ye, K., Mitchell, G. S., & Chang, Q. (2012). 7,8-Dihydroxyflavone exhibits therapeutic efficacy in a mouse model of Rett syndrome. *Journal of Applied Physiology, 112*, 704–710.

Jones, K. R., Farinas, I., Backus, C., & Reichardt, L. F. (1994). Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell, 76*, 989–999.

Kahn, M. A., Kumar, S., Liebl, D., Chang, R., Parada, L. F., & De Vellis, J. (1999). Mice lacking NT-3, and its receptor TrkC, exhibit profound deficiencies in CNS glial cells. *Glia, 26*, 153–165.

Kerschensteiner, M., Gallmeier, E., Behrens, L., Leal, V. V., Misgeld, T., Klinkert, W. E., Kolbeck, R., Hoppe, E., Oropeza-Wekerle, R. L., Bartke, I., Stadelmann, C., Lassmann, H., Wekerle, H., & Hohlfeld, R. (1999). Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: A neuroprotective role of inflammation? *The Journal of Experimental Medicine, 189*, 865–870.

Kumar, S., & de Vellis, J. (1996). Neurotrophin activates signal transduction in oligodendroglial cells: Expression of functional TrkC receptor isoforms. *Journal of Neuroscience Research, 44*, 490–498.

Kumar, S., Kahn, M. A., Dinh, L., & de Vellis, J. (1998). NT-3-mediated TrkC receptor activation promotes proliferation and cell survival of rodent progenitor oligodendrocyte cells in vitro and in vivo. *Journal of Neuroscience Research, 54*, 754–765.

Lappe-Siefke, C., Goebbels, S., Gravel, M., Nicksch, E., Lee, J., Braun, P. E., Griffiths, I. R., & Nave, K. A. (2003). Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. *Nature Genetics, 33*, 366–374.

Lee, D. H., Geyer, E., Flach, A. C., Jung, K., Gold, R., Flügel, A., Linker, R. A., & Lühder, F. (2011). Central nervous system rather than immune cell-derived BDNF mediates axonal protective effects early in autoimmune demyelination. *Acta Neuropathologica, 123*, 247–258.

Lee, F. S., & Chao, M. V. (2001). Activation of Trk neurotrophin receptors in the absence of neurotrophins. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 3555–3560.

Lee, F. S., Rajagopal, R., Kim, A. H., Chang, P. C., & Chao, M. V. (2002). Activation of Trk neurotrophin receptor signaling by pituitary adenylate cyclase-activating polypeptides. *Journal of Biological Chemistry, 277*, 9096–9102.

Lee, X., Yang, Z., Shao, Z., Rosenberg, S. S., Levesque, M., Pepinsky, R. B., Qiu, M., Miller, R. H., Chan, J. R., & Mi, S. (2007). NGF regulates the expression of axonal LINGO-1 to inhibit oligodendrocyte differentiation and myelination. *Journal of Neuroscience, 27*, 220–225.

Lee, Y., Morrison, B. M., Li, Y., Lengacher, S., Farah, M. H., Hoffman, P. N., Liu, Y., Tsingalia, A., Jin, L., Zhang, P. W., Pellerin, L., Magistretti, P. J., & Rothstein, J. D. (2012). Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature, 487*, 443–448.

Lewin, G. R., & Barde, Y. A. (1996). Physiology of the neurotrophins. *Annual Review of Neuroscience, 19*, 289–317.

Liebetanz, D., & Merkler, D. (2006). Effects of commissural de- and remyelination on motor skill behaviour in the cuprizone mouse model of multiple sclerosis. *Experimental Neurology, 202*, 217–224.

Liebl, D. J., Tessarollo, L., Palko, M. E., & Parada, L. F. (1997). Absence of sensory neurons before target innervation in brain-derived neurotrophic factor-, neurotrophin 3-, and TrkC-deficient embryonic mice. *Journal of Neuroscience, 17*, 9113–9121.

Linker, R. A., Lee, D. H., Demir, S., Wiese, S., Kruse, N., Siglienti, I., Gerhardt, E., Neumann, H., Sendtner, M., Luhder, F., & Gold, R. (2010). Functional role of brain-derived neurotrophic factor in neuroprotective autoimmunity: Therapeutic implications in a model of multiple sclerosis. *Brain, 133*, 2248–2263.

Liu, X., Chan, C. B., Jang, S. W., Pradoldej, S., Huang, J., He, K., Phun, L. H., France, S., Xiao, G., Jia, Y., Luo, H. R., & Ye, K. (2010). A synthetic 7,8-dihydroxyflavone derivative promotes neurogenesis and exhibits potent antidepressant effect. *Journal of Medicinal Chemistry, 53*, 8274–8286.

Liu, X., Chan, C. B., Qi, Q., Xiao, G., Luo, H. R., He, X., & Ye, K. (2012). Optimization of a small tropomyosin-related kinase B (TrkB) agonist 7,8-dihydroxyflavone active in mouse models of depression. *Journal of Medicinal Chemistry, 55*, 8524–8537.

Luttrell, L. M., Daaka, Y., & Lefkowitz, R. J. (1999). Regulation of tyrosine kinase cascades by G-protein-coupled receptors. *Current Opinion in Cell Biology, 11*, 177–183.

Makar, T. K., Bever, C. T., Singh, I. S., Royal, W., Sahu, S. N., Sura, T. P., Sultana, S., Sura, K. T., Patel, N., Dhib-Jalbut, S., & Trisler, D. (2009). Brain-derived neurotrophic factor gene delivery

in an animal model of multiple sclerosis using bone marrow stem cells as a vehicle. *Journal of Neuroimmunology, 210*, 40–51.

Massa, S. M., Xie, Y., Yang, T., Harrington, A. W., Kim, M. L., Yoon, S. O., Kraemer, R., Moore, L. A., Hempstead, B. L., & Longo, F. M. (2006). Small, nonpeptide p75NTR ligands induce survival signaling and inhibit proNGF-induced death. *Journal of Neuroscience, 26*, 5288–5300.

Massa, S. M., Yang, T., Xie, Y., Shi, J., Bilgen, M., Joyce, J. N., Nehama, D., Rajadas, J., & Longo, F. M. (2010). Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. *The Journal of Clinical Investigation, 120*, 1774–1785.

McDonald, W. I., & Sears, T. A. (1969). Effect of demyelination on conduction in the central nervous system. *Nature, 221*, 182–183.

McHugh, J. M., & McHugh, W. B. (2004). Diabetes and peripheral sensory neurons: What we don't know and how it can hurt us. *AACN Clinical Issues, 15*, 136–149.

Mi, S., Miller, R. H., Lee, X., Scott, M. L., Shulag-Morskaya, S., Shao, Z., Chang, J., Thill, G., Levesque, M., Zhang, M., Hession, C., Sah, D., Trapp, B., He, Z., Jung, V., McCoy, J. M., & Pepinsky, R. B. (2005). LINGO-1 negatively regulates myelination by oligodendrocytes. *Nature Neuroscience, 8*, 745–751.

Michailov, G. V., Sereda, M. W., Brinkmann, B. G., Fischer, T. M., Haug, B., Birchmeier, C., Role, L., Lai, C., Schwab, M. H., & Nave, K. A. (2004). Axonal neuregulin-1 regulates myelin sheath thickness. *Science, 304*, 700–703.

Miller, D. H. (1996). Demyelinating diseases. *Current Opinion in Neurology, 9*, 153–154.

Mitsumoto, H., Ikeda, K., Klinkosz, B., Cedarbaum, J. M., Wong, V., & Lindsay, R. M. (1994). Arrest of motor neuron disease in wobbler mice cotreated with CNTF and BDNF. *Science, 265*, 1107–1110.

Molliver, D. C., & Snider, W. D. (1997). Nerve growth factor receptor TrkA is down-regulated during postnatal development by a subset of dorsal root ganglion neurons. *The Journal of Comparative Neurology, 381*, 428–438.

Murray, S. S., Bartlett, P. F., & Cheema, S. S. (1999). Differential loss of spinal sensory but not motor neurons in the p75NTR knockout mouse. *Neuroscience Letters, 267*, 45–48.

Narayanan, S. P., Flores, A. I., Wang, F., & Macklin, W. B. (2009). Akt signals through the mammalian target of rapamycin pathway to regulate CNS myelination. *Journal of Neuroscience, 29*, 6860–6870.

Nave, K. A., & Salzer, J. L. (2006). Axonal regulation of myelination by neuregulin 1. *Current Opinion in Neurobiology, 16*, 492–500.

Obata, K., Katsura, H., Sakurai, J., Kobayashi, K., Yamanaka, H., Dai, Y., Fukuoka, T., & Noguchi, K. (2006). Suppression of the p75 neurotrophin receptor in uninjured sensory neurons reduces neuropathic pain after nerve injury. *Journal of Neuroscience, 26*, 11974–11986.

O'Leary, P. D., & Hughes, R. A. (1998). Structure-activity relationships of conformationally constrained peptide analogues of loop 2 of brain-derived neurotrophic factor. *Journal of Neurochemistry, 70*, 1712–1721.

O'Leary, P. D., & Hughes, R. A. (2003). Design of potent peptide mimetics of brain-derived neurotrophic factor. *Journal of Biological Chemistry, 278*, 25738–25744.

Oluich, L. J., Stratton, J. A., Xing, Y. L., Ng, S. W., Cate, H. S., Sah, P., Windels, F., Kilpatrick, T. J., & Merson, T. D. (2012). Targeted ablation of oligodendrocytes induces axonal pathology independent of overt demyelination. *Journal of Neuroscience, 32*, 8317–8330.

Pehar, M., Cassina, P., Vargas, M. R., Xie, Y., Beckman, J. S., Massa, S. M., Longo, F. M., & Barbeito, L. (2006). Modulation of p75-dependent motor neuron death by a small non-peptidyl mimetic of the neurotrophin loop 1 domain. *European Journal of Neuroscience, 24*, 1575–1580.

Pepinsky, R. B., Walus, L., Shao, Z., Ji, B., Gu, S., Sun, Y., Wen, D., Lee, X., Wang, Q., Garber, E., & Mi, S. (2011a). Production of a PEGylated Fab' of the anti-LINGO-1 Li33 antibody and

assessment of its biochemical and functional properties in vitro and in a rat model of remyelination. *Bioconjugate Chemistry, 22*, 200–210.

Pepinsky, R. B., Shao, Z., Ji, B., Wang, Q., Meng, G., Walus, L., Lee, X., Hu, Y., Graff, C., Garber, E., Meier, W., & Mi, S. (2011b). Exposure levels of anti-LINGO-1 Li81 antibody in the central nervous system and dose-efficacy relationships in rat spinal cord remyelination models after systemic administration. *Journal of Pharmacology and Experimental Therapeutics, 339*, 519–529.

Pittenger, G., & Vinik, A. (2003). Nerve growth factor and diabetic neuropathy. *Experimental Diabesity Research, 4*, 271–285.

Poduslo, J. F., & Curran, G. L. (1996). Permeability at the blood–brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. *Brain Research. Molecular Brain Research, 36*, 280–286.

Pradat, P. F., Kennel, P., Naimi-Sadaoui, S., Finiels, F., Orsini, C., Revah, F., Delaere, P., & Mallet, J. (2001). Continuous delivery of neurotrophin 3 by gene therapy has a neuroprotective effect in experimental models of diabetic and acrylamide neuropathies. *Human Gene Therapy, 12*, 2237–2249.

Rajagopal, R., Chen, Z. Y., Lee, F. S., & Chao, M. V. (2004). Transactivation of Trk neurotrophin receptors by G-protein-coupled receptor ligands occurs on intracellular membranes. *Journal of Neuroscience, 24*, 6650–6658.

Rodriguez, M. (2007). Effectors of demyelination and remyelination in the CNS: Implications for multiple sclerosis. *Brain Pathology, 17*, 219–229.

Rosenberg, S. S., Ng, B. K., & Chan, J. R. (2006). The quest for remyelination: A new role for neurotrophins and their receptors. *Brain Pathology, 16*, 288–294.

Rubio, N., Rodriguez, R., & Arevalo, M. A. (2004). In vitro myelination by oligodendrocyte precursor cells transfected with the neurotrophin-3 gene. *Glia, 47*, 78–87.

Ryden, M., Murray-Rust, J., Glass, D., Ilag, L. L., Trupp, M., Yancopoulos, G. D., McDonald, N. Q., & Ibanez, C. F. (1995). Functional analysis of mutant neurotrophins deficient in low-affinity binding reveals a role for p75LNGFR in NT-4 signalling. *EMBO Journal, 14*, 1979–1990.

Saini, H. S., Gorse, K. M., Boxer, L. M., & Sato-Bigbee, C. (2004). Neurotrophin-3 and a CREB-mediated signaling pathway regulate Bcl-2 expression in oligodendrocyte progenitor cells. *Journal of Neurochemistry, 89*, 951–961.

Schmid, D. A., Yang, T., Ogier, M., Adams, I., Mirakhur, Y., Wang, Q., Massa, S. M., Longo, F. M., & Katz, D. M. (2012). A TrkB small molecule partial agonist rescues TrkB phosphorylation deficits and improves respiratory function in a mouse model of Rett syndrome. *Journal of Neuroscience, 32*, 1803–1810.

Silos-Santiago, I., Molliver, D. C., Ozaki, S., Smeyne, R. J., Fagan, A. M., Barbacid, M., & Snider, W. D. (1995). Non-TrkA-expressing small DRG neurons are lost in TrkA deficient mice. *Journal of Neuroscience, 15*, 5929–5942.

Song, X. Y., Zhou, F. H., Zhong, J. H., Wu, L. L., & Zhou, X. F. (2006). Knockout of p75(NTR) impairs re-myelination of injured sciatic nerve in mice. *Journal of Neurochemistry, 96*, 833–842.

Stadelmann, C., Kerschensteiner, M., Misgeld, T., Bruck, W., Hohlfeld, R., & Lassmann, H. (2002). BDNF and gp145trkB in multiple sclerosis brain lesions: Neuroprotective interactions between immune and neuronal cells. *Brain, 125*, 11.

Taveggia, C., Zanazzi, G., Petrylak, A., Yano, H., Rosenbluth, J., Einheber, S., Xu, X., Esper, R. M., Loeb, J. A., Shrager, P., Chao, M. V., Falls, D. L., Role, L., & Salzer, J. L. (2005). Neuregulin-1 type III determines the ensheathment fate of axons. *Neuron, 47*, 681–694.

Teng, K. K., Felice, S., Kim, T., & Hempstead, B. L. (2010). Understanding proneurotrophin actions: Recent advances and challenges. *Developmental Neurobiology, 70*, 350–359.

Tessarollo, L., Tsoulfas, P., Donovan, M. J., Palko, M. E., Blair-Flynn, J., Hempstead, B. L., & Parada, L. F. (1997). Targeted deletion of all isoforms of the trkC gene suggests the use of

alternate receptors by its ligand neurotrophin-3 in neuronal development and implicates trkC in normal cardiogenesis. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 14776–14781.

Tomita, K., Kubo, T., Matsuda, K., Fujiwara, T., Yano, K., Winograd, J. M., Tohyama, M., & Hosokawa, K. (2007). The neurotrophin receptor p75NTR in Schwann cells is implicated in remyelination and motor recovery after peripheral nerve injury. *Glia, 55*, 1199–1208.

Trapp, B. D., Nishiyama, A., Cheng, D., & Macklin, W. (1997). Differentiation and death of premyelinating oligodendrocytes in developing rodent brain. *The Journal of Cell Biology, 137*, 459–468.

Tyler, W. A., Gangoli, N., Gokina, P., Kim, H. A., Covey, M., Levison, S. W., & Wood, T. L. (2009). Activation of the mammalian target of rapamycin (mTOR) is essential for oligodendrocyte differentiation. *Journal of Neuroscience, 29*, 6367–6378.

VonDran, M. W., Clinton-Luke, P., Honeywell, J. Z., & Dreyfus, C. F. (2010). BDNF+/− mice exhibit deficits in oligodendrocyte lineage cells of the basal forebrain. *Glia, 58*, 848–856.

VonDran, M. W., Singh, H., Honeywell, J. Z., & Dreyfus, C. F. (2011). Levels of BDNF impact oligodendrocyte lineage cells following a cuprizone lesion. *Journal of Neuroscience, 31*, 14182–14190.

Wan, L., Xia, R., & Ding, W. (2010). Short-term low-frequency electrical stimulation enhanced remyelination of injured peripheral nerves by inducing the promyelination effect of brain-derived neurotrophic factor on schwann cell polarization. *Journal of Neuroscience Research, 88*, 2578–2587.

Wiese, S., Jablonka, S., Holtmann, B., Orel, N., Rajagopal, R., Chao, M. V., & Sendtner, M. (2007). Adenosine receptor A2A-R contributes to motoneuron survival by transactivating the tyrosine kinase receptor TrkB. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 17210–17215.

Wiggins, R. C., Benjamins, J. A., & Morell, P. (1975). Appearance of myelin proteins in rat sciatic nerve during development. *Brain Research, 89*, 99–106.

Wong, A. W., Xiao, J., Kemper, D., Kilpatrick, T. J., & Murray, S. S. (2013). Oligodendroglial expression of TrkB independently regulates myelination and progenitor cell proliferation. *Journal of Neuroscience, 33*(11), 4947–4957

Woo, N. H., Teng, H. K., Siao, C. J., Chiaruttini, C., Pang, P. T., Milner, T. A., Hempstead, B. L., & Lu, B. (2005). Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nature Neuroscience, 8*, 1069–1077.

Woolley, A. G., Tait, K. J., Hurren, B. J., Fisher, L., Sheard, P. W., & Duxson, M. J. (2008). Developmental loss of NT-3 in vivo results in reduced levels of myelin-specific proteins, a reduced extent of myelination and increased apoptosis of schwann cells. *Glia, 56*, 306–317.

Xiao, J., Kilpatrick, T. J., & Murray, S. S. (2009a). The role of neurotrophins in the regulation of myelin development. *Neurosignals, 17*, 265–276.

Xiao, J., Wong, A. W., Willingham, M. M., Kaasinen, S. K., Hendry, I. A., Howitt, J., Putz, U., Barrett, G. L., Kilpatrick, T. J., & Murray, S. S. (2009b). BDNF exerts contrasting effects on peripheral myelination of NGF-dependent and BDNF-dependent DRG neurons. *Journal of Neuroscience, 29*, 4016–4022.

Xiao, J., Wong, A. W., Willingham, M. M., van den Buuse, M., Kilpatrick, T. J., & Murray, S. S. (2010). Brain-derived neurotrophic factor promotes central nervous system myelination via a direct effect upon oligodendrocytes. *Neurosignals, 18*, 186–202.

Xiao, J., Ferner, A. H., Wong, A. W., Denham, M., Kilpatrick, T. J., & Murray, S. S. (2012). Extracellular signal-regulated kinase 1/2 signaling promotes oligodendrocyte myelination in vitro. *Journal of Neurochemistry, 122*, 1167–1180.

Xiao, J., Hughes, R., Lim, J. Y., Ferner, A. H., Wong, A. W., Kilpatrick, T. J., & Murray, S. S. (2013). A small peptide mimetic of BDNF promotes peripheral myelination. *Journal of Neurochemistry, 125*(3), 386–398.

Yamauchi, J., Chan, J. R., & Shooter, E. M. (2003). Neurotrophin 3 activation of TrkC induces Schwann cell migration through the c-Jun N-terminal kinase pathway. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 14421–14426.

Yamauchi, J., Chan, J. R., & Shooter, E. M. (2004). Neurotrophins regulate Schwann cell migration by activating divergent signaling pathways dependent on Rho GTPases. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 8774–8779.

Yamauchi, J., Chan, J. R., Miyamoto, Y., Tsujimoto, G., & Shooter, E. M. (2005a). The neurotrophin-3 receptor TrkC directly phosphorylates and activates the nucleotide exchange factor Dbs to enhance Schwann cell migration. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 5198–5203.

Yamauchi, J., Miyamoto, Y., Tanoue, A., Shooter, E. M., & Chan, J. R. (2005b). Ras activation of a Rac1 exchange factor, Tiam1, mediates neurotrophin-3-induced Schwann cell migration. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 14889–14894.

Yan, H., & Wood, P. M. (2000). NT-3 weakly stimulates proliferation of adult rat O1(−)O4(+) oligodendrocyte-lineage cells and increases oligodendrocyte myelination in vitro. *Journal of Neuroscience Research, 62*, 329–335.

Yang, T., Knowles, J. K., Lu, Q., Zhang, H., Arancio, O., Moore, L. A., Chang, T., Wang, Q., Andreasson, K., Rajadas, J., Fuller, G. G., Xie, Y., Massa, S. M., & Longo, F. M. (2008). Small molecule, non-peptide p75 ligands inhibit Abeta-induced neurodegeneration and synaptic impairment. *PLoS One, 3*, e3604.

Yin, X., Baek, R. C., Kirschner, D. A., Peterson, A., Fujii, Y., Nave, K. A., Macklin, W. B., & Trapp, B. D. (2006). Evolution of a neuroprotective function of central nervous system myelin. *The Journal of Cell Biology, 172*, 469–478.

Zawadzka, M., & Franklin, R. J. (2007). Myelin regeneration in demyelinating disorders: new developments in biology and clinical pathology. *Current Opinion in Neurology, 20*, 294–298.

Zhang, J. Y., Luo, X. G., Xian, C. J., Liu, Z. H., & Zhou, X. F. (2000). Endogenous BDNF is required for myelination and regeneration of injured sciatic nerve in rodents. *European Journal of Neuroscience, 12*, 4171–4180.

Neurotrophic Factors and Ethanol Neurotoxicity

Margaret I. Davis

Contents

Abstract

Neurotrophins are essential for the growth, differentiation, and survival of neurons during development and in the adult. Considerable data have accumulated over the last few decades implicating classical neurotrophins, Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), and Neurotrophins 3 and 4 (NT-3/4), in many aspects of ethanol addiction, neurotoxicity, and repair after ethanol withdrawal. Genetic screens in *Drosophila* have identified novel neurotrophic factors and signaling intermediates involved in acute tolerance and pharmacodynamic adaptation to prolonged exposure in rodent. Ethanol modulates neurotrophic factor expression in vivo in a time- and

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region-specific fashion. Ethanol inhibits neurotrophin signaling acutely in vivo and in vitro in many brain regions. Conversely, acute and chronic ethanol exposure can upregulate neurotrophin-associated signaling pathways, particularly in brain nuclei associated with anxiety and addiction. Cell death induced by high concentrations of ethanol can be mitigated by exogenous neurotrophins indicating that neurotrophin induction in vivo may also be neuroprotective but ultimately fails over time. Neurotrophin levels in serum and plasma of patients with alcohol use disorders are dynamic and may serve as a surrogate for central nervous system levels. The kinetics suggest that increased levels during withdrawal may be involved in repair, but these analysis are complicated by genetic polymorphisms and the blood component analyzed, particularly with BDNF which is polymorphic in human populations and also produced by platelets. Neurotrophins are intricately involved in pharmacodynamic compensation with prolonged ethanol exposure, addiction-related plasticity, and neurotoxicity, but considerable work remains to be performed and replicated. Recent pharmacological advances targeting neurotrophins and neurotrophin signaling may ultimately be useful for treating ethanol-induced neurodegeneration and aberrant plasticity associated with addiction.

List of Abbreviations

AD	Alcohol dependence
ALK	Anaplastic lymphoma kinase
ARND	Alcohol-related neurodevelopmental disorders
BAC	Blood alcohol concentration
BDNF	Brain-derived neurotrophic factor
BFCN	Basal forebrain cholinergic nuclei
cAMP	3′-5′ cyclic adenosine monophosphate
ChAT	Choline acyltransferase
E	Embryonic
ERK	Extracellular signal regulated kinase
FAS	Fetal alcohol syndrome
GABA	Gamma amino butyric acid
GDNF	Glial-derived neurotrophic factor
GENSAT	Genetic nervous system Atlas
ICV	Intracerebroventricular
IGF	Insulin-like growth factor
JNK	Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MSN	Medium spiny neuron
NAc	Nucleus accumbens
NGF	Nerve growth factor
NMDA	*N*-Methyl D-aspartate
NT	Neurotrophin
PACAP	Pituitary adenylate cyclase activating peptide

PI3K	Phosphatidyl inositol 3-OH-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PN	Postnatal
RACK	Receptor for activated C kinase
Trk	Tropomyosin-related protein kinase
VTA	Ventral tegmental area

1 Introduction

The majority of heavy drinkers who consume ethanol daily for years to decades show some form of brain pathology either as a result of alcohol-associated neurodegeneration or secondary to nutritional deficiencies, vascular damage, and hepatotoxicity (see Harper 2009; Zahr et al. 2011; Welch 2011; de la Monte et al. 2012 for excellent reviews). Ethanol is even more detrimental to the developing fetus, with Fetal Alcohol Syndrome (FAS) and Alcohol-Related Neurodevelopmental Disorders (ARND) occurring at a combined rate of <0.7 % in the United States to as high as 5 % in places like South Africa with high per capita alcohol consumption (de Sanctis et al. 2011; May et al. 2009). Chronic and developmental ethanol exposure clearly causes neurodegeneration and cognitive deficits; therefore, it seems intuitive that factors which mediate neuronal survival would be involved in ethanol-induced neurotoxicity. In models of ARND/FAS and in the adult, neurotrophins play a role in cell death, aberrant synaptogenesis, perturbed migration, tolerance, and repair but may also regulate the selective plasticity that occurs with addiction (reviewed by Ghitza et al. 2010; Castren 2004). Whether neurotrophin expression is the result of or induces subsequent changes in synaptic activity is not always clear, but neurotrophins are integral to many ethanol-sensitive processes.

Neurotrophins are a family of proteins initially characterized for the ability to provide trophic support to developing neurons but also regulate synaptic plasticity and survival in mature neurons (reviewed by Reichardt 2006). Nerve Growth Factor (NGF) was the first neurotrophic factor described which supported the growth of chick sensory ganglia neurons (Levi-Montalcini and Cohen 1960). This was followed by isolation and cloning of a family of proteins that includes Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3), and NT-4/5 in mammals and their respective Trk receptors (Leibrock et al. 1989; Klein et al. 1989; Lamballe et al. 1991; Maisonpierre et al. 1990b). Since these initial discoveries, many other factors have been isolated that are also trophic for neurons, but these factors do not contain regions of significant homology with the NGF family. Trophic factors explored in alcohol toxicology belonging to the latter group include Insulin-like Growth Factor 1 (IGF-1), Pituitary Adenylate Cyclase Activating Peptide (PACAP), and Glial-Derived Neurotrophic Factor (GDNF).

Classical neurotrophins of the NGF family are cysteine knot proteins that function as dimers. Neurotrophins are expressed as pro-proteins and cleaved to form active proteins, primarily through activity-dependent secretion and protease activation. Neurotrophins can be target-derived, transported trans-synaptically in a retrograde or anterograde direction, or signal through local mechanisms, depending on the brain region and neurotrophin (see Ginty and Segal 2002; Chowdary et al. 2012; Ascano et al. 2012 for reviews). Classical neurotrophins bind to Tropomyosin Related Kinase (Trk) receptors with picomolar affinity. TrkA is the full-length receptor for NGF, TrkB for BDNF and NT-4, and TrkC is the NT-3 receptor, although neurotrophins can bind other Trks with reduced affinity or differential coupling to signaling pathways (Schecterson and Bothwell 2010; Reichardt 2006). Trks also exist as splice variants that result in truncated receptors without signaling capacity, or with only the Shc binding domain (reviewed by Schecterson and Bothwell 2010). Trk receptors can also be transactivated by G protein–coupled receptors, adding further complexity to the signaling repertoire, potential for cell specific signals, and cross-regulation. Activation leads to dimerization and autophosphorylation of tyrosine residues in the full-length Trk receptors, creating docking sites for signaling intermediates (reviewed by Reichardt 2006). Modulation of signaling by ethanol exposure has been demonstrated for many of the major signaling pathways downstream of these trophic factors either individually, sequentially, or in parallel, including Extracellular signal Regulated Kinase (ERK), phosphatidylinositol 3-kinase (PI3K), phospholipase C, Protein Kinase C (PKC), small molecular weight GTPases, calcium, Nuclear Factor (NF) kB, and cyclic AMP (reviewed my Ron and Messing 2013). Neurotrophins are subject to activity-dependent, promoter-specific regulation and therefore regulated by synaptic activity, which is also directly regulated by ethanol exposure. In addition, novel and growth factor-convergent pathways have been identified in *Drosophila* that regulate ethanol sensitivity, tolerance, and toxicity that remain to be fully explored in mammalian systems (Devineni et al. 2011; Bhandari et al. 2009). Collectively, these data implicate neurotrophins as integral mediators that are regulated by, or regulate, nearly all neuronal processes, albeit with relative selectivity.

Classical neurotrophins also bind p75 with lower affinity, but pro-neurotrophins bind p75 with high affinity (reviewed by Koshimizu et al. 2009). p75 dimerized with sortilin family members to regulate Rho, NF-kB, and Jun N-terminal Kinase (JNK)-p53-Bax pathways, resulting in both survival and apoptosis (reviewed by Friedman 2010). Apoptosis through p75 is mediated by JNK while survival is signaled via NFkB in some cell types (Yeiser et al. 2004). p75 expression declines with age, indicating an active role in development. p75 can also dimerize with Trks to regulate activity, acting as dominant-negative receptors or enhancers of Trk signaling depending on the context, cell type, and neurotrophin concentration (reviewed by Schecterson and Bothwell 2010). For example, intracerebroventricular (ICV) injection of pro-NGF induces degeneration of the cholinergic system (Fortress et al. 2011) and mediates hippocampal growth cone collapse through p75-sortilin signaling to Trio-GEF-mediated Rac inhibition (Deinhardt et al. 2011).

Pro-BDNF and cleavage-resistant BDNF induce apoptosis and inhibit migration in cerebellar granule cells (Koshimizu et al. 2009, 2010). Cleavage-resistant pro-BDNF reduces cholinergic hippocampal innervation and hippocampal dendritic spines but does not appear to induce apoptosis in hippocampal neurons (Koshimizu et al. 2009, 2010). Pro-BDNF also mediates long-term depression (Woo et al. 2005). Thus, the maturation state of the neurotrophin, which is activity-regulated, may dramatically influence the receptor selectivity and the subsequent response of the cell.

This chapter will review the background on neurotrophin signaling, expression in animal models of ethanol exposure, and in patients with alcohol dependence. Each section will provide the necessary background information to evaluate the data. While not exhaustive in detail, these sections will provide references to the primary literature and more extensive reviews. Many studies differ in the presentation of dose or concentration and that should be considered. The LD50 for a single intraperitoneal dose of ethanol is ~3.5 g/kg for most mouse strains and range from less than 0.4 g/dL for some rat studies (Prado Carvalho and Izquierdo 1977) to as high as 7–9 g/kg in others (Material Safety Data Sheet). Concentrations are also given in percent (g/dL or mg/dL) or as millimoles per liter depending on the laboratory. Humans become unconscious at Blood Alcohol Concentrations (BACs) ~0.35 g/dL, and death is likely at concentrations ~100 mM or 0.5 g/dL. Therefore, LD50 should be considered in experimental situations where tolerance is not a factor. Besides the obvious complexities of dose, duration, tolerance, and withdrawal, several experimental caveats in inconsistencies must also be considered when reviewing 30 years of data speckled on a background of exponential scientific discovery and technological advances. These concerns will be addressed as they arise. Current data collectively suggest that work remains to be performed and replicated to gain a better understanding of how temporal changes in neurotrophin expression regulate, and are regulated by, physiological responses within the nervous system and how these influence neurotoxicity, plasticity, and repair after ethanol exposure.

2 Neurotrophin and their Receptors after Ethanol Exposure In Vivo: Animal Models

2.1 Nerve Growth Factor

2.1.1 NGF-TrkA Expression and Distribution

NGF was the first neurotrophin to be examined in models of alcohol-induced neurodegeneration and fetal alcohol syndrome. NGF is expressed in targets of sympathetic neurons and throughout the periphery, with high levels submaxillary gland, heart, prostate, stomach, endocrine system, skin, mast cells, intestine, spleen, lung, skin, and kidney (Korsching and Thoenen 1983; Heumann et al. 1984; Shelton and Reichchardt 1986). Within the CNS, NGF is trophic for Basal Forebrain Cholinergic Neurons (BFCN) where it upregulates choline acyltransferase (ChAT)

activity with an EC50 of ~3 ng/mL (Honegger and Lenoir 1982; Hefti et al. 1984; Mobley et al. 1986; Korsching et al. 1985). Functional studies and developmental expression indicate an essential role for NGF in hippocampal-dependent learning and expression of cholinergic markers in afferent structures (Conner et al. 2009; Conner and Varon 1997; Houeland et al. 2010). NGF also enhances communication between the nucleus basalis and the amygdala (Moises et al. 1995). Functional studies and the expression pattern implicate NGF as a candidate for modulation of ethanol-induced toxicity in the BFCN, hippocampus, and striatum, leading to cognitive deficits and addiction.

NGF mRNA expression (Fig. 1, Allen Institute) and GFP expression from the NGF promoter (Fig. 1, GENSAT) show the general distribution of NGF-producing cells in the adult mouse brain. Expression is sparse in comparison to BDNF shown in the adjacent panels. The highest level of NGF mRNA and protein is detected in the hippocampus within the mossy fiber terminal fields (hilus and stratum lucidum of CA3 and CA2) followed by cortex and olfactory bulbs (Fig. 1, Allen Institute and Gibbs et al. 1989; Maisonpierre et al. 1990a; Conner and Varon 1992; Altar et al. 1997; Conner and Varon 1997). Within the hippocampus, mRNA is localized primarily to interneurons of the hilus, but is also observed scattered in dentate granule cells, scattered interneurons within the CA fields, and interneurons in distal pyramidal cell synaptic fields after colchicine treatment (Ceccatelli et al. 1991). NGF mRNA is also detected in anterior olfactory nucleus, magnocellular preoptic nucleus, nucleus of the horizontal limb of the diagonal band, supramammillary nucleus, striatal interneurons, bed nucleus of the stria terminalis, ventral pallidum, mediodorsal thalamic nucleus, paraventricular hypothalamic nucleus, supraoptic nucleus, lateral and medial septum, substantia innominata, and nucleus basalis. Within the brainstem, NGF is localized to oculomotor, nucleus, facial nucleus, ambiguus nucleus, gigantocellular reticular nucleus, gigantocellular, reticular nucleus (alpha part), inferior olive, reticular nucleus, nucleus of the solitary tract, and vestibular nucleus (Maisonpierre et al. 1990a; Conner and Varon 1992; Altar et al. 1997).

The exact concentration of NGF (and all neurotrophins) in brain regions when measured by ELISA is variable due to extraction procedures, buffer composition, pH, fraction assayed, and the presence or absence of tissue homogenates in the standard curve (see Larkfors et al. 1987; Hoener et al. 1996; Fawcett et al. 1999; Zhang et al. 2000; Soderstom and Ebendal 1995; Whittemore et al. 1986). Calculated value from the studies cited above estimate NGF protein levels at (ng/g wet weight): hippocampus 1.34–6, cortex 0.57–3.5, olfactory bulb 0.85–5.3, basal forebrain 0.672–4.77, cerebellum 0.42–1.86 and striatum 0.16–1.0. Protein levels increase during development in the CNS with a peak that is approximately twice the adult level at postnatal day (PN)14 in cortex, PN14-21 in the striatum and BFCN, and PN7 in the cerebellum (Whittemore et al. 1986; Das et al. 2001; Mobley et al. 1986).

NGF is notoriously sensitive to fixation, pH, and post-fixation times; therefore, many studies purporting to measure NGF by immunohistochemistry may not be measuring NGF. Earlier studies discussed above are replicated in the mouse

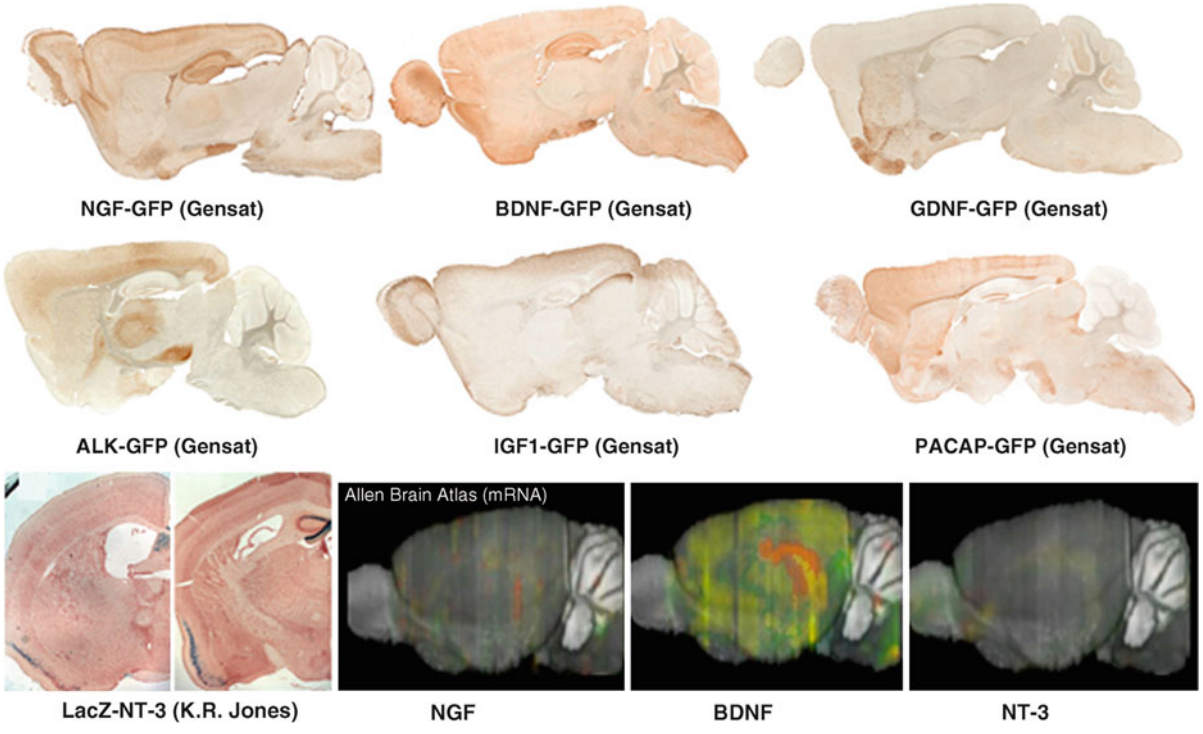

Fig. 1 Neurotrophic factor localization in the mouse brain using promoter-driven GFP expression and in situ hybridization. Immunohistochemistry for GFP in sagittal sections is shown for each neurotrophic factor to show the distribution of cells that produce the factors within the brain. Lac-Z expression from the NT-3 promoter in an adult mouse is shown in the *bottom left*. Rendered images from the Allen Brain Atlas mRNA series are shown for NGF, BDNF, and NT-3 in the *bottom row*. For more detail and immunohistochemical detection of each neurotrophin, see citations discussed in the text, GENSAT (www.gensat.org), and The Allen Brain Atlas (www.brain-map.org/) (Lac-Z images were generously provided by Dr. Kevin R. Jones and were generated as described in Vigers et al. [2000])

expressing GFP from the NGF promoter produced by GENSAT (Fig. 1). NGF immunoreactivity is particularly strong in hippocampal mossy fibers in mature and developing animals with transient production in other dentate lamina during development (Conner et al. 1992; Conner and Varon 1992, 1997). Cells in the stratum radiatum and stratum oriens contain NGF, while a few larger interneurons in the pyramidal cell layer contain NGF immunoreactivity (Conner and Varon 1992; Rocamora et al. 1996; Pascual et al. 1998). Cortical neurons immunoreactive for NGF were generally small (<12 um), but occasional NGF+ cells could be observed that were 13–17 μm in diameter, consistent with NGF expression primarily in interneurons (Conner and Varon 1992; Conner et al. 1992; Rocamora et al. 1996).

Regions of neurotrophin expression detected by mRNA frequently do not overlap with the protein distribution measured by immunohistochemistry. This is because NGF is produced in the target region or by local interneurons and retrogradely transported to the cell bodies of the "consumer" neurons (Seiler and Schwab 1984; Conner et al. 1992; Conner and Varon 1992). Colchicine treatment increases somatic immunoreactivity, allowing refined localization of NGF by allowing accumulation of protein in the cells that produce it and blocking retrograde transport to cells that stain for NGF because of uptake from postsynaptic sources. Under these conditions, NGF-expressing cells were detected in the olfactory bulb, tenia tecta, striatum, cortex, septum, BFCN, bed nucleus of the stria terminalis, diagonal band magnocellular preoptic region, ventral pallidum, and substantia innominata. Cholinergic cells with previous immunoreactivity in the septum and BFCN disappeared, thereby segregating the NGF "consumers" from "producers" (Conner and Varon 1992). Thus, many forebrain neurons will contain NGF protein but do not express NGF mRNA, instead obtaining NGF from cortical, hippocampal, and local interneurons (Conner et al. 1992; Conner and Varon 1992; Lauterborn et al. 1995; Whittemore et al. 1986). Similarly, much of the septal NGF is the result of retrograde transport from the hippocampus where it is expressed by cells in the target region for septohippocampal afferents (Conner and Varon 1992; Seiler and Schwab 1984). GFP expression from the NGF promoter also found NGF expression by Fananas astrocytes in the cerebellum and by cerebellar interneurons (Kawaja et al. 2011; GENSAT Fig. 1).

TrkA is widely expressed within the nervous system (see Sobreviela et al. 1994; Altar et al. 1991; Cohen-Cory et al. 1989 for detailed anatomy), including neurons of the striatum, ventral premammillary nucleus, mesencephalic trigeminal nucleus, vestibulocochlear ganglion, prepositus hypoglossal nucleus, raphe nucleus, nucleus ambiguus, glomeruli of the olfactory bulb, area postrema, and nucleus tractus solitarius. TrkA is not present in hippocampal pyramidal cells but is expressed by hippocampal interneurons. The suprachiasmic nucleus, olivary pretectal nucleus, and spinal trigeminal nuclei only contain TrkA immunoreactive fibers. TrkA immunoreactivity is also detected in the leptomeninges, ventricles, and ependymal cells (Gibbs et al. 1989). Iodinated NGF binding is highest in the interpeduncular nucleus and spinal trigeminal tract followed by striatum (higher in lateral striatum), amygdala, diagonal band, nucleus accumbens (NAc), olfactory tubercle, cerebellum, hippocampus, cortex, and subiculum. p75 is notably absent from striatal

cholinergic neurons that express TrkA (Sobreviela et al. 1994). Conversely, TrkA is not generally detected in mature cerebellar Purkinje cells that express p75 (Sobreviela et al. 1994; Larkfors et al. 1987, 1996). However, GFP expressed from the TrkA promoter does label a subset of Purkinje cells developmentally, suggesting that some transiently express TrkA. Interestingly, p75 regulates autophagy in Purkinje cells (Florez-McClure et al. 2004). NGF is also trophic for cholinergic interneurons of the striatal matrix in vivo (Van Vulpen and Van Der Kooy 1999) and in vitro (Studer et al. 1994) but has no effect on the survival of striatal medium spiny neuron (Ventimiglia et al. 1995), Purkinje cells (Larkfors et al. 1996), or hippocampal pyramidal cells (Ip et al. 1993).

Genetic deletion of NGF reduces acetylcholine esterase, causes a loss of sympathetic preganglionic neurons, reduces cholinergic septal and striatal neurons, leads to atrophy of BFCNs, and reduces hippocampal innervation by cholinergic afferents (Smeyne et al. 1994; Sanchez-Ortiz et al. 2012), while overexpression of NGF increases the size of septal neurons (Kawaja et al. 1998) and increases cholinergic neuron number and size without altering the innervation of the hippocampus (Fagan et al. 1997; Kawaja et al. 1998). Trk A knockout mice die by 1 month of age, exhibiting severe sensory neuropathies with smaller trigeminal, sympathetic, and dorsal root ganglia (Smeyne et al. 1994).

2.1.2 Ethanol and NGF Expression in Animal Models

NGF levels are sensitive to dose, duration, and withdrawal state, suggesting a dynamic role for NGF (and all neurotrophic factors) in intoxication, tolerance, and toxicity. Ethanol administered acutely via liquid diet causes an increase in NGF and TrkA protein and mRNA in the cerebellum (Wang et al. 2010). Chronic exposure via drinking water also produced a transient increase in NGF in the hippocampus, septum, striatum, cortex, BFCN, and olfactory bulbs (Gericke et al. 2006). After 9 months of exposure, there was a slight reduction in cortical NGF, but no differences in the other regions examined. Shorter exposures (2–4 weeks) of ethanol increased NGF in hippocampus and cortex with a return to control values after 3 months of ethanol exposure (Nakano et al. 1996). Chronic intermittent exposure produced an increase in hippocampal NGF protein levels (Miller 2004), but, in contrast to Gericke and colleagues, chronic exposure for 8 or 24 weeks reduced NGF in most cortical regions cortex and basal forebrain at most time points (Miller and Mooney 2004). Chronic ethanol exposure via liquid diet also reduced TrkA mRNA in the basal forebrain (Miller et al. 2002) and NGF can induce TrkA expression (Holtzman et al. 1992), suggesting failure of NGF to signal with chronic exposure. Combined with reduced basal forebrain TrkA (Miller et al. 2002), this increase in cortical and hippocampal NGF may reflect failure of NGF to transport or a neuroprotective induction that eventually fails with prolonged exposure. In contrast to chronic forced exposure, no differences in striatal NGF levels were observed by McGough and colleagues (2004) after 4 weeks of drinking using the 2 bottle choice model, suggesting some selectivity, depending on the model. The reason for these differences is unclear but may be the result of differential neurotoxicity, the choice to drink, or other environmental factors.

Bruns and Miller (2007) also observed an increase in NGF in the somatosensory cortex in layer V pyramidal cells, not interneurons, with repeated episodic exposure that correlated with the duration of exposure. NGF is generally not generally observed in pyramidal cells (Conner et al. 1992; Conner and Varon 1992; in contrast, see Pitts and Miller 1995; Pitts and Miller 2000), and only pyramidal cells were shown in the micrographs presented by Bruns and Miller; therefore, it is uncertain whether it is ethanol-specific ectopic expression, different pools of NGF, cellular uptake, or resulting from fixation procedures that were significantly different from Conner and colleagues (See Conner 2001 for detailed methods).

While the induction of NGF (i.e., Miller 2004; Gericke et al. 2006; Wang et al. 2010) may correlate with protective capacity and plasticity at early time points, many reports agree that long-term exposure to ethanol decreases NGF levels in the mature rodent hippocampus and cortex (De Simone and Aloe 1993; Aloe et al. 1993; Miller and Mooney 2004; Gericke et al. 2006). Furthermore, reduced hippocampal NGF after prolonged ethanol exposure is associated with atrophy cholinergic cells in the medial septum; similarly reduced cortical levels are associated with atrophy of septal neurons (De Simone and Aloe 1993; Aloe et al. 1993; Arendt et al. 1988; Cadete-Leite et al. 2003; Ehrlich et al. 2012). This atrophy and reduced NGF are accompanied by reduced ChAT activity in the hippocampus, septum, and striatum, which can be reversed by administered of NGF via osmotic pump (Cadete-Leite et al. 2003).

NGF-sensitive cells in the developing BFCNs and striatum show transient sensitivity to ethanol that parallel observations in the adult with variable resiliency. Initial studies used chick embryos to show that ethanol was toxic to cholinergic neurons, which was reversed by NGF, and established a window of vulnerability for these neurons (Rahman et al. 1993; Brodie and Vernadakis 1992). A similar window may exist in rodents; a single binge exposure on E15 resulted in a transient increase in hippocampal NGF at PN15 followed by a decline below control values in adulthood and parallel changes in ChAT (Angelucci et al. 1997, 1999).

Rats exposed prenatally to ethanol throughout gestation also have a transient reduction in ChAT activity in the BFCN, striatum, cortex, hippocampus, and septum neonatally (Swanson et al. 1995, 1996; De Simone and Aloe 1993; Fiore et al. 2009a, b). Reversal was associated with an increase in hippocampal NGF (transiently at PN7 and robustly at PN21) and repair in some studies (Heaton et al. 1996). In contrast, others (De Simone and Aloe 1993; Tsuji et al. 2008; Fattori et al. 2008) did not detect any differences in NGF in cortex with prenatal or postnatal exposure but reported a transient decrease in NGF in the hippocampus at PN7 that correlated with reduced cholinergic activity. Similarly, constant gestational exposure to ethanol (but not wine) reduced hippocampal and cortical NGF (Fiore et al. 2009a, b). This was correlated with a reduction in cholinergic neurons in the septum. The survival of cholinergic neurons and NGF induction differs with exposure paradigms, but each outcome is associated changes in NGF levels correlated with cholinergic neuron activity. Whether repair succeeds or fails may depend on the dose and duration of exposure, with binge exposure at a defined window being more toxic to developing BFCNs. As with the pattern in the adult where the ability to

upregulate NGF with intermittent exposure may be protective, variability in these data may be the result of the populations that survive the insult and their ability to produce NGF.

NGF levels are acutely increased in the cerebellum in mature mice by ethanol, suggesting a role in adaptation or repair (Wang et al. 2010), but NGF-ethanol interactions have primarily been addressed in the developing cerebellum. Cerebellar Purkinje cells are particularly vulnerable to ethanol toxicity during the first postnatal week but resistant to ethanol-induced cell death during the second postnatal week (West 1993), a period that coincides with the rise to the peak of developmental NGF expression (Das et al. 2001; Maisonpierre et al. 1990a). High dose ethanol (6.6 g/kg/day, peak BAC 336 mg/dL, cyclic exposure) PN4-10 via artificial rearing did not change NGF levels as detected by ELISA in neonatal cerebellar homogenates but reduced both p75 and TrkA in Purkinje cell dendrites and in cerebellar homogenates (Dohrman et al. 1997; also see Moore et al. 2004a). When this window was further subdivided, Heaton and colleagues (2003) found a transient reduction in NGF only when animals were exposed to ethanol at PN4 (correlating with the developmental period when Purkinje cells are sensitive to ethanol) but an increase at PN7 immediately after exposure and a delayed decrease at 12 h. Surprisingly, exposure on PN7-8 did not change NGF levels, suggesting some form of compensation with prolonged exposure, as was noted in the cholinergic populations. (The levels reported in this study (2003c) are two orders of magnitude greater than previously reported by this lab (i.e., Heaton et al. 2000c) and higher than classical reports. It is possible that this is calculation error, but no correction has been issued.) Furthermore, NGF overexpression from the GFAP promoter rescued Purkinje cells from ethanol toxicity in the neonatal cerebellum without hypertrophy (Heaton et al. 2000b). Purkinje cell number was slightly reduced in the control transgenic mice, suggesting that NGF overexpression alone in a non-activity dependent way may reduce Purkinje cell viability since hypertrophy resulting in fewer neurons per area is excluded but this was not evaluated. NGF overexpression induces aberrant cholinergic sprouting in the cerebellum that might also contribute to the increased survival in this paradigm through cholinergic systems positively coupled to survival pathways and independent of direct NGF effects on Purkinje cells (Kawaja and Crutcher 1997). Furthermore, the locus of NGF action in these studies has not been adequately established and again may suggest robust, dynamic changes in interneurons (Kawaja et al. 2011) or cerebellar afferent fibers since which then modulate Purkinje cell activity. Purkinje cells have not traditionally shown TrkA-mediated survival (Sobreviela et al. 1994; Larkfors et al. 1996) but this does not exclude a role for p75.

Upregulation of NGF at earlier time points after withdrawal or from low levels of ethanol may represent an attempt to compensate for the effect of ethanol on synaptic transmission that fails as producer neurons degenerate. Alternatively, the NGF measured may represent increased pro-NGF, leading to neurodegeneration. In addition, the effect of ethanol on the NGF system specifically in hippocampal and cortical interneurons has not been selectively evaluated in this context, but collectively these data suggest that interneurons may be a central mediator of

ethanol effects on NGF-mediated processes, perhaps secondary to reduction in synaptic activity, or death, with prolonged ethanol exposure. As discussed above, neurotrophic activity cannot be examined independent of the chronic and pharmacodynamic effects of ethanol on neural activity and circuit function.

2.1.3 NGF and In Vitro Models of Ethanol Exposure

Cell culture models have provided insight into ethanol pharmacology and toxicology, but many of the in vitro studies discussed below require supraphysiological concentrations of ethanol to induce cell death. These concentrations are generally more than 100 mM or 0.4 g/dL. Many dose–response curves in the studies discussed below range from 0.4 to 6 g/dL and require high concentrations of ethanol to decrease neurite outgrowth and induce cell death. Ethanol impairs neuronal function in vivo at much lower concentrations; therefore, the events leading to cell death in vivo are possibly more subtle, linked to developmental events such as migration and synaptogenesis, may be distinct from the overt toxicity observed over 24–48 h in vitro, and take longer to develop. Therefore, cell death may not be the result of immediate toxicity but may occur secondary to more subtle impairment in the developmental program that leads to cell death at a later developmental stage as the result of aberrant synapse formation or impaired migration. This caveat applies to similar sections on direct toxicity throughout this chapter.

As the prototypic neurotrophin, NGF was the first to be examined for direct modulation of ethanol toxicity in vitro. Chick sympathetic ganglia developed fewer neurites in response to ethanol in combination with NGF in vitro (Dow and Riopelle 1985) and impaired development of NGF-supported structures in ovo that could be reversed by exogenous NGF (Brodie et al. 1991; Brodie and Vernadakis 1992; Rahman et al. 1993). Dow and Riopelle (1985) used ethanol at concentrations ranging from 0.01 to 0.25 g/dL and evaluated neurite formation as a measure of toxicity but found no evidence for cell death at these concentrations. Follow-up in vitro studies replicated the decrease in neurites but extended the concentrations to include doses up to 6 g/dL (Heaton et al. 1993). Ethanol (0.25 g/dL) combined with NGF caused a slight (∼20 %) increase in processes in this model but higher concentrations reduced process formation and concentrations above 5 g/dL were required to kill dorsal root ganglia neurons. Similarly, cell death in cultured hippocampal and septal neurons did not occur at concentrations below 2.4 and 1.8 g/dL, respectively (Heaton et al. 1994). NGF was protective against toxicity, even at these extreme concentrations. When cultures were prepared from embryos exposed to ethanol in vivo, they were resistant to cell death at 1.8 g/dL but not 2.4 g/dL (Heaton et al. 1995), suggesting that exposure to ethanol increased resistance to subsequent exposure. In cortical organotypic cultures, NGF has region-specific effects, with neuroprotection only evident within the lower cortical layer developmentally (Mooney and Miller 2007). NGF-mediated neuroprotection is more dramatic in dispersed cultures of cortical, hippocampal, and cerebellar granule neurons (Heaton et al. 2000a; Mitchell et al. 1998; Seabold et al. 1998; Luo et al. 1997; Bonthius et al. 2003), although pyramidal cells and cerebellar granule cells do not respond to NGF through TrkA (Ip et al. 1993; Collazo et al. 1992;

Segal et al. 1992; Barker-Gibb et al. 2001; Courtney et al. 1997; Nonomura et al. 1996; Larkfors et al. 1996). These effects may instead be mediated by p75 in granule cells (Courtney et al. 1997). Alternatively, this may also be due to the relatively high concentration of NGF used, which can nonspecifically activate other Trks. While many neurotrophins are capable of increasing survival in ethanol-exposed cultures, some may be increasing baseline survival without a specific protective effect from ethanol (Bonthius et al. 2003).

PC-12 cells, an immortalized pheochromocytoma cell line with sympathetic neuron-like properties, express TrkA and show enhanced neurite formation, increased microtubule content, and enhanced differentiation in response to 100 mM ethanol (~0.48 g/dL) and NGF (Wooten and Ewald 1991; Messing et al. 1991; Roivainen et al. 1993; Roivainen et al. 1995). Enhancement was not seen with forskolin and ethanol, but FGF-induced neurite outgrowth was enhanced (Messing et al. 1991). This was accompanied by an increase in Thy-1 expression and subsequently determined to be mediated by PKC- and ERK-dependent pathways (Roivainen et al. 1993, 1995). PKC epsilon, a calcium-independent PKC isoform, was shown to mediate this enhancement of ERK (Hundle et al. 1997), while PKC delta, not epsilon, is responsible for the increase in polymerized microtubule content (Reiter-Funk and Dohrman 2005). Interestingly, PKC epsilon can induce neurite formation independent of catalytic activity (Zeidman et al. 1999) and inactive kinase induces more neurites than wild type PKC epsilon (Ling et al. 2004). Cell cycle arrest and enhanced differentiation were also observed in PC-12 cells, as determined by an increase in neuron-specific enolase expression, a decrease in ^{3}H-thymidine incorporation, and increased acetylcholine esterase expression with 87 mM ethanol in combination with NGF (Wooten and Ewald 1991). NGF-differentiated PC-12 cells are less sensitive to ethanol-induced cell death in the absence of serum (Oberdoerster and Rabin 1999) and, when specifically induced to differentiate with NGF, became less sensitive to ethanol inhibition of calcium channels (Mullikin-Kilpatrick and Treistman 1995). Therefore, ethanol causes cell cycle arrest and cell death in mitotic PC-12 cells, but once induced to differentiate by NGF, the signaling to ERK is enhanced by ethanol and neurite formation is enhanced, potentially protecting the cell from toxicity but with a potentially detrimental outcome if this were to occur in differentiating neurons.

Recent work using explant cultures of the nucleus basalis suggests more subtle effects than cell death in cholinergic neurons in response to ethanol (Ehrlich et al. 2012). Ethanol reduced ChAT immunoreactivity in neonatal explants without causing cell death or inflammatory cytokine induction which was reversible upon withdrawal. NGF (10 ng/mL) reversed the 100 mM ethanol-induced reduction in ChAT but did not reverse the reduction observed with 50 mM ethanol. Induction of trophic factors with the lower concentration could occlude an NGF effect since NGF was used near the EC50 in these experiments. Perhaps the ability of the cells to produce their own trophic factors is impaired at 100 mM, unmasking the effect. Furthermore, Erlich and colleagues implicate the nitric oxide synthase-p38 MAPK pathway in the reduction in ChAT with both 50 and 100 mM ethanol. These are

intriguing findings and support a role for NGF in repair after prolonged (7 days) ethanol exposure. The time course of NGF inductions varies significantly between studies and duration of ethanol exposure, but collectively, these data suggest induction of NGF that may mitigate some of the toxic effects of ethanol. NGF can be trophic, but if it is induced without concurrent neuronal activity and not cleaved, then pro-NGF can signal cytoskeletal changes or apoptosis through p75. Replicative experiments could focus on determining levels of pro and mature NGF, take advantage of newer technologies to determine the contribution of ethanol-induced changes in NGF and NGF signaling in survival, adaptive plasticity, addiction, and apoptosis.

2.1.4 NGF in Patients with Alcohol Dependence

Genetic polymorphisms and haplotypes in neurotrophins and their receptors in psychiatric and neurodegenerative disorders is an area of active research. The role of neurotrophin polymorphisms in neuropsychiatric disorders is controversial, and many initial association studies were not replicated. Others have reported no association with the underlying disorder but instead indicate a role for genotype in response to treatment (see Table 1). A few definitive associations of neurotrophin mutations with overt neurological disorders have been made, while most associations are weak or only relevant in subsets of patients. This applies to BDNF and other neurotrophic factors as well. Mutations and polymorphisms in NGF or TrkA have been associated with cancer (Scaruffi et al. 1999; George et al. 1998), hypertension (Kapuscinski et al. 1996), Alzheimer's disease (Di Maria et al. 2012; Nagata et al. 2011), anxiety (Lang et al. 2008), white matter structure (Braskie et al. 2012) and congenital insensitivity to pain (Mardy et al. 2001); however, unlike BDNF, no genetic associations with NGF polymorphisms have been reported with alcohol dependence.

Neurotrophins can be measured in the periphery and may be proportional to central levels as well as a surrogate for sympathetic dysfunction and peripheral neuronal activity. Table 1 details measurements of plasma and serum neurotrophin levels in patients with alcohol dependence and during withdrawal. The majority of the reports detailed in Table 1 indicate that ethanol increases peripheral NGF, regardless of the source (plasma vs. serum), in patients still drinking and during early withdrawal (Aloe et al. 1996; Lee et al. 2009; Heberlein et al. 2008; Jockers-Scherubl et al. 2007). NGF levels were found to be an order of magnitude higher in patients that had previously suffered delirium tremens and were not correlated with Korsakoff's Syndrome (Jockers-Scherubl et al. 2007). NGF levels decline toward normal during prolonged withdrawal but remain elevated for as long as 8 months (Aloe et al. 1996; Heberlein et al. 2008; Jockers-Scherubl et al. 2007). Furthermore, downregulation of NGF toward control levels was associated with methylation of the NGF promoter (Heberlein et al. 2011). In contrast, Yoon and colleagues (2006) reported decreased NGF after only 3 months of withdrawal. Ethanol produced a more dramatic decrease in NGF in patients with a family history of alcohol dependence. The patient demographics may contribute to these disparate

Table 1 Measurements of blood neurotrophin levels in patients with alcohol dependence

Study	Serum/ Plasma	NGF	BDNF	GDNF	Kit
Lee et al. (2009)	P	112 pg/mL C	861 pg/mL C		DuoSet ELISA
		137 pg/mL AD[a]	3502 pg/mL AD[a]		R&D
Huang et al. (2008)	S		15.8 ng/mL C		EMax ELISA
			13.9 ng/mL AD		Promega
			15.4 ng/mL 7d WD		
Joe et al. (2007)	P		822 pg/mL C		DuoSet ELISA
			384 pg/mL AD		R&D
			+FH 247 pg/mL		
			−FH 583 pg/mL		
Yoon et al. (2006)	P	110 pg/mL C			DuoSet ELISA
		71.9 pg/mL AD,			R&D
		+FH 64.7			
		−FH 83.3			
		>3 mos WD			
Heberlein et al. (2008)	P	19.87 pg/mL C			
		61.77 pg/mL AD			
		81.8 pg/mL AD[a]			
		21.68 pg/mL 1d WD			
		42.73 pg/mL 7d WD			
Jockers-Scherubl et al. (2007)	S	42 pg/mL			
		401 AD no DT			
		3292 AD + DT			
		225 1d WD			
		108 8d WD			
		144 3 m WD			
		128 8 m WD			
Costa et al. (2011)	S		24.2 pg/mL C		EMax ELISA
			23 pg/mL AD		Promega
			27 pg/mL AD[a]		
			32 pg/mL Abstinent		
Umene-Nakano et al. (2009)	S	21 ng/mL C			EMax ELISA
		9 ng/mL D			Promega
		9.8 ng/mL D + AD			
Meng et al. (2011)	S				Chemi-kine
					Millipore

(*continued*)

Table 1 (continued)

Study	Serum/ Plasma	NGF	BDNF	GDNF	Kit
Zanardini et al. (2011)	S		S-4.08 ng/mL C		Quanti-kine R&D
	P		S-4.77 ng/mL AD		
			P-41.7 ng/mL C		
			P-35.97 ng/mL AD upon admission		
Heberlein et al. (2010) Assoc with thrombocyte count and GGT levels	S		535 pg/mL C	196 ng/mL C	DuoSet ELISA R&D
			629 pg/mL AD 1d WD	82.7 pg/mL AD 1d WD	
			548 pg/mL AD 7d WD	93.5 pg/mL AD 7d WD	
			614 pg/mL AD 14d WD	81.4 pg/mL AD 14d WD	
Huang et al. (2011)	S		14.8 ng/mL C		EMax ELISA Promega
			12.3 No DT		
			6.2 DT		
			13.4 No DT, 7d WD		
			8.9 DT, 7d WD		
Aloe et al. (1996)	P[b]	32 C			Sandwich ELISA
		37 pg/mL Acute 1 hr			
		284 pg/mL AD			
		320 pg/mL Late WD			
		239 pg/mL Early WD			
D'Sa (2012)	P		S-26.6 ng/mL C		DuoSet ELISA R&D
	S		S-35.3 ng/mL AD		
			P ~ 1.3 ng/mL C		
			P ~ 1.4 ng/mL AD		

AD alcohol dependent, *C* control, *D* depressed, *WD* withdrawal, *FH* family history, *d* day, *m* month, *S* serum, *P* plasma

[a]Still drinking

[b]Results indicate plasma but methods state "No heparin, EDTA, or protease inhibitors were used"

observations. Patients were European in the first three studies, while the patients were Asian in the Yoon study and the control population varied by 11 years in age. While peripheral sources, concurrent anticonvulsant therapy, and systemic inflammation contribute to serum and plasma NGF levels, these do not appear to be significant covariates when measuring NGF levels in alcohol-dependent patients, nor does the blood component assayed. The well-established association between

stress and peripheral NGF levels (reviewed by Berry et al. 2012) also provides an intriguing link to alcohol dependence and NGF induction. The relative consistency among these studies implies global effects involving elevated NGF in homeostasis, withdrawal hyperexcitability, and repair after prolonged alcohol exposure that parallel observations in rodent models.

2.2 BDNF

2.2.1 BDNF-TrkB Expression and Distribution

While BDNF was the second neurotrophin to be characterized, BDNF distribution and functions are more widespread than NGF. Figure 1 shows the wide distribution of BDNF promoter–driven GFP expression and mRNA in the mature mouse. BDNF expression is regulated by multiple promoters that are differentially methylated and transcribed developmentally, regionally, by activity, pathologically, and upon exposure to xenobiotics (Pruunsild et al. 2007; Aid et al. 2007; Timmusk et al. 1993). BDNF mRNA targeting and translation are also regulated by 5′ and 3′ UTR sequences (Tongiorgi et al. 2006). BNDF expression level coincides developmentally with synaptogenesis and begins weakly at E13 in the brain (reviewed by Bartkowska et al. 2010). Levels of BDNF remain low until the second postnatal week which coincides with a peak in apoptosis for many structures (Das et al. 2001; Maisonpierre et al. 1990a; Katoh-Semba et al. 1997). BDNF levels are highest in the hippocampus (5.4 ng/g wet weight) followed by hypothalamus (4.33). All other brain regions contain less than 1.5 ng/g wet weight.

BDNF is expressed at high levels by cells in the cortex and hippocampus but is notably absent in the striatum, NAc, and striatal-derived amygdaloid structures (see Maisonpierre et al. 1990a; Ernfors et al. 1990a, b; Conner et al. 1997; Altar et al. 1997 for detailed expression and anatomy). Within the hippocampus, CA3 pyramidal cells and granule cells show the highest mRNA expression with lower expression in CA1 while hilar polymorph cells produce moderate levels of BDNF. BDNF-immunoreactive fibers populate the hippocampal strata oriens, radiatum, lucidum, and lacunosum moleculare, avoiding the stratum pyramidale. High levels of BDNF mRNA are also detected in the thalamus while both immunoreactive fibers and cell bodies containing mRNA are detected in the cortex, septum, BFCN, and the lateral and basal nuclei of the amygdala (Altar et al. 1997; Conner et al. 1997). As with NGF, BDNF is often synthesized in one area and transported, frequently anterograde (Conner et al. 1997; Altar et al. 1997; Li et al. 2012), but also retrograde as is observed in cerebellar granule cells (Bhattacharyya et al. 1997). Notable in addiction research, the medial habenula, striatum, NAc, central amygdala, and bed nucleus of the stria terminalis do not contain significant amounts of BDNF mRNA detected by in situ hybridization or BDNF-immunoreactive cell bodies but contain BDNF-immunoreactive fibers and cells expressing TrkB, consistent with anterograde transport to these (and other) regions (Altar et al. 1997; Conner et al. 1997; Baquet et al. 2004; Li et al. 2012),

The BDNF receptor TrkB is a family of proteins with thirty-six potential splice variants (Luberg et al. 2010), three of which have been well-characterized. TrkB.FL refers to the full-length receptor with tyrosine kinase activity. TrkB-Shc is truncated but retains Shc binding and coupling to the ERK and PI3K pathways. The truncated TrkB.T1 lacks tyrosine kinase activity and was thought to serve as a "decoy" to sequester and inhibit BDNF signaling, but TrkB.T1 coupling to Rho also regulates cytoskeletal dynamics (reviewed by Fenner 2012). TrkB splice variants are also believed to differentially regulate signaling intermediates, with full-length TrkB coupling to transcription through CREB and truncated variants signaling to the cytoskeleton. Both full-length and TrkB.T1 receptors are expressed throughout the brain, but the full-length receptor is exclusively associated with neurons, while TrkB.T1 is expressed by neurons, glia, choroid plexus, and ependymal cells (Altar et al. 1994). Iodinated BDNF and NT-4/5 binding sites were widely distributed similar to in situ hybridization (Altar et al. 1994). The highest levels of TrkB are in hippocampus, cerebellum, and cortex. Diencephalic, hypothalamic, and midbrain monoaminergic neurons express TrkB, and BDNF increases neurotransmitter synthesis and survival in these cells (Altar et al. 1992, 1994; Madhav et al. 2001; Akbarian et al. 2002; Baquet et al. 2004). TrkB is expressed on the majority of mesencephalic dopaminergic neurons, while many of these neurons also produce BDNF (Li et al. 2012; Numan and Seroogy 1999).

Homozygous BDNF knockout mice are not viable and die as neonates. Heterozygous mice exhibit a motor/balance phenotype by 2 weeks of age due to loss of vestibular ganglia (Klein 1994; Ernfors et al. 1994a; Jones et al. 1994). TrkB knockout mice do not eat and also die at birth (Klein et al. 1992). Reduced BDNF impairs taste (Liebl et al. 1997), resulting in abnormal appetitive behaviors in BDNF haploinsufficient mice (Kernie et al. 2000; see Krimm 2007) and, while controlled for and not observed in all experimental cohorts (Hensler et al. 2003; McGough et al. 2004), could contribute to increased alcohol consumption by BDNF haploinsufficient mice in some substrains. Deletion of BDNF also reduces neuropeptide expression in cortical interneurons but does not decrease parvalbumin- and NPY-expressing interneuron numbers (Jones et al. 1994). Recent work has employed region-specific Cre-recombinase-mediated gene deletion strategies and drug-inducible promoters to circumvent the developmental lethality of the full knockout. Combined, both of these strategies validate that deletion of, or reduction in, BDNF impairs hippocampal function, decreases striatal neuron survival, decreases spine density, and induces hyperactivity and hyperphagia (Lyons et al. 1999; Kernie et al. 2000; Rios et al. 2001; Zorner et al. 2003; Gray et al. 2006; Gorski et al. 2003; Li et al. 2012; Baydyuk et al. 2011). Cortex-specific and nigra-cortex double deletion of BDNF causes degeneration of striatal medium spiny neurons due to the loss of anterograde trophic support (Baquet et al. 2004; Strand et al. 2007; Li et al. 2012). Selective deletion of TrkB from medium spiny neurons impairs motor function, reduces striatal volume, MSN size, spine density, and impairs MSN dendritic development that is accompanied by a reduction in dopaminergic innervation (Baydyuk et al. 2011; Li et al. 2012). Interestingly, a human mutation in the TrkB gene is associated with developmental delay and severe obesity, a phenotype quite similar to the deficient mice (Yeo et al. 2004).

2.2.2 BDNF and Ethanol in Animal Models

A detailed review of ethanol and BDNF interactions in addiction and anxiety was recently published (Davis 2008); therefore, this section will concentrate on toxicity and recent controversial reports. BDNF has been extensively studied in the mature brain after acute and chronic ethanol exposure, but the results have varied significantly, often with disparate results in the same species, with the same exposure paradigm and over the same time course. For example, a few studies report no change in hippocampal BDNF levels (Miller et al. 2002; Okamoto et al. 2006), while most find a decrease in hippocampal BDNF with chronic exposure (MacLennan et al. 1995; Miller and Mooney 2004; Tapia-Arancibia et al. 2001; Hauser et al. 2011; Rueda et al. 2012). Still others report a transient increase in hippocampal BDNF with shorter exposure models (Kulkarny et al. 2011; Kalev-Zylinska and During 2007; McGough et al. 2004) and others see increased BDNF during withdrawal (Tapia-Arancibia et al. 2001) or with chronic intermittent exposure (Miller 2004). Disparate results were observed in BDNF mRNA in the hippocampus with intraperitoneal injection, the simplest of exposure paradigms. McGough and colleagues (2004) found an increase at 30 min while Raivio and colleagues (2012) examined levels at 3 h, implicating time as a factor; however, McGough did not observe a change in BDNF mRNA at this time point but found a reduction at 24 and 48 h. Raivio and colleagues conversely did not observe any differences in hippocampal BDNF at 24 h. Much of the discordant data are clustered around the acute time course as most studies agree that doses and exposure times sufficient to cause degeneration decrease BDNF but periods of withdrawal with intermittent exposure may be associated with attempts at repair (Miller 2004). These disparate results are observed in the same brain region and over the same time course, but collectively suggest that BDNF induction eventually fails in the hippocampus with prolonged exposure while upregulation during withdrawal may contribute to excitotoxicity and withdrawal-associated seizures.

Observations are inconclusive in other regions as well. Both increased and decreased BDNF protein levels have been observed in the cortex after chronic ethanol exposure and vary with region and pattern of exposure (Pandey et al. 1999; Miller and Mooney 2004; Miller 2004; Rueda et al. 2012). BDNF protein and mRNA levels in the cerebellum are acutely reduced by ethanol (Wang et al. 2010). BDNF mRNA levels in amygdala and NAc were also acutely reduced in rats after a single intraperitoneal dose of 2.5 g/kg of ethanol (peak BAC 58 mM); levels remained reduced in the frontal cortex at 24 h but returned to control values in the amygdala and hippocampus. The acute phase of this response is consistent with a decrease in excitatory transmission. BDNF mRNA in the NAc was initially reduced but increased in the ventral tegmental area (VTA) at 3 h, rebounding to increase at 24 h in both structures (Raivio et al. 2012). Kerns and colleagues (2005) also observed an increase in BDNF in NAc by microarray with acute exposure that was mouse strain specific. The acute increase in the VTA could result from direct excitation, reduced inhibition, or accumulation of mRNA that fails to transport but suggest that the VTA may be a source for accumbal BDNF mRNA levels since cortical levels remained lower. Microarray analysis also identified BDNF as a gene

with reduced expression in the prefrontal cortex after chronic intermittent ethanol exposure (Melendez et al. 2012). Interestingly, the array analysis also detected increased Oprl1 and extensive modulation of mRNA species belonging to the actin dynamics and MAPK signaling nodes consistent with a pharmacodynamic process. Consistent with a decrease in cortical BDNF mRNA, Bosse and Mathews (2011) measured BDNF protein levels in the striatum after acute ethanol exposure in both wild type and BDNF haploinsufficient mice. Both exhibited a decrease in BDNF; furthermore, the deficient mice did not respond to ethanol with a robust increase in dopamine when measured by in vivo microdialysis.

This is in contrast to work from the Ron/Janak laboratory that did not detect changes in cortical or NAc BDNF with acute exposure or self-administration (Logrip et al. 2009; McGough et al. 2004). Hippocampal BDNF was also elevated by ethanol through a Receptor for Activated C Kinase (RACK1) dependent pathway after both acute and prolonged (4 weeks) ethanol exposure (McGough et al. 2004; Jeanblanc et al. 2009, 2006; He et al. 2010). The data contrast the observations discussed above in extended striatum, implicating an acute increase in dorsolateral striatal BDNF that is negatively correlated with ethanol consumption (McGough et al. 2004; Logrip et al. 2009; Jeanblanc et al. 2009). This is supported by the observation that BDNF haploinsufficient mice voluntarily consume more ethanol than wild type litter mates, but these cohorts do not exhibit generalized hyperphagia, as discussed above (Hensler et al. 2003; McGough et al. 2004). Furthermore, viral mediated expression of BDNF siRNA in the dorsolateral striatum clearly transduced medium spiny neurons identified with GFP and replicated the phenotype of increased drinking (Jeanblanc et al. 2009). RACK1 mediated the ethanol-induced BDNF expression since Tat-RACK1 administration increased BDNF, reduced ethanol intake through TrkB, and reversed the phenotype in haploinsufficient mice when administered in the dorsolateral striatum (McGough et al. 2004; Jeanblanc et al. 2006). BDNF induction by ethanol was also observed in vitro with immunofluorescence in striatal neurons (Logrip et al. 2008), neuroblastoma cells (He et al. 2010), and in hippocampal pyramidal cells (McGough et al. 2004; He et al. 2010), requiring RACK1 for gene expression in each cell type. RACK1 activation of BDNF transcription is purported to be mediated by cAMP (Yaka et al. 2003b, c; He et al. 2010; Neasta et al. 2012), 14-3-3 association (Neasta et al. 2012) and epigenetic modulation of BDNF promoter IV (He et al. 2010). Interestingly, cAMP signaling via PACAP also appears to use this pathway to induce BDNF (Yaka et al. 2003a, b). Cyclic AMP causes dissociation of RACK1 from the NMDA/Fyn complex, thereby increasing Fyn phosphorylation of the NMDA receptor and increased NMDA activity. Collectively, these data place cAMP upstream of many key events involved in adaptation to ethanol exposure but implicate RACK1 and not CREB as the key transacting factor in this pathway.

Similarly, BDNF induction was observed in MSNs of the central and medial amygdala by Pandey and colleagues, where interference with BDNF expression using antisense oligonucleotides increased anxiety-like behavior and ethanol consumption (Pandey et al. 2005). Furthermore, alcohol-preferring rats produced less central amygdala and BNST BDNF mRNA and protein and exhibited higher

anxiety-like behavior (Moonat et al. 2011; Prakash et al. 2008). In this case, CREB appears to be a key intermediate in the behavioral response but Pandey and colleagues place CREB and ERK downstream of BDNF induction. While Pandey's group did not detect strain differences in BDNF in the NAc, Yan and colleagues found a reduction in accumbal BDNF in the preferring strain (Yan et al. 2005). Together, these data implicate reduced BDNF in the basal ganglia in alcohol appetite, but the anatomical locus is not resolved. As with MSNs in the striatum, BDNF protein should be delivered to the central amygdala via afferents (Conner et al. 1997) but the source of the mRNA and efficacy of BDNF siRNA and antisense oligonucleotides were unequivocally shown to be locally mediated within medium spiny neurons in the dorsal striatum and striatal-like nuclei of the amygdala. This was not concluded by Riovio (2012) and colleagues whose data and discussion suggest afferent or rare local sources in the accumbens. These data are difficult to reconcile and will require further anatomical, pharmacological, and molecular investigations.

Variable results have also been reported in developing animals with BDNF expression, likely amplified by the low levels of BDNF in neonates and stress. Many fetal alcohol experiments focus on the developing cerebellum because the ontogeny of specific neuronal populations, their migration, synaptogenesis, and the factors modulating these processes have been well-characterized (reviewed by Sotelo 2004). The majority of studies examining BDNF levels, TrkB, or BDNF signaling have shown that ethanol inhibits the neurotrophic activity of BDNF in the cerebellum, and this may contribute to cerebellar apoptosis and impaired foliation. Ge and colleagues (2004) performed a detailed dose–response, time-course analysis and found a rapid decrease in BDNF and TrkB mRNA in the cerebellum after exposure on PN4 but not PN9, suggesting a role for BDNF in the selective vulnerability at this time point when levels are low. In a follow-up study (Light et al. 2002), TrkB was detected in cerebellar Purkinje cells during the vulnerable first postnatal week; ethanol decreased TrkB immunoreactivity as well as TrkB.T1 and TrkB mRNA in these experiments. Moore and colleagues found an increase in TrkB in the cerebellum of females exposed prenatally but no other alterations in cerebellar TrkB with prenatal or postnatal exposure (Moore et al. 2004a, b). Moreover, Heaton and colleagues did not observe significant regulation of BDNF protein levels in cerebellum with numerous exposure windows that span postnatal development but subsequently reported a transient increase in BDNF immediately after exposure on PN4 that normalized by 2 h (Heaton et al. 1999, 2003a). Exposure on PN7 increased BDNF levels at 2 h and decreased levels at 12 h in this study. Observations made in juvenile rats after a single binge of ethanol indicate no changes in cerebellar BDNF mRNA (Kulkarny et al. 2011), suggesting modulation may be more dynamic in younger animals.

Multiple lines of evidence support a role for BDNF expression and signaling in the cortex and hippocampus in the development of FASD, but the sequence and mechanisms have not been fully resolved. A few reports have suggested an increase in BDNF (and related TrkB agonist NT-4) immediately following exposure. Increased BDNF/NT-4 levels were measured in combined "cortex/striatum" at

PN1 after prenatal exposure and at PN10 after PN4 -10 (Heaton et al. 2000c). Surprisingly there was no significant developmental increase detected in BDNF between PN10 and PN21. By contrast, Heaton and colleagues (2003a) reported bidirectional changes in BDNF cortex after exposure on PN7 and increased BDNF on PN10 after neonatal exposure that resolved by PN21. It should be noted that the levels of BDNF measured in these assays were very low (50–80 pg/g wet weight) and may have been below linear detection with the dilutions given in the text (100-fold). Others have found that fetal exposure decreases BDNF in the cortex (Climent et al. 2002; Fattori et al. 2008; Feng et al. 2005; Caldwell et al. 2008). In addition, Caldwell and colleagues (2008) found decreased BDNF and specific decreases in transcripts III, IV, and VI in the medial frontal cortex and hippocampus.

Similar differences were observed in the hippocampus where Heaton and colleagues (2000c) find a transient increase in hippocampal BDNF at PN10 with neonatal exposure and no change with prenatal exposure. However, Fattori found a decrease in BDNF after PN5-8 exposure. Feng and colleagues (2005) and Caldwell colleagues (2008) also report a decrease in BDNF after gestational exposure. Miki et al. (2008) exposed animals from PN10-15 via vapor inhalation and also noted a transient increase in hippocampal BDNF mRNA from PN16-PN30 that varied in duration depending upon the control group comparison. These observations were less robust in older animals and complicated by differential BDNF expression between the maternally reared control group and the separation control group such that BDNF levels were reduced when compared to the separation control animals but unchanged relative to the maternally reared animals at 60 days of age (Miki et al. 2008). Similarly, separation of PN5 pups increased PKC activity in the hippocampus, which correlated with increased ERK signaling (Davis et al. 1999). These observations illustrate how stress significantly affects signaling in the developing brain and may be another variable contributing to the differential effects of ethanol on neurotrophin levels and signaling between laboratories and exposure paradigms. While it is difficult to draw firm conclusions from the current data, combined, these studies suggest that ethanol may acutely and transiently increase BDNF in some brain regions developmentally. Correlations with cell death suggest that the increase may reflect an attempt at repair, but may ultimately lead to a reduction in BDNF production in the mature structure if administered during a vulnerable window.

Ethanol also produces variable effects on TrkB that may be dependent not only on timing but also on gender (Moore et al. 2004a, b). However, in spite of this overwhelming confound, others have found that prenatal exposure increases TrkB/TrkB-T1 ratios as neonates (Climent et al. 2002). Baseline neurotrophin levels and BDNF levels are responsive to multiple environmental and experimental factors that can make accurate measurements difficult. More experiments examining acute effects on signaling during exposure and compensatory regulation over time and after in specific cell types are required. It is also unclear whether these changes result in a decrease in activity-dependent BDNF release or represent differences in synaptic complexity. Examination of BDNF signaling (discussed below) suggests that ethanol causes a decrease in the ability of TrkB to transduce signal and desensitize, which may lead to compensatory regulation of both BDNF and TrkB

in vivo. There is considerable data to indicate a role for aberrant BDNF modulation during post-exposure development, but this may be secondary to impaired synaptogenesis, activity, and neuronal apoptosis (Bhave et al. 1999; Feng et al. 2005; Miki et al. 2008; Caldwell et al. 2008; Climent et al. 2002).

2.2.3 BDNF and In Vitro Models of Ethanol Exposure

As with NGF in the previous section, BDNF diminishes ethanol-induced hippocampal and cerebellar granule cell death in vitro (Heaton et al. 2000b; Bonthius et al. 2003). The neuroprotective effects of NMDA in cerebellar granule neurons are also mediated by BDNF induction (Marini et al. 1998; Bhave et al. 1999) and likely involve PI3K since the survival-promoting effects of BDNF were blocked by PI3K inhibition (Bhave et al. 1999). Ethanol also decreased Akt phosphorylation in the developing, but not adult, brain (Chandler and Sutton 2005), implicating PI3K/Akt in the selective vulnerability of the developing brain to ethanol.

Neurotrophins also reverse many of the effects of ethanol on synaptic activity. Exogenous BDNF reverses ethanol attenuation of GABA-A responses in cerebellar granule cells (Ericson et al. 2003). Ethanol also reverses BDNF-mediated long-term plasticity at developing hippocampal CA3 GABAergic synapses through inhibition of voltage-gated calcium channels (Zucca and Valenzuela 2010). At excitatory synapses, ethanol blocks BDNF enhancement of NMDA (NR2B-containing), but not AMPA, responses in hippocampal neurons (Kolb et al. 2005). As a control, the authors show that NGF and NT3 do not alter synaptic transmission, indicating cell type selectivity in the ion channels modulated by specific neurotrophins as would be expected from the anatomical distribution discussed above. Kolb and colleagues further dissected the effect of ethanol on the channel from the BDNF enhancement by using ethanol concentrations that did not have an impact on NMDA signaling, indicating that the effect of the effect of ethanol is independent of NMDA receptor inhibition.

In contrast to the experiments discussed above where chronic ethanol enhanced NGF-stimulated ERK activity in PC-12 cells, acute and chronic ethanol administration decreases activation of ERK in primary neurons and in the rodent brain (Davis et al. 1999; Kalluri and Ticku 2002, 2003; Tsuji et al. 2003; Chandler and Sutton 2005; Han et al. 2006; Ohrtman et al. 2006; Fattori et al. 2008 however, see Chen and Charness 2012 and Acquaah-Mensah et al. 2001). Regulation of ERK by ethanol also contributes to reduction in hippocampal LTP (Roberto et al. 2003). Ethanol acutely reduces TrkB signaling to ERK in cerebellar granule cells (Li et al. 2004; Ohrtman et al. 2006), but does not change TrkB phosphorylation state (Li et al. 2004), indicating an intracellular site of action downstream of the receptor. Neonatal hippocampal slices stimulated with BDNF displayed reduced nuclear translocation of phosphorylated ERK in hippocampal pyramidal cells in CA1 (Davis et al. 1999). Inhibition of BDNF-stimulated ERK phosphorylation by ethanol in cerebellar granule cells is dependent upon BDNF concentration and not modified by NMDA receptor antagonists (Ohrtman et al. 2006). Low concentrations of ethanol inhibit ERK activation only when BDNF was present at concentrations in the rising portion of the high affinity concentration–response curve (<5 ng/mL), while high concentrations of ethanol also block the desensitization of the ERK response at longer times (30–60 min), and at high BDNF

concentrations (>10 ng/mL). Thus, there was no apparent inhibition with 50 ng/mL of BDNF after 20 min, but the peak was never reached in the ethanol-exposed cells while the control curve is declining (Ohrtman et al. 2006). This was also reported recently by Chen and Charness (2012) who did not observe ethanol inhibition of BDNF-stimulated ERK activity with 50 ng/mL of BDNF, but this study did not perform the same pharmacological analysis as Ohrtman and colleagues. Both PKA and PKC regulate ERK, and reduced ERK activity correlates inversely with PKA activity and positively with a concurrent decrease in calcium-sensitive protein kinase C activity in the developing hippocampus (Davis et al. 1999); therefore, acute inhibition of TrkB signaling to ERK may be independent of Trk activation and ultimately cross-regulated by other pathways with different concentrations of ethanol.

In addition to mediating survival, BDNF also regulates migration and synaptogenic processes. Mutations that either upregulate or downregulate signaling through growth factor pathways and their associated GTPases cause a myriad of neurodevelopmental disorders resulting from accelerated or decelerated development (Stornetta and Zhu 2011). Perturbation of signaling that results in subtle changes in the rate of migration and synaptogenesis might also contributes to the development of alcohol-related neurodevelopmental disorders without inducing overt cell death. Numerous reports indicate that concentrations of ethanol as low as 25 mM inhibit neuronal migration neurite outgrowth through the adhesion molecule L1 (Charness et al. 1994; Bearer et al. 1999), and inhibition of ERK and Src and implicated in inhibition of L1-mediated neurite formation by ethanol (Tang et al. 2006; Yearney et al. 2009). In contrast, a few studies report increased migration and increased neurite formation in response to ethanol, as discussed above, and increased basal spines on pyramidal cells treated with chronic intermittent ethanol (Kroener et al. 2012). Developmentally, Powrozek and Olson (2012) observed increased dendrite formation in cortical explant cultures treated with ethanol. Proliferating neuroepithelial precursors exposed to ethanol display a differentiation-associated increase in migration (Camarillo and Miranda 2008) and ethanol increased the rate of axon formation in hippocampal pyramidal cells (Lindsley et al. 2011). This overall increase in axon growth rate was associated with ethanol inhibition of BDNF-stimulated Rac1/Cdc42 activation and increased Rho activity in hippocampal axonal growth cones, leading to increased growth cone surface area (Lindsley et al. 2011). Although ethanol increased the surface area of growth cones, the levels of active Rho GTPases in axonal growth cones were not affected in the absence of exogenous BDNF. These observations are in contrast to a recent study from Chen and Charness (2012), detailing reduced axon growth that was not mediated by BDNF and was dependent on Src, albeit with different substrates. We have also observed an increase in BDNF-stimulated migration/dendrite formation in cerebellar granule cells using a Boyden chamber assay (Hassoun et al. 2007a; See Hassoun et al. 2007b for detailed methods). As discussed above, ethanol also enhances differentiation in some cells, therefore cell cycle arrest, possible through ERK inhibition in cycling cells, and premature differentiation would increase the pool of migration-competent precursors if administered during the correct developmental window. This does not necessarily have to increase the rate

of migration, which has been shown to be reduced in the cerebellum after ethanol exposure (Jiang et al. 2008), but may simply increase the number of cells that migrate toward BDNF.

Oxidative stress, also known to modulate neurotrophin signaling, has recently been implicated in the etiology of some forms of autism (Rossignol and Frye 2012; Sajdel-Sulkowska et al. 2011) as well as the development of FAS/ARND (Brocardo et al. 2011). A target that has been suggested to mediate the toxic effects of ethanol is cJun N-terminal Kinase (JNK), a member of the mitogen-activated protein kinase family, which is induced by ethanol, genotoxicity, and oxidative stress (reviewed by Weston and Davis 2007). p75 also activates the JNK pathway (Koshimizu et al. 2010). JNK, as the name implies, phosphorylates c-Jun, a member of the AP-1 (Fos/Jun) transcription factor complex. "JNK" represents 3 families and as many as 16 splice variant isozymes that do not always correlate with molecular weight but is generally divided into 3 molecular weight categories of 58, 54, and 46 kDa. Suppression of Akt activates JNK (Shimoke et al. 1999), but apoptosis in cerebellar granule cells induced by growth factor withdrawal, glutamate, and beta amyloid is not mediated by JNK (Gunn-Moore and Tavare 1998). Some reports suggest that JNK with an apparent molecular weight near 60 kDa is involved in ethanol-induced apoptosis and ethanol-induced oxidative stress, but only one isoform was observed by western blot (Heaton et al. 2003a, 2012). Han and colleagues (2006) observed ethanol-induced increases in p46 and p54 phosphorylation that are associated with apoptosis in PN7 rat pups. However, when phosphorylated cJun is measured as a surrogate for JNK activity after ethanol exposure, there is no overlap between cells undergoing apoptosis and phospho-cJun in immunostained brain sections (Young et al. 2008). Moreover, JNK activation induces genes associated with protection in *Drosophila* in response to oxidative stress (Wang et al. 2003) and mutations that enhance JNK signaling in Drosophila can extend lifespan (Karpac and Jasper 2009). BDNF stimulation of AP1 DNA binding activity is also reduced by ethanol in cerebellar granule cells (Li et al. 2004). The effects of JNK activation may therefore be isozyme or splice variant, specific as these data suggest reduced genomic signaling through Fos/Jun after ethanol in cerebellar granule cells and a non-genomic, possibly protective, isoform-specific, role for JNK in ethanol toxicity which has not been explored.

The JNK pathway stimulates both dendrite and axon formation through phosphorylation of proteins such as MARKS and doublecortin that are associated with cytoskeletal dynamics (Oliva et al. 2006; Bjorkblom et al. 2012; Jin et al. 2010). JNK1 is associated with microtubules where it regulates axodendritic length (Tararuk et al. 2006), and genetic deletion of JNK or intermediates in the JNK pathway leads to axonal growth defects (Chang et al. 2003; Koushika 2008). Immunohistochemical staining for phospho-JNK in the neonatal mouse brain labels primarily developing axon pathways and not apoptotic cell populations (Davis, unpublished observations). A role for JNK regulating migration and axon outgrowth in response to ethanol is further supported by the observations that ethanol increases integrin and NCAM levels (Vangipuram et al. 2008; Miller et al. 2006), increases laminins (Vangipuram et al. 2008), increases Fyn activity (Yaka et al. 2003b), increases PKC, and increases FAK phosphorylation in cerebellar granule cells (Sivaswamy et al. 2010), which all regulate

cytoskeletal dynamics (Becchetti and Arcangeli 2010). JNK may therefore be an overlooked contributor to ethanol effects on the neuronal cytoskeleton and may serve protective as well as apoptotic functions.

2.2.4 BDNF in Patients with Alcohol Dependence

Variability in peripheral BDNF levels in alcohol-dependent patients is more extreme than NGF, making it difficult to deduce trends from the current data. The levels of BDNF reported in Table 1 are variable, often with standard deviations greater than the mean. Stress, depression, hematologic parameters, xenobiotics, age, and gender are all known to influence peripheral BDNF levels (reviewed by Autry and Monteggia 2012; Lommatzsch et al. 2005). BDNF levels detailed in Table 1 do not correlate with the source (plasma vs. serum), method used, or duration of abstinence. The source of the variability could be innate, due to simultaneous use of anticonvulsants during withdrawal, or due to sample preparation. Many experiments used serum, which includes BDNF released by thrombocytes, and is orders of magnitude higher than the plasma concentration (Lommatzsch et al. 2005). Two studies state different sources in the methods compared to the results section, although the levels reported suggest the source (ng vs. pg concentrations). In other studies, it is not clear that the centrifugation steps are sufficient to remove the thrombocytes, while others use sedimentation forces likely to rupture the cells. Zanardini and colleagues (2011) and D'Sa and colleagues (2012) directly compared serum and plasma BDNF. The first study reported a small increase in both components, while the second study only detected an increase in BDNF in serum in alcohol-dependent patients. Heberlein and colleagues (2010) also addressed this confound by simultaneously measuring thrombocyte activity and serum BDNF levels, observing a positive correlation between serum BDNF levels and both gamma glutamyl transferase levels and thrombocyte function. In addition, Costa and colleagues (2011) found a nonsignificant but suggestive association of BDNF levels with platelet count. Alcohol dependence and withdrawal are known to alter thrombocyte levels and coagulation cascades with robust rebound upon withdrawal (Schmitt et al. 1999; Chanarin 1982). BDNF is also produced and released by the vascular endothelium (Nakahashi et al. 2000), further complicating any correlation between central function and peripheral levels in patients with underlying cardiovascular disease.

Peripheral levels may provide valuable information since the production and release mechanisms regulating plasma and serum levels are likely to be similar to mechanisms governing central production and release, even if they are not the same pools of BDNF. Interestingly, this was predicted by Chinarin in 1982: "After platelet aggregation the platelets contract and squeeze various metabolites out of the platelet; this is called the 'release reaction'. This phase is also curtailed or even absent in platelets from alcoholics". BDNF polymorphisms also have the potential to regulate peripheral levels, but these reports are similarly variable and primarily examine serum BDNF. The Met allele of the Val66Met BDNF polymorphism decreases neuronal BDNF secretion (Egan et al. 2003; Chen et al. 2004, 2006). Some evidence supports an association between the homozygosity for the Met or

Val allele with lower or higher BDNF levels, respectively (Ozan et al. 2010; Cirulli et al. 2011). In contrast, no association between genotype and serum levels were found in the majority of patient populations examined thus far (Yoshimura et al. 2011; Trajkovska et al. 2007; Terracciano et al. 2011) and, paradoxically, patients with the Met allele were even found to have higher serum BDNF concentrations (Lang et al. 2009). Thus, factors other than genotype may be responsible for the majority of the variability in serum and plasma BDNF.

BDNF is highly polymorphic and linkage analysis identified an alcoholism susceptibility gene that mapped near the BDNF locus, 11p13 (Uhl et al. 2001). The human BDNF gene is encoded by 11 exons and 9 promoters (Pruunsild et al. 2007) and is polymorphic between different human populations, further complicating association analysis (Petryshen et al. 2010). Three polymorphisms have been examined in alcohol-dependent patients; these polymorphisms change BDNF secretion and activity-dependent processing (Egan et al. 2003; Chen et al. 2004, 2006). The first is a missense change (G196A, rs6265) that results in a valine to methionine substitution (Val66Met). The Met allele shows impaired trafficking and activity-dependent secretion, and humans homozygous for the Met allele have reduced hippocampal and amygdalar volume, increased anxiety, increased heart rate, and poor working memory (Egan et al. 2003; Hariri et al. 2003). Humans homozygous for the Met allele have recently been reported to have higher anxiety, increased cortisol, and consume more alcohol (Colzato et al. 2011). Abstinent Met/Val and Val/Val patients differ in their rate and locus of recovery measured by MRI, with Val/Val homozygotes showing more gray matter recovery while heterozygotes had more recovery in white matter measures (Mon et al. 2012). Transgenic mice carrying the human Met allele and their human counterparts were also impaired in models of extinction learning, with human Met/Met homozygotes demonstrating impaired fronto-amygdaloid activity by functional magnetic resonance imaging (Soliman et al. 2010). A second polymorphism (C270T) is present in the 5′ noncoding region that can regulate transcription or translation. The third genotype examined is a G712A polymorphism that has not been extensively investigated molecularly but may be associated with Parkinson's Disease (Chen et al. 2011). Initial studies suggested BDNF polymorphisms were associated with psychiatric disorders; however, subsequent studies and meta-analysis have failed to support associations for most major neuropsychiatric disorders, while studies contain too few patients to asses validity in less common disorders at this point (reviewed by Hong et al. 2011).

Anxiety is a precipitating factor for alcohol abuse. Association between the Met allele and higher anxiety-like behavior has been observed in both humans and transgenic mice expressing the human Met allele (Jiang et al. 2005; Montag et al. 2010; Chen et al. 2006). BDNF polymorphisms have been examined by several groups and the associations with alcohol dependence have been weak and variable in spite of promising initial observations. Matsushita et al. 2004 found that G196A (Val66Met) BDNF polymorphism frequency did not differ significantly from controls but alcohol-dependent patients with violent tendencies and a history of delirium tremens had an increased frequency of Met/Met genotype. Antisocial

alcohol behavior and alcohol dependence may also segregate with a TrkB variant (Xu et al. 2007). The Met allele was also associated with earlier disease onset in this study. Met carriers were subsequently shown to consume more alcohol than Val66Val or Val66Met patients which segregated with alcohol use disorders (Shin et al. 2010; Colzato et al. 2011). Reduction of BDNF is associated with aggression in mice (Chen et al. 2006), but the genetic association between aggression, Met/Met, and alcoholism was not observed in the majority of replicate analyses (Tsai et al. 2005; Muschler et al. 2011; Wojnar et al. 2009; Grzywacz et al. 2010). In a subgroup analysis, the Met allele was overrepresented in alcohol-dependent patients with depression compared to controls or dependent patients without depression (Su et al. 2011). While no association was found with dependence, Wojnar and colleagues (2009) found an association between the Val66Val genotype and relapse that was greater when restricted to analysis of those with a positive family history of alcohol use disorders. In support of this phenotypic association, the Val/Val is also associated with impulsivity (Gatt et al. 2009), which can contribute to relapse. A modest association between SNP G712A and substance dependence was also described but did not specifically examine the alcohol-dependent subgroup (Zhang et al. 2006). These data do not suggest a strong association between the BDNF polymprphisms and alcohol dependence but may identify a subgroup of patients and indicate response to treatment. Interestingly, over 60 polymorphisms in the BDNF gene have been identified to date, so lack of association at two loci does not exclude a role for BDNF genotype as a modifying factor in alcohol abuse, dependence, and toxicity.

2.3 NT-3

2.3.1 NT-3-TrkC Expression and Distribution

NT-3 is widely expressed in the periphery, with high levels in the kidney, spleen, heart, and muscle (Maisonpierre et al. 1990a; Ernfors et al. 1990b). As with NGF and BDNF, NT-3 supports survival and neurite outgrowth from peripheral ganglia (Maisonpierre et al. 1990a; Ernfors et al. 1990b). With the exception of hippocampal dentate granule cells, NT-3 mRNA is not highly expressed in the mature CNS (Fig. 1, Allen Institute and LazC expression from the NT-3 promoter generously provided by Dr. Kevin Jones at the University of Colorado). NT-3 is present at low levels in mature animals in the indusium griseum, tenia tecta, olfactory bulb, substantia nigra, medial locus coeruleus, and scattered paraventricular (IV) brain stem nuclei (Ernfors et al. 1990b; Vigers et al. 2000). Other structures such as the retrosplenial and visual cortices with low NT-3 expression and a developmental peak were also identified in a mouse strain expressing LacZ from the NT-3 promoter (Vigers et al. 2000). LacZ expression is more sensitive than in situ hybridization, but this essentially replicated the earlier mRNA studies with respect to relative expression levels, including lack of NT-3 expression by cells in striatum and globus pallidus (Maisonpierre et al. 1990b; Friedman et al. 1991).

Adult NT-3 protein levels range from 1 to 1.5 ng/g wet weight with hippocampus, cerebellum, and piriform cortex having the highest levels and superficial and deep cortical layers containing the greatest immunoreactivity (Anderson et al. 1994). Acid treatment has been shown to increase NT-3 detection by 30–50 %, depending on the region (Okragly and Haak-Frendscho 1997). NT-3 is detected in the internal granule cell layer of the cerebellum and ELISA estimates the concentration is around 500 pg/g wet weight in mature rats (Söderström and Ebendal 1995). Levels in neonatal animals reach 9 ng/g wet weight transiently in cingulate cortex (2.5 ng/g in hippocampus) and peak expression in the hippocampus, cerebellum, piriform cortex, and entorhinal cortex occurs at birth and declines over the first 2 postnatal weeks (Ernfors et al. 1990b; Söderström and Ebendal 1995; Das et al. 2001).

NT-3 regulates the development of discrete neuronal populations during early, middle, and late periods of CNS neurogenesis and synaptogenesis. NT-3 regulates neural crest development and is expressed at the earliest steps in neurulation (Maisonpierre et al. 1990b). NT-3 has a developmental peak in mRNA expression that coincides with the arrival of perforant path afferents into the hippocampus (Maisonpierre et al. 1990b, Ernfors 1990b; Friedman et al. 1991; Lauterborn et al. 1994), and NT-3 is transiently expressed in the hilus of the hippocampus at birth (Friedman et al. 1999). Similarly, late expression of NT-3 mRNA in the visual cortex and lateral geniculate nucleus coincides with eye opening and decreases after the critical period for ocular dominance column formation to undetectable levels in the adult (Lein et al. 2000). Follow-up studies using Cre-mediated deletion in cortex establish a role for NT-3 in thalamocortical innervation (Ma et al. 2002). NT-3 expression in the thalamus is transient, and targeted deletion of NT-3 in the mouse cortex selectively perturbs the development of thalamocortical axons projecting to retrosplenial and visual cortex (Ma et al. 2002). In organotypic cultures, NT-3 induces cortical, axon fiber branching and is an attractive signal for layer 6 axons while repelling layers 2/3 axons (Castellani and Bolz 1999).

Cerebellar granule neurons switch trophism from BDNF to NT-3 after they enter the internal granule layer (Segal et al. 1992). Conditional deletion of NT-3 results in a dramatic reduction in foliation of the cerebellum with normal Purkinje cell arborization, normal EGL proliferation, and normal lamination (Bates et al. 1999). Deletion of IGF-1 receptor in the cerebellum reduces NT-3 and results in a similar foliation phenotype (de la Monte et al. 2011). In addition to scattered pre- and post-migratory cerebellar granule cells expressing mRNA (Rocamora et al. 1996; Ernfors et al. 1990a), NT-3 immunoreactivity is detected in cerebellar Purkinje cells, cerebellar interneurons, deep cerebellar nuclei, medial vestibular nuclei, and the inferior olive (Friedman et al. 1991; Zhou and Rush 1994). Purkinje cells have not been shown to express NT-3 mRNA (Ceccatelli et al. 1991; Ernfors et al. 1994a), suggesting that NT-3 may accumulate in Purkinje cells via retrograde or anterograde transport.

TrkC has limited distribution in the CNS of mature animals but is highly expressed early in development. TrkC binding, TrkC mRNA, and NT-3 expression are similar, with the highest receptor levels in superficial cortical layers, stratum oriens, stratum radiatum, the molecular layer of the dentate gyrus, the nucleus of the

lateral olfactory tract, entorhinal cortex, the anterior olfactory nucleus, and the anteromedial thalamic nucleus. Neocortical deep layers, striatum, amygdala, dorsal root ganglia, and the central gray of spinal cord show moderate iodinated-NT-3 binding and TrkC expression, while expression is low in the cerebellum (Altar et al. 1993, 1994; Tessarollo et al. 1993).

2.3.2 NT-3 and Ethanol: Animal Models

It is not surprising that few studies have examined the effect of ethanol on NT-3/TrkC expression after ethanol exposure in mature animals, given the low, limited, and discrete regional expression in the adult. Recent studies of human postmortem tissue have suggested a role for ectopic induction of NT-3 induced by oxidative stress in the etiology of autism, particularly in cerebellar dysfunction (Sajdel-Sulkowska et al. 2011), but studies on NT-3 in developing and adult animals have been equivocal. NT-3 administered into the ventricles did not rescue vasoactive intestinal peptide-immunoreactive neurons in the suprachiasmic nucleus of withdrawn animals (Paula-Barbosa et al. 2003). Similarly, Baek and colleagues (1996) did no observe any changes in hippocampal NT-3 mRNA normalized to cyclophilin A after 28 weeks of constant ethanol exposure via liquid diet. Miller (2004) used a repeated episodic vapor exposure model, while Miller and Mooney (2004) exposed rats chronically to ethanol via liquid diet for 8 or 24 weeks and measured NT-3 levels by ELISA in parietal and entorhinal cortices, hippocampus, septum, and basal forebrain. Episodic exposure increased hippocampal NT-3 levels (from ~100 to 155–195 pg/mg protein) and reduced in basal forebrain NT-3 (from ~500 pg/mg protein to ~200 pg/mg). Chronic exposure increased NT-3 in entorhinal cortex and septum at both 8 and 24 weeks but transiently increased levels in basal nuclei and hippocampus. Both Miller and Mooney (2004) and Miller (2004) report the highest NT-3 content in the septum (600–700 pg/mg protein) with hippocampal levels ranging from 100 to 150 pg/mg protein and entorhinal cortex slightly higher at ~200 pg/mg protein. These data are given normalized to total protein but assuming 10 % protein, a value of 100 pg/mg is roughly 10 ng/g wet weight and regions such as septum contain 45+ ng/g wet weight. It should be noted that these concentrations are higher and not in the regional proportions previously observed in the adult, even upon acid treatment (see above) but may be the result of different sample preparation methods or, alternatively, significant transport.

When measured in homogenates after a variety of exposure paradigms, ethanol exposure either prenatally or postnatally changed NT-3 mRNA (Fattori et al. 2008; Light et al. 2001; Tsuji et al. 2008) and protein levels measured by ELISA (Heaton et al. 2000c). Heaton and colleagues (2000c) showed a trend toward increased "cortex/striatum" NT-3 on PN10 with PN4-10 vapor exposure (~90 pg/g wet weight to ~140 pg/g wet weight) in pups, but this did not reach statistical significance and NT-3 levels did not differ in other regions examined. When the telencephalon was further dissected (Heaton et al. 2003b), exposure to ethanol at PN14, but not the vulnerable PN3 exposure, increased NT-3 from ~8 ng/g to ~14 ng/g of striatal wet weight in rats at 24 h after exposure; NT-3 levels did not vary between

PN3 and PN14 (Heaton et al. 2003b). These levels are perplexing, given the variable concentrations observed between experiments and the lack of a developmental decrease in NT-3 postnatally (i.e., Das et al. 2001). NT-3 mRNA and NT-3 promoter–driven LacZ is not detected in the striatum in developing or mature animals (Maisonpierre et al. 1990b; Vigers et al. 2000; Soderstrom and Ebendal 1995; Das et al. 2001; Ernfors 1990b; Friedman et al. 1991; Lauterborn et al. 1994). Moreover, mRNA and LacZ expression also indicate low level of expression from the resident cells in the septum and basal forebrain. Therefore, the source of striatal NT-3 is unclear in these experiments as well. As discussed above, NT-3 is transiently produced in many structures including the thalamus, visual, cingulate, and retrosplenial cortex. NT-3 also regulates thalamocortical axon development during the critical stage with a peak in mRNA expression at PN8 (Ma et al. 2002). Again, NT-3 protein measured in striatum could be in retrograde transit through the striatum since the PN3 and PN14 time points in Heaton (2003b) flank the peak at PN5-8 but levels have not been shown to vary by orders of magnitude within a few days developmentally.

When the cerebellum is subdivided or immunoreactivity evaluated in situ, NT-3 and TrkC are sensitive to ethanol exposure in selected regions. TrkC immunoreactivity is reduced when examined specifically on Purkinje cells in pups exposed to ethanol during the vulnerable PN4-6 window (Light et al. 2002), but global mRNA for TrkC, likely representing mRNA from mature granule cells as well, was unchanged (Light et al. 2001). When the vermis was selectively assayed by ELISA, Parks and colleagues (2008) found increased cerebellar NT-3 and an increase in hippocampal NT-3 in mature animals after prenatal exposure, which could reflect compensation within the system. The high dose (6 g/kg/day by gavage) increased NT-3 in both regions while only the vermis was sensitive to the lower dose (4 g/kg/day). In addition, NT-3 levels in the vermis were higher in animals exposed to an enriched environment after prenatal alcohol exposure at both doses, again suggestive of a role in plasticity. The NT-3 concentrations measured were again higher than earlier reports in normal rats, but the relative levels, with the hippocampus having the highest NT-3 content, followed by cerebellum, were consistent.

The discrepancy between these studies and the classical descriptions is unclear as is the source of the high NT-3 levels measured in tissues not shown previously to have high levels of NT-3, suggesting the source of the NT-3 may be external to the structure. Acid treatment has been shown to increase NT-3 detection, but this should be uniform and not change the relative levels from region to region. Neutralization can also cause the reformation of NGF protein complexes. The samples in Miller and Mooney (2004) and Miller (2004) were fractionated by centrifugation, which may have concentrated specific pools of NT-3, and acid treated prior to assay; the samples in the Heaton studies were also acid treated. Parks and colleagues report higher levels than previously observed, but ratios consistent with the classical studies and those using in acid treated/neutralized samples in normal rodents.

2.3.3 NT-3 and Ethanol: In Vitro Models

The role of NT-3 in development is similarly time- and population-selective, and the effect of NT-3 on ethanol toxicity is similarly selective. Seabold and colleagues (1998) did not observe a protective effect of NT-3 on differentiated cortical neurons treated in vitro with a high (400 mg/dL) concentration of ethanol. Earlier in development, exposure of embryo cultures to ethanol for 3–10 h in vitro reduced proliferation of cephalic neural crest cells that was prevented by the addition of NT-3 to the medium (Jaurena et al. 2011). These studies are consistent with the limited cortical responsiveness to NT-3 and the early developmental peak in expression (see Sect. 2.3). Recent studies indicate that NT-3 may have a larger role in cerebellar development than previously appreciated. Cerebellar foliation is impaired when the insulin or IGF-1 system is perturbed in the cerebellum by siRNA; NT-3 levels are also reduced (de la Monte et al. 2011). This treatment reduces cerebellar foliation, suggesting role for NT-3 in this process, and developmental ethanol exposure similarly impairs cerebellar foliation (Cragg and Phillips 1985; Sakata-Haga et al. 2001).

3 Nonclassical Neurotrophic Factors: IGF-1, GDNF, and PACAP

3.1 The Insulin Family: IGF and ALK

IGF-1 is known to be involved in ethanol toxicity both centrally and peripherally. IGF-1 is produced in the liver in response to growth hormone secretion from the pituitary and thereby links IGF-1 levels to ethanol-induced growth restriction. Circulating IGF-1 levels in adults may also be involved in ethanol-induced vascular damage due to a lack of IGF-1-mediated repair, either directly by inhibiting signaling or secondary to hepatotoxicity, thereby compromising the blood–brain barrier. IGF-1 is bound in vivo to IGF Binding Proteins (IGFBPs) that further regulate bioavailability. Acute ethanol exposure results in a dramatic increase in IGFBP-1 and a decline in IGF-1 in healthy adults, while chronic ethanol exposure decreases IGF-1 bioavailability and growth hormone levels in patients (Passilta et al. 1999; Rojdmark et al. 2000; Rojdmark and Brismar 2001). After 4 days of withdrawal, IGF levels increase and IGFBP-1 levels decrease (Passilta et al. 1998). IGF-I and IGF-II are both upregulated in children exposed prenatally to ethanol, indicative of compensatory induction and a potential biomarker for FAS/ARND (Aros et al. 2011). The significance of alterations in peripheral IGF-1 to brain pathology is uncertain, but IGF-1 crosses the blood–brain barrier (Pan and Kastin 2000) and transport is activity dependent (Nishijima et al. 2010). Moreover, reduced IGF-1 levels are associated with depressive-like behavior (Mitschelen et al. 2011). Circulating IGF-1 levels are sensitive to hepatic function, thereby providing a link between hepatotoxicity and neuronal toxicity and repair processes. The GH-IGF-1 axis, neural activity, vascular integrity, and hepatic function are intimately linked, particularly during development.

IGF-1 is active in the early stages of neurogenesis, regulates stem cell proliferation, and enhances survival after ethanol exposure (Tateno et al. 2004). Ethanol blocks IGF-1-stimulated proliferation in vitro (Resnicoff et al. 1996) and ERK activation (Hallak et al. 2001) but, while trophic, does not significantly protect cerebellar granule cells from ethanol toxicity (Bonthius et al. 2003). Interestingly, in vivo siRNA-mediated reduction in insulin or IGF-1 receptor signaling produces cerebellar malformations that resemble fetal alcohol syndrome (de la Monte et al. 2011). McClure and colleagues (2011) used *Drosophila* to model fetal alcohol syndrome and implicate *Drosophila* insulin-like peptide and reduced insulin receptor expression in the teratogenic effects of ethanol. Another related molecule, ALK, was also recently suggested to be ethanol-sensitive in fly and mouse experiments (Lasek et al. 2011a, b). ALK is proapoptotic in the absence of ligand, but signals survival in presence of ligand (Yanagisawa et al. 2010). ALK promotes sensitization and cocaine reward (Lasek et al. 2011a) in flies and is involved in ethanol tolerance and reward in mice (Lasek et al. 2011a, b). ALK knockout mice show enhanced loss of righting reflex in response to high doses of ethanol, and this is not explained by reduced ethanol metabolism. ALK expression in striatum is negatively correlated with ethanol sensitivity. While knockout mice were more sensitive to the sedating effects, they were less sensitive to ataxia. These data implicate multiple intermediates in the IGF pathway as potential modulators of ethanol sensitivity and toxicity.

3.2 GDNF

GDNF is also a target derived factor that was originally described as a trophic factor for dopaminergic, sensory, and motor neurons (reviewed by Pascual 2011). GDNF is a member of a larger family that includes Artemin, Neurturin, and Persephin. While trophic for dopaminergic neurons, genetic ablation of GDNF does not affect genesis or survival of dopaminergic neurons (Sanchez et al. 1996; Jain et al. 2006), but Ret deletion cause late degeneration of the substantia nigra pars compacta (Kramer et al. 2007). GDNF is also produced in peripheral tissues, with high levels detected in kidney, ovary, testes, stomach, skin, and lung (Trupp et al. 1995). Like neurotrophins, GDNF signals through tyrosine kinase coupled pathway. GFR-alpha 1 binds GDNF and recruits the Ret tyrosine kinase; neurturin preferentially activates alpha 2, and artemin preferentially activates alpha 3 (reviewed by Mason 2000). Ret activation leads to ERK, Akt, phospholipase C, and Src activation to promote survival and differentiation. GDNF is also a potent regulator of anti-apoptosis pathways and NFkB. As with classical neurotrophins, alternative signaling pathways also exist for GDNF. GDNF can signal through NCAM to activate Fyn and focal adhesion kinase which mediate neurite outgrowth and survival (Sariola and Saarma 2003). Homophilic transsynaptic binding of GFR-alpha has also been described during hippocampal synaptogenesis (Ledda 2007).

GDNF is expressed at high levels in the striatal fast spiking interneurons, olfactory bulb, ventral mesencephalon, and septum with lower expression in the cerebellum and colliculi (see Fig. 1 and Hidalgo-Figueroa et al. 2012;

Pochon et al. 1997; Golden et al. 1999 for detailed anatomy). Receptors for GDNF are more widely expressed with Ret mRNA detected at high levels in diencephalic structures, cerebellum, brain stem, and olfactory bulb. Ret expression is low in the hippocampus, cortex, and striatum. GFR-alpha 1 is expressed at high levels in the septum, hippocampus, and colliculi, but at lower levels in the olfactory bulb, diencephalon, and brainstem. GFR-alpha 2 is high in cortex, hypothalamus, and colliculi, with low expression in the cerebellum and brainstem. GFR-alpha 3 expression is limited to the developing CNS and cerebellum (Masure et al. 1998) but is expressed in peripheral tissues such as heart, lung, and trigeminal ganglia. Interestingly, the striatal FSIs, not medium spiny neurons, produce GDNF while the receptors are produced by, and GDNF is accumulated in, mesencephalic dopamine neurons (Hidalgo-Figueroa et al. 2012; Barroso-Chinea et al. 2005). As with other neurotrophins, the differential distribution of GDNF and its receptors is consistent with target-derived factors and the consumer-producer model of neurotrophin action.

As with BDNF, GDNF was also identified for having the ability to reduce alcohol consumption in rodents (Carnicella et al. 2008, 2009a, b). The locus for this effect is the VTA since infusion of GDNF shRNA into the VTA blocks the effect (Barak et al. 2011) so it does not appear to involve fast spiking interneuron in the striatum, which also produce GDNF. Examination of the effects of ethanol on GDNF-mediated survival is limited. Fiore and colleagues (2009b) observed a decrease in cortical GDNF after a diet of chronic red wine (but not ethanol). Hippocampal GDNF does not appear sensitive to ethanol in mature animals (Okamoto et al. 2006).

Developmental studies in vivo indicate that GDNF protects developing chick spinal motor neurons from ethanol, but ethanol exposure did not reduce muscle GDNF levels in rats (Bradley et al. 1999; Barrow Heaton et al. 1999). However, Wentzel and Ericksson (2009) found a decrease in GDNF in trunk, but not cranial, neural crest–derived cells from the E10 rat embryo. Interestingly, this paper also reported a decrease in GAPDH mRNA in cranial neural crest cells exposed to ethanol. McAlhany and colleagues (1999) also found a reduction in GDNF protein, but not mRNA, with developmental ethanol exposure but a paradoxical increase in GDNF signaling to Shc. Neonatal brain growth restriction in response to high dose (5 g/kg gavage) ethanol during the postnatal brain growth spurt (PN6-8) is similarly correlated with a transient reduction in GDNF (Tsuji et al. 2008). (Exposure on PN 2–4 or PN 13–15 also reduced BDNF levels, but this did not correlate with microcephaly, as discussed above.) Interestingly, the ERK pathway was similarly sensitive regardless of age but inhibition of the Akt pathway was only observed with PN6-8 exposure, correlating with the temporal decrease in GDNF in these experiments.

In vitro, GDNF reverses Purkinje cell ethanol toxicity in cerebellar explants (McAlhany et al. 1997, 1999) and ethanol blocks GDNF-stimulated process outgrowth in granule cells (Chen and Charness 2012). GDNF reversed ethanol-induced JNK activation in SK-N-SH neuroblastoma cells and associated cell death (McAlhany et al. 2000). Interestingly, these cells underwent DNA fragmentation at 34 and 68 mM ethanol but displayed signs of necrosis at concentrations above 100 mM. GDNF can also protect glial-type transformed cells from ethanol toxicity

in vitro (Villegas et al. 2006). In contrast, Mitchell and colleagues (1999) found no neuroprotective effects of GDNF against cell death in hippocampal cultures, induced by high concentrations (>100 mM, 0.4–1.6 g/dl) of ethanol combined with hypoglycemia and hypoxia.

3.3 PACAP

PACAP is a member of the vasoactive intestinal peptide/glucagon/growth hormone releasing hormone superfamily and is active in stress, anxiety, consummatory behavior, metabolism, and thermoregulation (Adams et al. 2008). PACAP receptors exists as splice variants that stimulate adenylate cyclase and phosphoinositol hydrolysis with different kinetics (Spengler et al. 1993). PACAP also signals through Ca^{++}, PCK; Src family kinases, and by transactivation of Trks through the PAC1 receptor, with Gq-coupled pathways predominating in the hippocampus (Macdonald et al. 2005; Rajagopal et al. 2004; Shi et al. 2010). PACAP may also regulate BDNF levels through receptor-coupled activation of RACK1 through cAMP (Yaka et al. 2003a) placing BDNF downstream of PACAP. PACAP-deficient mice display hyperlocomotion, decreased body mass, novelty-seeking behavior, jumping, impaired hippocampal associative learning, and mossy fiber LTP. PACAP in the arcuate nucleus of the hypothalamus stimulates NPY neurons to regulate carbohydrate intake (Nakata et al. 2004) but inhibits food intake via the POMC arcuate neurons (Mounien et al. 2009). Peripherally, PACAP is coupled to catecholamine secretion from adrenal chromaffin, providing a peripheral link to the stress response (Kuri et al. 2009).

The initial evidence for involvement of PACAP in ethanol sensitivity came from *Drosophila* where an allele of the *Drosophila* PACAP homologue amnesiac called cheapdate was identified as a gene regulating ethanol sensitivity (Moore et al. 1998). Rutabaga, another cAMP pathway mutant, is required in mushroom bodies for ethanol consumption in *Drosophila* (Xu et al. 2012). As with flies, PACAP knockout mice are less sensitive to the hypothermic and hypnotic effects of ethanol (Tanaka et al. 2004). Cerebellar granule cells from PACAP knockout mice are more sensitive to ethanol and peroxide toxicity, while addition of PACAP or dibutryl cyclic AMP reversed this effect (Vaudry et al. 2005). PACAP administered to neonatal rats mitigated the ethanol-induced loss of righting and apoptosis, possibly through caspase 3 and cJun inhibition (Botia et al. 2011). As with BDNF, PACAP also protected cerebellar granule cells from ethanol toxicity by the activation of PI3K (Bhave and Hoffman 2004).

3.4 Nonconventional Neurotrophic Factors in Patients with Alcohol Dependence

To date only one study has examined peripheral GDNF levels in alcohol-dependent patients. Heberlein and colleagues (2010) found GDNF levels significantly

depressed in the serum of alcohol-dependent patients which remain decreased for up to 2 weeks after withdrawal. Interestingly, serum levels were also inversely related to subjective assessment of tolerance. Those with high serum GDNF levels reported lower tolerance and may therefore be more likely to limit their alcohol intake. This is consistent with observations in rodents, suggesting that GDNF negatively regulates ethanol consumption (Carnicella et al. 2009a, b).

4 Neurotrophic Therapies in Alcoholism, FAS, and ARND

Neurotrophins-based therapies for both addiction and repair of ethanol-induced brain damage may be on the horizon. Small interfering RNA and other expression-based strategies have been used experimentally to dissect the pathways involved in ethanol self-administration in rodents, but this strategy will not be immediately useful for treating patients. Direct activation of Trks with ICV neurotrophin infusion has been used experimentally. For example, neurotrophin infusion can maintain ethanol tolerance (Szabò and Hoffman 1995) and infusion of NGF can restore cholinergic function in the mature brain after chronic ethanol exposure in rodents (Cadete-Leite et al. 2003; Lukoyanov et al. 2003). Unfortunately, ICV infusion of NGF is also not feasible for human treatment, but intranasal delivery of IGF-1 is being investigated for ameliorating ischemia and hypoxia-induced damage in developing mice (Lin et al. 2009, 2011) and in adult stroke models (Kooijman et al. 2009). Intranasal IGF-1 also protects Purkinje cells in spinocerebellar ataxia type 1 and improves roto-rod function (Vig et al. 2006). Given the trophic role of IGF-1 in the CNS, IGF-1 might also be useful in FAS/ARND and it readily crosses the blood–brain barrier. While not protective against the insult, IGF-1 may enhance recovery in the child.

Neurotrophic peptides and peptidomimetics are also being developed, which may reverse ethanol-induced neurodegeneration and withdrawal-associated anxiety. Cyclotraxin-B was recently isolated from a peptidomimetic screen as a TrkB allosteric antagonist that reduces anxiety-like behavior (Cazorla et al. 2010, 2011). Such molecules may reduce anxiety-associated drinking and withdrawal hyperexcitability but may also pose a toxicity risk. Conversely, mimetic and small molecule agonists of TrkA and TrkB reverse cell death and enhance recovery in experimental models (Scarpi et al. 2012; Massa et al. 2010; Jang et al. 2010) and may therefore be useful for treating ethanol-induced neurodegeneration and cognitive dysfunction. Activity-Dependent Neurotrophic Factor and related peptides enhance survival in moderate (Zhou et al. 2008) and extreme (Incerti et al. 2010) fetal alcohol models. Interestingly, the ADNF peptides SAL and NAP reversed the deficits observed after a single exposure on day 8 of gestation to a dose of ethanol that produces BACs in excess of 500 mg/dL. This protective effect was attributed to normalization of ethanol-induced upregulation of BDNF at E9 and E18. Thus, NAP and SAL can influence events prior to the onset of CNS neurogenesis and normalize BDNF expression without off target teratogenesis; however, recent studies have excluded a direct role for BDNF in NAP effects on axon formation

(Chen and Charness 2012). The efficacy of NAP and related peptides may therefore be related to cytoskeletal interactions (Gozes 2011), or inhibition of apoptosis (Zemlyak et al. 2009) and not a direct interaction with BDNF signaling.

Small molecule pharmacological strategies that indirectly modulate neurotrophin levels have also been proposed and tested in rodent models. Antidepressants increase BDNF in the hippocampus and can reverse ethanol-induced reductions in BDNF in the hippocampus (Hauser et al. 2011), which may increase recovery after long-term ethanol exposure. The anti-addiction compounds Ibogaine and noribogaine may also interact with GDNF to reduce ethanol drinking (He et al. 2005; Carnicella et al. 2010). Neurotrophin-based therapeutics need not involve administration of a drug, however. Exercise in mature animals can induce hippocampal neurogenesis and increase BDNF levels, which ameliorates many of the effects of prenatal ethanol exposure (Boehme et al. 2011). Antioxidants provide neuroprotection in fetal alcohol models, in part through modulation of neurotrophins (reviewed by Brocardo et al. 2011). Dietary supplementation with omega-3 fatty acids also reverses behavioral deficits in fetal alcohol models and increases BDNF, possibly through antioxidant modulation (Patten et al. 2012). Similarly, choline supplementation modulates neurotrophins and choline is being examined in preclinical models as a treatment for alcohol-related birth defects (Ryan et al. 2008; Monk et al. 2012). These interventions, while subtle, may increase neurogenesis and plasticity, ultimately involving regulation of neurotrophins. The strategies vary but all ultimately increase neurotrophic activity to influence survival and plasticity.

5 Conclusion

Alterations in neurotrophins and neurotrophin signaling mediate the acute toxic effects, plasticity that occurs with pharmacodynamic tolerance, plasticity that occurs with addiction, and repair that occurs after withdrawal. The temporal and cell specific activation and inhibition of neurotrophins and their associated pathways have not been conclusively determined, and the data on ethanol and neurotrophic factors summarized in this chapter clearly do not outline a cohesive story. Neurotrophin levels can increase, decrease, or not change depending on the brain region, time of exposure, and presence or absence of drug upon analysis. Neurotrophin signaling is dependent upon concentration, with full-length receptors desensitizing, high concentrations activating p75, and related receptors being nonspecifically activated at higher concentrations. In addition, neurotrophin levels and signaling to not occur in isolation; therefore, it is important to consider the physiological changes that result from neurotrophin action. For example, an increase in glutamatergic synaptic activity during withdrawal would be expected to increase BDNF but the place of BDNF in this sequence is not well defined in most studies. Does BDNF drive these changes or, more likely, does BDNF increase as a result of excess NMDA receptor activation? The sequence is different early in exposure and with low ethanol concentrations where ethanol can reduce signaling

in BDNF independent of effects on ion channels (Kolb et al. 2005; Ohrtman et al. 2006). In this case, neurotrophin signaling may be directly involved in the regulation of subsequent events and not vice versa. When concentrations of ethanol become too high, none of these adaptive responses are likely to occur, however, as is observed with suprapharmacological concentrations. More subtle effects are likely to contribute to ethanol-induced teratogenesis and chronic toxicity.

The expression data for receptors and neurotrophin levels measured after ethanol exposure also frequently do not agree. It is possible that ethanol induces ectopic expression in specific cell types, but this has not been specifically addressed since the control cells in these regions also frequently show expression of neurotrophins they are not believed to express. This may be partially due to the fixation procedures used to detect immunoreactivity in situ since neurotrophins are difficult to detect with immunohistochemistry. In situ hybridization and protein detection with and without colchicine treatment and global mapping may also help resolve some of these discrepancies and locate the "producer" neurons where neurotrophins are sensitive to ethanol treatment. The availability of genetically tagged mice will also aid in dissecting these issues anatomically, functionally, and allow for separation of specific populations by cell sorting. In addition, Cre-recombinase-mediated deletion in specific cell types, for example, BDNF deletion in cortex versus medium spiny neurons, could be combined with self-administration behavioral experiments to define the loci for specific effects. The pharmacological tools described in the previous section will aid in dissection of the actions of neurotrophins in ethanol toxicity, tolerance, addiction, withdrawal, and the development of FAS/ARND.

References

Acquaah-Mensah, G. K., Leslie, S. W., & Kehrer, J. P. (2001). Acute exposure of cerebellar granule neurons to ethanol suppresses stress-activated protein kinase-1 and concomitantly induces AP-1. *Toxicology and Applied Pharmacology, 175*, 10–18.

Adams, B. A., Gray, S. L., Isaac, E. R., Bianco, A. C., Vidal-Puig, A. J., & Sherwood, N. M. (2008). Feeding and metabolism in mice lacking pituitary adenylate cyclase-activating polypeptide. *Endocrinology, 149*(4), 1571–1580.

Aid, T., Kazantseva, A., Piirsoo, M., Palm, K., & Timmusk, T. (2007). Mouse and rat BDNF gene structure and expression revisited. *Journal of Neuroscience Research, 85*, 525–535.

Akbarian, S., Rios, M., Liu, R. J., Gold, S. J., Fong, H. F., Zeiler, S., et al. (2002). Brain-derived neurotrophic factor is essential for opiate-induced plasticity of noradrenergic neurons. *The Journal of Neuroscience, 22*, 4153–4162.

Aloe, L., Bracci-Laudiero, L., & Tirassa, P. (1993). The effect of chronic ethanol intake on brain NGF level and on NGF-target tissues of adult mice. *Drug and Alcohol Dependence, 31*(2), 159–167.

Aloe, L., Tuveri, M. A., Guerra, G., Pinna, L., Tirassa, P., Micera, A., & Alleva, E. (1996). Changes in human plasma nerve growth factor level after chronic alcohol consumption and withdrawal. *Alcohol Clinical and Experimental Research, 20*(3), 462–465.

Altar, C. A., Burton, L. E., Bennett, G. L., & Dugich-Djordjevic, M. (1991). Recombinant human nerve growth factor is biologically active and labels novel high-affinity binding sites in rat brain. *Proceedings of the National Academy of Sciences of the United States of America, 88*(1), 281–285.

Altar, C. A., Boylan, C. B., Jackson, C., Hershenson, S., Miller, J., Wiegand, S. J., et al. (1992). Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover in vivo. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 11347–11351.

Altar, C. A., Criden, M. R., Lindsay, R. M., & DiStefano, P. S. (1993). Characterization and topography of high-affinity ^{125}I-neurotrophin-3 binding to mammalian brain. *The Journal of Neuroscience, 13*(2), 733–743.

Altar, C. A., Siuciak, J. A., Wright, P., Ip, N. Y., Lindsay, R. M., & Wiegand, S. J. (1994). In situ hybridization of trkB and trkC receptor mRNA in rat forebrain and association with high-affinity binding of [^{125}I]BDNF, [^{125}I]NT-4/5 and [^{125}I]NT-3. *The European Journal of Neuroscience, 6*, 1389–1405.

Altar, C. A., Cai, N., Bliven, T., Juhasz, M., Conner, J. M., Acheson, A. L., et al. (1997). Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature, 389*, 856–860.

Anderson, K. D., Alderson, R. F., Altar, C. A., DiStefano, P. S., Corcoran, T. L., Lindsay, R. M., & Wiegand, S. J. (1995). Differential distribution of exogenous BDNF, NGF, and NT-3 in the brain corresponds to the relative abundance and distribution of high-affinity and low-affinity neurotrophin receptors. *The Journal of Comparative Neurology, 357*(2), 296–317.

Angelucci, F., Cimino, M., Balduini, W., Piltillo, L., & Aloe, L. (1997). Prenatal exposure to ethanol causes differential effects in nerve growth factor and its receptor in the basal forebrain of preweaning and adult rats. *Journal of Neural Transplantation & Plasticity, 6*(2), 63–71.

Angelucci, F., Fiore, M., Cozzari, C., & Aloe, L. (1999). Prenatal ethanol effects on NGF level, NPY and ChAT immunoreactivity in mouse entorhinal cortex: A preliminary study. *Neurotoxicology and Teratology, 21*(4), 415–425.

Arendt, T., Henning, D., Gray, J. A., & Marchbanks, R. (1988). Loss of neurons in the rat basal forebrain cholinergic projection system after prolonged intake of ethanol. *Brain Research Bulletin, 21*(4), 563–569.

Aros, S., Mills, J. L., Iñiguez, G., Avila, A., Conley, M. R., Troendle, J., Cox, C., & Cassorla, F. (2011). Effects of prenatal ethanol exposure on postnatal growth and the insulin-like growth factor axis. *Hormone Research in Pædiatrics, 75*(3), 166–173.

Ascano, M., Bodmer, D., & Kuruvilla, R. (2012). Endocytic trafficking of neurotrophins in neural development. *Trends in Cell Biology, 22*(5), 266–273.

Autry, A. E., & Monteggia, L. M. (2012). Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacological Reviews, 64*(2), 238–258.

Baek, J. K., Heaton, M. B., & Walker, D. W. (1996). Up-regulation of high-affinity neurotrophin receptor, trk B-like protein on western blots of rat cortex after chronic ethanol treatment. *Brain Research. Molecular Brain Research, 40*, 161–164.

Baquet, Z. C., Gorski, J. A., & Jones, K. R. (2004). Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. *The Journal of Neuroscience, 24*, 4250–4258.

Barak, S., Ahmadiantehrani, S., Kharazia, V., & Ron, D. (2011). Positive autoregulation of GDNF levels in the ventral tegmental area mediates long-lasting inhibition of excessive alcohol consumption. *Translational Psychiatry, 1*, pii, e60.

Barker-Gibb, A. L., Dougherty, K. D., Einheber, S., Drake, C. T., & Milner, T. A. (2001). Hippocampal tyrosine kinase A receptors are restricted primarily to presynaptic vesicle clusters. *The Journal of Comparative Neurology, 430*(2), 182–199.

Barroso-Chinea, P., Cruz-Muros, I., Aymerich, M. S., Rodriguez-Diaz, M., Afonso-Oramas, D., Lanciego, J. L., & Gonzalez-Hernandez, T. (2005). Striatal expression of GDNF and differential vulnerability of midbrain dopaminergic cells. *The European Journal of Neuroscience, 21*(7), 1815–1827.

Barrow Heaton, M. B., Kidd, K., Bradley, D., Paiva, M., Mitchell, J., & Walker, D. W. (1999). Prenatal ethanol exposure reduces spinal cord motoneuron number in the fetal rat but does not affect GDNF target tissue protein. *Developmental Neuroscience, 21*(6), 444–452.

Bartkowska, K., Turlejski, K., & Djavadian, R. L. (2010). Neurotrophins and their receptors in early development of the mammalian nervous system. *Acta Neurobiologiae Experimentalis (Wars), 70*(4), 454–467.

Bates, B., Rios, M., Trumpp, A., Chen, C., Fan, G., Bishop, J. M., & Jaenisch, R. (1999). Neurotrophin-3 is required for proper cerebellar development. *Nature Neuroscience, 2*(2), 115–117.

Baydyuk, M., Nguyen, M. T., & Xu, B. (2011). Chronic deprivation of TrkB signaling leads to selective late-onset nigrostriatal dopaminergic degeneration. *Experimental Neurology, 228*(1), 118–125.

Bearer, C. F., Swick, A. R., O'Riordan, M. A., & Cheng, G. (1999). Ethanol inhibits L1-mediated neurite outgrowth in postnatal rat cerebellar granule cells. *Journal of Biological Chemistry, 274*(19), 13264–13270.

Becchetti, A., & Arcangeli, A. (2010). Integrins and ion channels in cell migration: Implications for neuronal development, wound healing and metastatic spread. *Advances in Experimental Medicine and Biology, 674*, 107–123.

Berry, A., Bindocci, E., & Alleva, E. (2012). NGF, brain and behavioral plasticity. *Neural Plasticity, 2012*, 784040.

Bhandari, P., Kendler, K. S., Bettinger, J. C., Davies, A. G., & Grotewiel, M. (2009). An assay for evoked locomotor behavior in *Drosophila* reveals a role for integrins in ethanol sensitivity and rapid ethanol tolerance. *Alcoholism, Clinical and Experimental Research, 33*(10), 1794–1805.

Bhattacharyya, A., Watson, F. L., Bradlee, T. A., Pomeroy, S. L., Stiles, C. D., & Segal, R. A. (1997). Trk receptors function as rapid retrograde signal carriers in the adult nervous system. *The Journal of Neuroscience, 17*(18), 7007–7016.

Bhave, S. V., & Hoffman, P. L. (2004). Phosphatidylinositol 3′-OH kinase and protein kinase A pathways mediate the anti-apoptotic effect of pituitary adenylyl cyclase-activating polypeptide in cultured cerebellar granule neurons: Modulation by ethanol. *Journal of Neurochemistry, 88*(2), 359–369.

Bhave, S. V., Snell, L. D., Tabakoff, B., & Hoffman, P. L. (1999). Ethanol sensitivity of NMDA receptor function in developing cerebellar granule neurons. *European Journal of Pharmacology, 369*, 247–259.

Bjorkblom, B., Padzik, A., Mohammad, H., Westerlund, N., Komulainen, E., Hollos, P., Parviainen, L., Papageorgiou, A. C., Iljin, K., Kallioniemi, O., Kallajoki, M., Courtney, M. J., Magard, M., James, P., & Coffey, E. T. (2012). c-Jun N-terminal kinase phosphorylation of MARCKSL1 determines actin stability and migration in neurons and in cancer cells. *Molecular and Cellular Biology, 32*(17), 3513–3526.

Boehme, F., Gil-Mohapel, J., Cox, A., Patten, A., Giles, E., Brocardo, P. S., & Christie, B. R. (2011). Voluntary exercise induces adult hippocampal neurogenesis and BDNF expression in a rodent model of fetal alcohol spectrum disorders. *The European Journal of Neuroscience, 33*(10), 1799–1811.

Bonthius, D. J., Karacay, B., Dai, D., & Pantazis, N. J. (2003). FGF-2, NGF and IGF-1, but not BDNF, utilize a nitric oxide pathway to signal neurotrophic and neuroprotective effects against alcohol toxicity in cerebellar granule cell cultures. *Brain Research. Developmental Brain Research, 140*, 15–28.

Bosse, K. E., & Mathews, T. A. (2011). Ethanol-induced increases in extracellular dopamine are blunted in brain-derived neurotrophic factor heterozygous mice. *Neuroscience Letters, 489*(3), 172–176.

Botia, B., Jolivel, V., Burel, D., Le Joncour, V., Roy, V., Naassila, M., Banard, M., Fournier, A., Vaudry, H., & Vaudry, D. (2011). Neuroprotective effects of PACAP against ethanol-induced toxicity in the developing rat cerebellum. *Neurotoxicity Research, 19*(3), 423–434.

Bradley, D. M., Beaman, F. D., Moore, D. B., Kidd, K., & Heaton, M. B. (1999). Neurotrophic factors BDNF and GDNF protect embryonic chick spinal cord motoneurons from ethanol neurotoxicity in vivo. *Brain Research. Developmental Brain Research, 112*(1), 99–106.

Braskie, M. N., Jahanshad, N., Stein, J. L., Barysheva, M., Johnson, K., McMahon, K. L., de Zubicaray, G. I., Martin, N. G., Wright, M. J., Ringman, J. M., Toga, A. W., & Thompson, P. M. (2012). Relationship of a variant in the NTRK1 gene to white matter microstructure in young adults. *The Journal of Neuroscience, 32*(17), 5964–5972.

Brocardo, P. S., Gil-Mohapel, J., & Christie, B. R. (2011). The role of oxidative stress in fetal alcohol spectrum disorders. *Brain Research Reviews, 67*(1–2), 209–225.

Brodie, C., & Vernadakis, A. (1992). Ethanol increases cholinergic and decreases GABAergic neuronal expression in cultures derived from 8-day-old chick embryo cerebral hemispheres: Interaction of ethanol and growth factors. *Brain Research. Developmental Brain Research, 65*(2), 253–257.

Brodie, C., Kentroti, S., & Vernadakis, A. (1991). Growth factors attenuate the cholinotoxic effects of ethanol during early neuroembryogenesis in the chick embryo. *International Journal of Developmental Neuroscience, 9*(3), 203–213.

Bruns, M. B., & Miller, M. W. (2007). Neurotrophin ligand-receptor systems in somatosensory cortex of adult rat are affected by repeated episodes of ethanol. *Experimental Neurology, 204*, 680–692.

Cadete-Leite, A., Pereira, P. A., Madeira, M. D., & Paula-Barbosa, M. M. (2003). Nerve growth factor prevents cell death and induces hypertrophy of basal forebrain cholinergic neurons in rats withdrawn from prolonged ethanol intake. *Neuroscience, 119*(4), 1055–1069.

Caldwell, K. K., Sheema, S., Paz, R. D., Samudio-Ruiz, S. L., Laughlin, M. H., Spence, N. E., Roehlk, M. J., Alcon, S. N., & Allan, A. M. (2008). Fetal alcohol spectrum disorder-associated depression: Evidence for reductions in the levels of brain-derived neurotrophic factor in a mouse model. *Pharmacology, Biochemistry, and Behavior, 90*(4), 614–624.

Carnicella, S., Kharazia, V., Jeanblanc, J., Janak, P. H., & Ron, D. (2008). GDNF is a fast-acting potent inhibitor of alcohol consumption and relapse. *Proceedings of the National Academy of Sciences of the United States of America, 105*(23), 8114–8119.

Carnicella, S., Ahmadiantehrani, S., Janak, P. H., & Ron, D. (2009a). GDNF is an endogenous negative regulator of ethanol-mediated reward and of ethanol consumption after a period of abstinence. *Alcoholism, Clinical and Experimental Research, 33*(6), 1012–1024.

Carnicella, S., Amamoto, R., & Ron, D. (2009b). Excessive alcohol consumption is blocked by glial cell line-derived neurotrophic factor. *Alcohol, 43*(1), 35–43.

Carnicella, S., He, D. Y., Yowell, Q. V., Glick, S. D., & Ron, D. (2010). Noribogaine, but not 18-MC, exhibits similar actions as ibogaine on GDNF expression and ethanolself-administration. *Addiction Biology, 15*(4), 424–433.

Castellani, V., & Bolz, J. (1999). Opposing roles for neurotrophin-3 in targeting and collateral formation of distinct sets of developing cortical neurons. *Development, 126*(15), 3335–3345.

Castren, E. (2004). Neurotrophins as mediators of drug effects on mood, addiction, and neuroprotection. *Molecular Neurobiology, 29*(3), 289–302.

Cazorla, M., Jouvenceau, A., Rose, C., Guilloux, J. P., Pilon, C., Dranovsky, A., & Premont, J. (2010). Cyclotraxin-B, the first highly potent and selective TrkB inhibitor, has anxiolytic properties in mice. *PLoS One, 5*(3), e9777.

Cazorla, M., Premont, J., Mann, A., Girard, N., Kellendonk, C., & Rognan, D. (2011). Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice. *The Journal of Clinical Investigation, 121*(5), 1846–1857.

Ceccatelli, S., Ernfors, P., Villar, M. J., Persson, H., & Hockfelt, T. (1991). Expanded distribution of mRNA for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 in the rat brain after colchicine treatment. *Proceedings of the National Academy of Sciences of the United States of America, 88*(22), 10352–10356.

Chandler, L. J., & Sutton, G. (2005). Acute ethanol inhibits extracellular signal-regulated kinase, protein kinase B, and adenosine 3′:5′-cyclic monophosphate response element binding protein activity in an age- and brain region-specific manner. *Alcoholism, Clinical and Experimental Research, 29*, 672–682.

Chang, L., Jones, Y., Ellisman, M. H., Goldstein, L. S., & Karin, M. (2003). JNK1 is required for maintenance of neuronal microtubules and controls phosphorylation of microtubule-associated proteins. *Developmental Cell, 4*(4), 521–533.

Chen, S., & Charness, M. E. (2012). Ethanol disrupts axon outgrowth stimulated by netrin-1, GDNF, and L1 by blocking their convergent activation of Src family kinase signaling. *Journal of Neurochemistry, 123*(4), 602–612.

Chen, Z. Y., Patel, P. D., Sant, G., Meng, C. X., Teng, K. K., Hempstead, B. L., et al. (2004). Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *The Journal of Neuroscience, 24*, 4401–4411.

Chen, Z. Y., Jing, D., Bath, K. G., Ieraci, A., Khan, T., Siao, C. J., et al. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science, 314*, 140–143.

Chowdary, P. D., Che, D. L., & Cui, B. (2012). Neurotrophin signaling via long-distance axonal transport. *Annual Review of Physical Chemistry, 63*, 571–594.

Climent, E., Pascual, M., Renau-Piqueras, J., & Guerri, C. (2002). Ethanol exposure enhances cell death in the developing cerebral cortex: Role of brain-derived neurotrophic factor and its signaling pathways. *Journal of Neuroscience Research, 68*, 213–225.

Cohen-Cory, S., Dreyfus, C. F., & Black, I. B. (1989). Expression of high- and low-affinity nerve growth factor receptors by Purkinje cells in the developing rat cerebellum. *Experimental Neurology, 105*(1), 104–109.

Collazo, D., Takahashi, H., & McKay, R. D. (1992). Cellular targets and trophic functions of neurotrophin-3 in the developing rat hippocampus. *Neuron, 9*(4), 643–656.

Colzato, L. S., Van der Does, A. J., Kouwenhoven, C., Elzinga, B. M., & Hommel, B. (2011). BDNF Val66Met polymorphism is associated with higher anticipatory cortisol stress response, anxiety, and alcohol consumption in healthy adults. *Psychoneuroendocrinology, 36*(10), 1562–1569.

Conner, J. M. (2001). Localization of neurotrophin proteins within the central nervous system by immunohistochemistry. *Methods in Molecular Biology, 169*, 3–19.

Conner, J. M., & Varon, S. (1992). Distribution of nerve growth factor-like immunoreactive neurons in the adult rat brain following colchicine treatment. *The Journal of Comparative Neurology, 326*(3), 347–362.

Conner, J. M., & Varon, S. (1997). Developmental profile of NGF immunoreactivity in the rat brain: A possible role of NGF in the establishment of cholinergic terminal fields in the hippocampus and cortex. *Brain Research. Developmental Brain Research, 101*(1–2), 67–79.

Conner, J. M., Muir, D., Varon, S., Hagg, T., & Manthorpe, M. (1992). The localization of nerve growth factor-like immunoreactivity in the adult rat basal forebrain and hippocampal formation. *The Journal of Comparative Neurology, 319*(3), 454–462.

Conner, J. M., Lauterborn, J. C., Yan, Q., Gall, C. M., & Varon, S. (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: Evidence for anterograde axonal transport. *The Journal of Neuroscience, 17*, 2295–2313.

Conner, J. M., Franks, K. M., Titterness, A. K., Russell, K., Merrill, D. A., Christie, B. R., Sejnowski, T. J., & Tuszynski, M. H. (2009). NGF is essential for hippocampal plasticity and learning. *The Journal of Neuroscience, 29*(35), 10883–10889.

Costa, M. A., Girard, M., Dalmay, F., & Malauzat, D. (2011). Brain-derived neurotrophic factor serum levels in alcohol-dependent subjects 6 months after alcohol withdrawal. *Alcoholism, Clinical and Experimental Research, 35*(11), 1966–1973.

Courtney, M. J., Akerman, K. E., & Coffey, E. T. (1997). Neurotrophins protect cultured cerebellar granule neurons against the early phase of cell death by a two-component mechanism. *The Journal of Neuroscience, 17*(11), 4201–4211.

Camarillo, C., & Miranda R. C. (2008). Ethanol exposure during neurogenesis induces persistent effects on neural maturation: Evidence from an ex vivo model of fetal cerebral cortical neuroepithelial progenitor maturation. *Gene Expression, 14*(3), 159–171.

Charness, M. E., Safran, R. M., & Perides, G. (1994). Ethanol inhibits neural cell-cell adhesion. *Journal of Biological Chemistry, 269*(12), 9304–9309.

Chanarin, I. (1982). Haemopoiesis and alcohol. *British Medical Bulletin, 38*(1), 81–86.

Chen, L., Wang, Y., Xiao, H., Wang, L., Wang, C., Guo, S., Zhao, Y., Hua, P., Liu, W., & Zhang, N. (2011). The 712A/G polymorphism of brain-derived neurotrophic factor is associated with Parkinson's disease but not Major Depressive Disorder in a Chinese Han population. *Biochemical and Biophysical Research Communications, 408*(2), 318–321.

Cirulli, F., Reif, A., Herterich, S., Lesch, K. P., Berry, A., Francia, N., Aloe, L., Barr, C. S., Suomi, S. J., & Alleva, E. (2011). A novel BDNF polymorphism affects plasma protein levels in interaction with early adversity in rhesus macaques. *Psychoneuroendocrinology, 36*(3), 372–379.

Cragg, B., & Phillips, S. (1985). Natural loss of Purkinje cells during development and increased loss with alcohol. *Brain Research, 325*(1–2), 151–160.

Davis, M. I. (2008). Ethanol-BDNF interactions: Still more questions than answers. *Pharmacology and Therapeutics, 118*(1), 36–57.

Dohrman, D. P., West, J. R., & Pantazis, N. J. (1997). Ethanol reduces expression of the nerve growth factor receptor, but not nerve growth factor protein levels in the neonatal rat cerebellum. *Alcohol Clinical and Experimental Research, 21*(5), 882–893.

D'Sa, C., Dileone, R. J., Anderson, G. M., & Sinha, R. (2012). Serum and plasma brain-derived neurotrophic factor (BDNF) in abstinent alcoholics and social drinkers. *Alcohol, 46*(3), 253–259.

Das, K. P., Chao, S. L., White, L. D., Haines, W. T., Harry, G. J., Tilson, H. A., & Barone, S., Jr. (2001). Differential patterns of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 mRNA and protein levels in developing regions of rat brain. *Neuroscience, 103*(3), 739–761.

Davis, M. I., Szarowski, D., Turner, J. N., Morrisett, R. A., & Shain, W. (1999). In vivo activation and in situ BDNF-stimulated nuclear translocation of mitogen-activated/extracellular signal-regulated protein kinase is inhibited by ethanol in the developing rat hippocampus. *Neuroscience Letters, 272*, 95–98.

de la Monte, S. M., Tong, M., Bowling, N., & Moskal, P. (2011). si-RNA inhibition of brain insulin or insulin-like growth factor receptors causes developmental cerebellar abnormalities: Relevance to fetal alcohol spectrum disorder. *Molecular Brain, 4*, 13.

de la Monte, S., Derdak, Z., & Wands, J. R. (2012). Alcohol, insulin resistance and the liver-brain axis. *Journal of Gastroenterology and Hepatology, 27*(Suppl 2), 33–41.

de Sanctis, L., Memo, L., Pichini, S., Tarani, L., & Vagnarelli, F. (2011). Fetal alcohol syndrome: New perspectives for an ancient and underestimated problem. *The Journal of Maternal-Fetal & Neonatal Medicine, 24*(Suppl 1), 34–37.

De Simone, R., & Aloe, L. (1993). Influence of ethanol consumption on brain nerve growth factor and its target cells in developing and adult rodents. *Annali dell'Istituto Superiore di Sanità, 29*(1), 179–183.

Deinhardt, K., Kim, T., Spellman, D. S., Mains, R. E., Eipper, B. A., Neubert, T. A., Chao, M. V., & Hempstead, B. L. (2011). Neuronal growth cone retraction relies on proneurotrophin receptor signaling through Rac. *Science Signaling, 4*(202), ra82.

Devineni, A. V., McClure, K. D., Guarnieri, D. J., Corl, A. B., Wolf, F. W., Eddison, M., & Heberlein, U. (2011). The genetic relationships between ethanol preference, acute ethanol sensitivity, and ethanol tolerance in *Drosophila melanogaster*. *Fly (Austin), 5*(3), 191–199.

Di Maria, E., Giorgio, E., Uliana, V., Bonvicini, C., Faravelli, F., Cammarata, S., Novello, M. C., Galimberti, D., Scarpini, E., Zanetti, O., Gennarelli, M., & Tabaton, M. (2012). Possible influence of a non-synonymous polymorphism located in the NGF precursor on susceptibility to late-onset Alzheimer's disease and mild cognitive impairment. *Journal of Alzheimer's Disease, 29*(3), 699–705.

Dow, K. E., & Riopelle, R. J. (1985). Ethanol neurotoxicity: Effects on neurite formation and neurotrophic factor production in vitro. *Science, 228*(4699), 591–593.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., et al. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257–269.

Ehrlich, D., Pirchl, M., & Humpel, C. (2012). Ethanol transiently suppresses choline-acetyltransferase in basal nucleus of Meynert slices. *Brain Research, 1459*, 35–42.

Ericson, M., Haythornthwaite, A. R., Yeh, P. W., & Yeh, H. H. (2003). Brain-derived neurotrophic factor mitigates chronic ethanol-induced attenuation of gamma-aminobutyric acid responses in cultured cerebellar granule cells. *Journal of Neuroscience Research, 73*(5), 722–730.

Ernfors, P., Ibanez, C. F., Ebendal, T., Olson, L., & Persson, H. (1990a). Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: Developmental and topographical expression in the brain. *Proceedings of the National Academy of Sciences of the United States of America, 87*(14), 5454–5458.

Ernfors, P., Wetmore, C., Olson, L., & Persson, H. (1990b). Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. *Neuron, 5*(4), 511–526.

Ernfors, P., Lee, K. F., & Jaenisch, R. (1994a). Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature, 368*, 147–150.

Ernfors, P., Lee, K. F., Kucera, J., & Jaenisch, R. (1994b). Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. *Cell, 77*(4), 503–512.

Fagan, A. M., Garber, M., Barbacid, M., Silos-Santiago, I., & Holtzman, D. M. (1997). A role for TrkA during maturation of striatal and basal forebrain cholinergic neurons in vivo. *The Journal of Neuroscience, 17*(20), 7644–7654.

Fattori, V., Abe, S. I., Kobayashi, K., Costa, L. G., & Tsuji, R. (2008). Effects of postnatal ethanol exposure on neurotrophic factors and signal transduction pathways in rat brain. *Journal of Applied Toxicology, 28*, 370–376.

Fawcett, J. R., Chen, X., Rahman, Y. E., & Frey, W. H. (1999). Previously reported nerve growth factor levels are underestimated due to an incomplete release from receptors and interaction with standard curve media. *Brain Research, 842*(1), 206–210.

Feng, M. J., Yan, S. E., & Yan, Q. S. (2005). Effects of prenatal alcohol exposure on brain-derived neurotrophic factor and its receptor tyrosine kinase B in offspring. *Brain Research, 1042*, 125–132.

Fiore, M., Laviola, G., Aloe, L., di Fausto, V., Mancinelli, R., & Ceccanti, M. (2009a). Early exposure to ethanol but not red wine at the same alcohol concentration induces behavioral and brain neurotrophin alterations in young and adult mice. *Neurotoxicology, 30*(1), 59–71.

Fiore, M., Mancinelli, R., Aloe, L., Laviola, G., Sornelli, F., Vitali, M., & Ceccanti, M. (2009b). Hepatocyte growth factor, vascular endothelial growth factor, glial cell-derived neurotrophic factor and nerve growth factor are differentially affected by early chronic ethanol or red wine intake. *Toxicology Letters, 188*(3), 208–213.

Florez-McClure, M. L., Linseman, D. A., Chu, C. T., Barker, P. A., Bouchard, R. J., Le, S. S., Laessig, T. A., & Heidenreich, K. A. (2004). The p75 neurotrophin receptor can induce autophagy and death of cerebellar Purkinje neurons. *The Journal of Neuroscience, 24*(19), 4498–4509.

Fortress, A. M., Buhusi, M., Helke, K. L., & Granholm, A. C. (2011). Cholinergic degeneration and alterations in the TrkA and p75NTR balance as a result of Pro-NGF injection into aged rats. *Journal of Aging Research, 2011*, 460543.

Friedman, W. J. (2010). Proneurotrophins, seizures, and neuronal apoptosis. *The Neuroscientist, 16*(3), 244–252.

Friedman, W. J., Ernfors, P., & Persson, H. (1991). Transient and persistent expression of NT-3/HDNF mRNA in the rat brain during postnatal development. *The Journal of Neuroscience, 11*(6), 1577–1584.

Fenner, B. M. (2012). Truncated TrkB: Beyond a dominant negative receptor. *Cytokine Growth Factor Reviews, 23*(1–2), 15–24.

Gatt, J. M., Nemeroff, C. B., Dobson-Stone, C., Paul, R. H., Bryant, R. A., Schofield, P. R., Gordon, E., Kemp, A. H., & Williams, L. M. (2009). Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Molecular Psychiatry, 14*(7), 681–695.

Ge, Y., Belcher, S. M., & Light, K. E. (2004). Alterations of cerebellar mRNA specific for BDNF, p75NTR, and TrkB receptor isoforms occur within hours of ethanol administration to 4-day-old rat pups. *Brain Research. Developmental Brain Research, 151*, 99–109.

George, D. J., Suzuki, H., Bova, G. S., & Isaacs, J. T. (1998). Mutational analysis of the TrkA gene in prostate cancer. *Prostate, 36*(3), 172–180.

Gericke, C. A., Schulte-Herbrüggen, O., Arendt, T., & Hellweg, R. (2006). Chronic alcohol intoxication in rats leads to a strong but transient increase in NGF levels in distinct brain regions. *Journal of Neural Transmission, 113*(7), 813–820.

Ghitza, U. E., Zhai, H., Wu, P., Airavaara, M., Shaham, Y., & Lu, L. (2010). Role of BDNF and GDNF in drug reward and relapse: A review. *Neuroscience and Biobehavioral Reviews, 35*(2), 157–171.

Gibbs, R. B., McCabe, J. T., Buck, C. R., Chao, M. V., & Pfaff, D. W. (1989). Expression of NGF receptor in the rat forebrain detected with in situ hybridization and immunohistochemistry. *Brain Research. Molecular Brain Research, 6*(4), 275–287.

Ginty, D. D., & Segal, R. A. (2002). Retrograde neurotrophin signaling: Trk-ing along the axon. *Current Opinion in Neurobiology, 12*, 268–274.

Golden, J. P., DeMaro, J. A., Osborne, P. A., Milbrandt, J., & Johnson, E. M., Jr. (1999). Expression of neurturin, GDNF, and GDNF family-receptor mRNA in the developing and mature mouse. *Experimental Neurology, 158*(2), 504–528.

Gorski, J. A., Balogh, S. A., Wehner, J. M., & Jones, K. R. (2003). Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. *Neuroscience, 121*, 341–354.

Gozes, I. (2011). Microtubules (tau) as an emerging therapeutic target: NAP (davunetide). *Current Pharmaceutical Design, 17*(31), 3413–3417.

Gray, J., Yeo, G. S., Cox, J. J., Morton, J., Adlam, A. L., Keogh, J. M., et al. (2006). Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. *Diabetes, 55*, 3366–3371.

Grzywacz, A., Samochowiec, A., Ciechanowicz, A., & Samochowiec, J. (2010). Family-based study of brain-derived neurotrophic factor (BDNF) gene polymorphism in alcohol dependence. *Pharmacological Reports, 62*(5), 938–941.

Gunn-Moore, F. J., & Tavare, J. M. (1998). Apoptosis of cerebellar granule cells induced by serum withdrawal, glutamate or beta-amyloid, is independent of Jun kinase or p38 mitogen activated protein kinase activation. *Neuroscience Letters, 250*(1), 53–56.

Hallak, H., Seiler, A. E., Green, J. S., Henderson, A., Ross, B. N., & Rubin, R. (2001). Inhibition of insulin-like growth factor-I signaling by ethanol in neuronal cells. *Alcoholism, Clinical and Experimental Research, 25*(7), 1058–1064.

Han, J. Y., Jeong, J. Y., Lee, Y. K., Roh, G. S., Kim, H. J., Kang, S. S., et al. (2006). Suppression of survival kinases and activation of JNK mediate ethanol-induced cell death in the developing rat brain. *Neuroscience Letters, 398*, 113–117.

Hariri, A. R., Goldberg, T. E., Mattay, V. S., Kolachana, B. S., Callicott, J. H., Egan, M. F., et al. (2003). Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *The Journal of Neuroscience, 23*, 6690–6694.

Harper, C. (2009). The neuropathology of alcohol-related brain damage. *Alcohol and Alcoholism, 44*(2), 136–140.

Hassoun, A. T., Lovinger, D. M., & Davis, M. I. (2007). A rapid screening method for interactions between substrates, motogens, and developmental neurotoxins. *Alcoholism, Clinical and Experimental Research, 31*(S6), 99A.

Hauser, S. R., Getachew, B., Taylor, R. E., & Tizabi, Y. (2011). Alcohol induced depressive-like behavior is associated with a reduction in hippocampal BDNF. *Pharmacology, Biochemistry, and Behavior, 100*(2), 253–258.

He, D. Y., McGough, N. N., Ravindranathan, A., Jeanblanc, J., Logrip, M. L., Phamluong, K., Janak, P. H., & Ron, D. (2005). Glial cell line-derived neurotrophic factor mediates the desirable actions of the anti-addiction drug ibogaine against alcohol consumption. *The Journal of Neuroscience, 25*(3), 619–628.

He, D. Y., Neasta, J., & Ron, D. (2010). Epigenetic regulation of BDNF expression via the scaffolding protein RACK1. *The Journal of Biological Chemistry, 285*(25), 19043–19050.

Heaton, M. B., & Bradley, D. M. (1995). Ethanol influences on the chick embryo spinal cord motor system: Analyses of motoneuron cell death, motility, and target trophic factor activity and in vitro analyses of neurotoxicity and trophic factor neuroprotection. *Journal of Neurobiology, 26*(1), 47–61.

Heaton, M. B., Paiva, M., Swanson, D. J., & Walker, D. W. (1993). Modulation of ethanol neurotoxicity by nerve growth factor. *Brain Research, 620*(1), 78–85.

Heaton, M. B., Paiva, M., Swanson, D. J., & Walker, D. W. (1994). Responsiveness of cultured septal and hippocampal neurons to ethanol and neurotrophic substances. *Journal of Neuroscience Research, 39*(3), 305–318.

Heaton, M. B., Paiva, M., Swanson, D. J., & Walker, D. W. (1995). Alterations in responsiveness to ethanol and neurotrophic substances in fetal septohippocampal neurons following chronic prenatal ethanol exposure. *Brain Research. Developmental Brain Research, 85*(1), 1–13.

Heaton, M. B., Swanson, D. J., Paiva, M., & Walker, D. W. (1996). Influence of prenatal ethanol exposure on cholinergic development in the rat striatum. *The Journal of Comparative Neurology, 364*(1), 113–120.

Heaton, M. B., Mitchell, J. J., & Paiva, M. (1999). Ethanol-induced alterations in neurotrophin expression in developing cerebellum: Relationship to periods of temporal susceptibility. *Alcoholism, Clinical and Experimental Research, 23*, 1637–1642.

Heaton, M. B., Kim, D. S., & Paiva, M. (2000a). Neurotrophic factor protection against ethanol toxicity in rat cerebellar granule cell cultures requires phosphatidylinositol 3-kinase activation. *Neuroscience Letters, 291*(2), 121–125.

Heaton, M. B., Mitchell, J. J., & Paiva, M. (2000b). Overexpression of NGF ameliorates ethanol neurotoxicity in the developing cerebellum. *Journal of Neurobiology, 45*(2), 95–104.

Heaton, M. B., Mitchell, J. J., Paiva, M., & Walker, D. W. (2000c). Ethanol-induced alterations in the expression of neurotrophic factors in the developing rat central nervous system. *Brain Research. Developmental Brain Research, 121*(1), 97–107.

Heaton, M. B., Moore, D. B., Paiva, M., Madorsky, I., Mayer, J., & Shaw, G. (2003a). The role of neurotrophic factors, apoptosis-related proteins, and endogenous antioxidants in the differential temporal vulnerability of neonatal cerebellum to ethanol. *Alcoholism, Clinical and Experimental Research, 27*, 657–669.

Heaton, M. B., Paiva, M., Madorsky, I., Mayer, J., & Moore, D. B. (2003b). Effects of ethanol on neurotrophic factors, apoptosis-related proteins, endogenous antioxidants, and reactive oxygen species in neonatal striatum: Relationship to periods of vulnerability. *Brain Research. Developmental Brain Research, 140*, 237–252.

Heaton, M. B., Paiva, M., Madorsky, I., & Shaw, G. (2003c). Ethanol effects on neonatal rat cortex: Comparative analyses of neurotrophic factors, apoptosis-related proteins, and oxidative processes during vulnerable and resistant periods. *Brain Research. Developmental Brain Research, 145*, 249–262.

Heaton, M. B., Paiva, M., Kubovic, S., Kotler, A., Rogozinski, J., Swanson, E., Madorsky, V., & Posados, M. (2012). Differential effects of ethanol on c-jun N-terminal kinase, 14-3-3 proteins, and Bax in postnatal day 4 and postnatal day 7 rat cerebellum. *Brain Research, 1432*, 15–27.

Heberlein, A., Bleich, S., Bayerlein, K., Frieling, H., Gröschl, M., Kornhuber, J., & Hillemacher, T. (2008). NGF plasma levels increase due to alcohol intoxication and decrease during withdrawal. *Psychoneuroendocrinology, 33*(7), 999–1003.

Heberlein, A., Muschler, M., Wilhelm, J., Frieling, H., Lenz, B., Groschl, M., Kornhuber, J., Bleich, S., & Hillemacher, T. (2010). BDNF and GDNF serum levels in alcohol-dependent patients during withdrawal. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 34*(6), 1060–1064.

Heberlein, A., Muschler, M., Frieling, H., Behr, M., Eberlein, C., Wilhelm, J., Gröschl, M., Kornhuber, J., Bleich, S., & Hillemacher, T. (2011). Epigenetic down regulation of nerve growth factor during alcohol withdrawal. *Addiction Biology, 18*(3), 508–510. doi: 10.1111/j.1369-1600.2010.00307.x.

Hefti, F., Dravid, A., & Hartikka, J. (1984). Chronic intraventricular injections of nerve growth factor elevate hippocampal choline acetyltransferase activity in adult rats with partial septo-hippocampal lesions. *Brain Research, 293*(2), 305–311.

Hellmann, J. D., Rommelspacher, H., & Wernicke, C. (2009). Long-term ethanol exposure impairs neuronal differentiation of human neuroblastoma cells involving neurotrophin-mediated intracellular signaling and in particular protein kinase C. *Alcoholism, Clinical and Experimental Research, 33*(3), 538–550.

Hensler, J. G., Ladenheim, E. E., & Lyons, W. E. (2003). Ethanol consumption and serotonin-1A (5-HT1A) receptor function in heterozygous BDNF (+/−) mice. *Journal of Neurochemistry, 85*, 1139–1147.

Heumann, R., Korsching, S., Scott, J., & Thoenen, H. (1984). Relationship between levels of nerve growth factor (NGF) and its messenger RNA in sympathetic ganglia and peripheral target tissues. *The EMBO Journal, 3*(13), 3183–3189.

Hidalgo-Figueroa, M., Bonilla, S., Gutierrez, F., Pascual, A., & Lopez-Barneo, J. (2012). GDNF is predominantly expressed in the PV + neostriatal interneuronal ensemble in normal mouse and after injury of the nigrostriatal pathway. *The Journal of Neuroscience, 32*(3), 864–872.

Hirai, S., de Cui, F., Miyata, T., Ogawa, M., Kiyonari, H., Suda, Y., et al. (2006). The c-Jun N-terminal kinase activator dual leucine zipper kinase regulates axon growth and neuronal migration in the developing cerebral cortex. *The Journal of Neuroscience, 26*, 11992–12002.

Hoener, M. C., & Varon, S. (1997). Reversible sedimentation and masking of nerve growth factor (NGF) antigen by high molecular weight fractions from rat brain. *Brain Research, 772*(1–2), 1–8.

Hoener, M. C., Hewitt, E., Conner, J. M., Costello, J. W., & Varon, S. (1996). Nerve growth factor (NGF) content in adult rat brain tissues is several-fold higher than generally reported and is largely associated with sedimentable fractions. *Brain Research, 728*(1), 47–56.

Hofer, M., Pagliusi, S. R., Hohn, A., Leibrock, J., & Barde, Y. A. (1990). Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *The EMBO Journal, 9*(8), 2459–2464.

Holtzman, D. M., Li, Y., Parada, L. F., Kinsman, S., Chen, C. K., Valletta, J. S., Zhou, J., et al. (1992). p140trk mRNA marks NGF-responsive forebrain neurons: Evidence that trk gene expression is induced by NGF. *Neuron, 9*, 465–478.

Honegger, P., & Lenoir, D. (1982). Nerve growth factor (NGF) stimulation of cholinergic telencephalic neurons in aggregating cell cultures. *Brain Research, 255*(2), 229–238.

Houeland, G., Romani, A., Marchetti, C., Amato, G., Capsoni, S., Cattaneo, A., & Marie, H. (2010). Transgenic mice with chronic NGF deprivation and Alzheimer's disease-like pathology display hippocampal region-specific impairments in short- and long-term plasticities. *The Journal of Neuroscience, 30*(39), 13089–13094.

Huang, M. C., Chen, C. H., Chen, C. H., Liu, S. C., Ho, C. J., Shen, W. W., & Leu, S. J. (2008). Alterations of serum brain-derived neurotrophic factor levels in early alcohol withdrawal. *Alcohol and Alcoholism, 43*(3), 241–245.

Huang, M. C., Chen, C. H., Liu, H. C., Chen, C. C., Ho, C. C., & Leu, S. J. (2011). Differential patterns of serum brain-derived neurotrophic factor levels in alcoholic patients with and without delirium tremens during acute withdrawal. *Alcoholism, Clinical and Experimental Research, 35*(1), 126–131.

Hundle, B., McMahon, T., Dadgar, J., Chen, C. H., Mochly-Rosen, D., & Messing, R. O. (1997). An inhibitory fragment derived from protein kinase Cepsilon prevents enhancement of nerve growth factor responses by ethanol and phorbol esters. *The Journal of Biological Chemistry, 272*(23), 15028–15035.

Hassoun, A. T., Erdélyi, F., Szabó, G., & Davis M. I. (2007b). A rapid screening method for population-specific neuronal motogens, substrates and associated signaling pathways. *Journal of Neuroscience Methods, 166*(2), 178–194.

Hoener, M. C., & Varon, S. (1997). Reversible sedimentation and masking of nerve growth factor (NGF) antigen by high molecular weight fractions from rat brain. *Brain Research, 772* (1–2), 1–8.

Hong, C. J., Liou, Y. J., & Tsai, S. J. (2011). Effects of BDNF polymorphisms on brain function and behavior in health and disease. *Brain Research Bulletin, 86*(5–6), 287–297.

Incerti, M., Vink, J., Roberson, R., Benassou, I., Abebe, D., & Spong, C. Y. (2010). Prevention of the alcohol-induced changes in brain-derived neurotrophic factor expression using neuroprotective peptides in a model of fetal alcohol syndrome. *American Journal of Obstetrics and Gynecology, 202*(5), 457.e1-4.

Ip, N. Y., Li, Y., Yancopoulos, G. D., & Lindsay, R. M. (1993). Cultured hippocampal neurons show responses to BDNF, NT-3, and NT-4, but not NGF. *The Journal of Neuroscience, 13*, 3394–3405.

Jain, S., Golden, J. P., Wozniak, D., Pehek, E., Johnson, E. M., Jr., & Milbrandt, J. (2006). RET is dispensable for maintenance of midbrain dopaminergic neurons in adult mice. *The Journal of Neuroscience, 26*(43), 11230–11238.

Jang, S. W., Liu, X., Yepes, M., Shepherd, K. R., Miller, G. W., Liu, Y., Wilson, W. D., Xiao, G., Blanchi, B., Sun, Y. E., & Ye, K. (2010). A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. *Proceedings of the National Academy of Sciences of the United States of America, 107*(6), 2687–2692.

Jaurena, M. B., Carri, N. G., Battiato, N. L., & Rovasio, R. A. (2011). Trophic and proliferative perturbations of in vivo/in vitro cephalic neural crest cells after ethanol exposure are prevented by neurotrophin 3. *Neurotoxicology and Teratology, 33*(3), 422–430.

Jeanblanc, J., He, D. Y., McGough, N. N., Logrip, M. L., Phamluong, K., Janak, P. H., & Ron, D. (2006). The dopamine D3 receptor is part of a homeostatic pathway regulating ethanol consumption. *Journal of Neuroscience, 26*(5), 1457–1464.

Jeanblanc, J., He, D. Y., Carnicella, S., Kharazia, V., Janak, P. H., & Ron, D. (2009). Endogenous BDNF in the dorsolateral striatum gates alcohol drinking. *The Journal of Neuroscience, 29*(43), 13494–13502.

Jiang, X., Xu, K., Hoberman, J., Tian, F., Marko, A. J., Waheed, J. F., et al. (2005). BDNF variation and mood disorders: A novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. *Neuropsychopharmacology, 30*, 1353–1361.

Jiang, Y., Kumada, T., Cameron, D. B., & Komuro, H. (2008). Cerebellar granule cell migration and the effects of alcohol. *Developmental Neuroscience, 30*(1–3), 7–23.

Jin, J., Suzuki, H., Hirai, S., Mikoshiba, K., & Ohshima, T. (2010). JNK phosphorylates Ser332 of doublecortin and regulates its function in neurite extension and neuronal migration. *Developmental Neurobiology, 70*(14), 929–942.

Jockers-Scherubl, M. C., Bauer, A., Kuhn, S., Reischies, F., Danker-Hopfe, H., Schmidt, L. G., Rentzsch, J., & Hellweg, R. (2007). Nerve growth factor in serum is a marker of the stage of alcohol disease. *Neuroscience Letters, 419*(1), 78–82.

Joe, K. H., Kim, Y. K., Kim, T. S., Roh, S. W., Choi, S. W., et al. (2007). Decreased plasma brain derived neurotrophic factor levels in patients with alcohol dependence. *Alcoholism, Clinical and Experimental Research, 31*, 1–6.

Jones, K. R., Farinas, I., Backus, C., & Reichardt, L. F. (1994). Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell, 76*, 989–999.

Kalev-Zylinska, M. L., & During, M. J. (2007). Paradoxical facilitatory effect of low-dose alcohol consumption on memory mediated by NMDA receptors. *The Journal of Neuroscience, 39*, 10456–10467.

Kalluri, H. S., & Ticku, M. K. (2002). Ethanol-mediated inhibition of mitogen-activated protein kinase phosphorylation in mouse brain. *European Journal of Pharmacology, 439*, 53–58.

Kalluri, H. S., & Ticku, M. K. (2003). Regulation of ERK phosphorylation by ethanol in fetal cortical neurons. *Neurochemical Research, 28*, 765–769.

Kapuscinski, M., Charchar, F., Innes, B., Mitchell, G. A., Norman, T. L., & Harrap, S. B. (1996). Nerve growth factor gene and hypertension in spontaneously hypertensive rats. *Journal of Hypertension, 14*(2), 191–197.

Karpac, J., & Jasper, H. (2009). Insulin and JNK: Optimizing metabolic homeostasis and lifespan. *Trends in Endocrinology and Metabolism, 20*(3), 100–106.

Kawaja, M. D., & Crutcher, K. A. (1997). Sympathetic axons invade the brains of mice overexpressing nerve growth factor. *The Journal of Comparative Neurology, 383*(1), 60–72.

Kawaja, M. D., Walsh, G. S., Tovich, P. R., & Julien, J. P. (1998). Effects of elevated levels of nerve growth factor on the septohippocampal system in transgenic mice. *The European Journal of Neuroscience, 10*(7), 2207–2216.

Kawaja, M. D., Smithson, L. J., Elliott, J., Trinh, G., Crotty, A. M., Michalski, B., & Fahnestock, M. (2011). Nerve growth factor promoter activity revealed in mice expressing enhanced green fluorescent protein. *The Journal of Comparative Neurology, 519*(13), 2522–2545.

Kernie, S. G., Liebl, D. J., & Parada, L. F. (2000). BDNF regulates eating behavior and locomotor activity in mice. *The EMBO Journal, 19*, 1290–1300.

Kerns, R. T., Ravindranathan, A., Hassan, S., Cage, M. P., York, T., Sikela, J. M., et al. (2005). Ethanol-responsive brain region expression networks: Implications for behavioral responses to acute ethanol in DBA/2 J versus C57BL/6 J mice. *The Journal of Neuroscience, 25*, 2255–2266.

Klein, R. (1994). Role of neurotrophins in mouse neuronal development. *The FASEB Journal, 8*, 738–744.

Klein, R., Lamballe, F., Bryant, S., & Barbacid, M. (1992). The trkB tyrosine protein kinase is a receptor for neurotrophin-4. *Neuron, 8*(5), 947–956.

Kolb, J. E., Trettel, J., & Levine, E. S. (2005). BDNF enhancement of postsynaptic NMDA receptors is blocked by ethanol. *Synapse, 55*, 52–57.

Kooijman, R., Sarre, S., Michotte, Y., & De Keyser, J. (2009). Insulin-like growth factor I: A potential neuroprotective compound for the treatment of acute ischemic stroke? *Stroke, 40*(4), e83-8.

Korsching, S., & Thoenen, H. (1983). Nerve growth factor in sympathetic ganglia and corresponding target organs of the rat: Correlation with density of sympathetic innervation. *Proceedings of the National Academy of Sciences of the United States of America, 80*(11), 3513–3516.

Korsching, S., Auburger, G., Heumann, R., Scott, J., & Thoenen, H. (1985). Levels of nerve growth factor and its mRNA in the central nervous system of the rat correlate with cholinergic innervation. *The EMBO Journal, 4*(6), 1389–1393.

Koshimizu, H., Kiyosue, K., Hara, T., Hazama, S., Suzuki, S., Uegaki, K., Nagappan, G., Zaitsev, E., Hirokawa, T., Tatsu, Y., Ogura, A., Lu, B., & Kojima, M. (2009). Multiple functions of precursor BDNF to CNS neurons: Negative regulation of neurite growth, spine formation and cell survival. *Molecular Brain, 2*, 27.

Koshimizu, H., Hazama, S., Hara, T., Ogura, A., & Kojima, M. (2010). Distinct signaling pathways of precursor BDNF and mature BDNF in cultured cerebellar granule neurons. *Neuroscience Letters, 473*(3), 229–232.

Koushika, S. P. (2008). "JIP"ing along the axon: The complex roles of JIPs in axonal transport. *Bioessays, 30*(1), 10–14.

Kramer, E. R., Aron, L., Ramakers, G. M., Seitz, S., Zhuang, X., Beyer, K., Smidt, M. P., & Klein, R. (2007). Absence of Ret signaling in mice causes progressive and late degeneration of the nigrostriatal system. *PLoS Biology, 5*(3), e39.

Krimm, R. F. (2007). Factors that regulate embryonic gustatory development. *BMC Neuroscience, 8*(Suppl 3), S4.

Kroener, S., Mulholland, P. J., New, N. N., Gass, J. T., Becker, H. C., & Chandler, L. J. (2012). Chronic alcohol exposure alters behavioral and synaptic plasticity of the rodent prefrontal cortex. *PLoS One, 7*(5), e37541.

Kulkarny, V. V., Wiest, N. E., Marquez, C. P., Nixon, S. C., Valenzuela, C. F., & Perrone-Bizzozero, N. I. (2011). Opposite effects of acute ethanol exposure on GAP-43 and BDNF expression in the hippocampus versus the cerebellum of juvenile rats. *Alcohol, 45*(5), 461–471.

Kuri, B. A., Chan, S. A., & Smith, C. B. (2009). PACAP regulates immediate catecholamine release from adrenal chromaffin cells in an activity-dependent manner through a protein kinase C-dependent pathway. *Journal of Neurochemistry, 110*(4), 1214–1225.

Katoh-Semba, R., Takeuchi, I. K., Semba, R., & Kato, K. (1997). Distribution of brain-derived neurotrophic factor in rats and its changes with development in the brain. *Journal of Neurochemistry, 69*, 34–42.

Lang, U. E., Hellweg, R., Sander, T., & Gallinat, J. (2009). The Met allele of the BDNF Val66Met polymorphism is associated with increased BDNF serum concentrations. *Molecular Psychiatry, 14*(2), 120–122.

Lin, S., Rhodes, P. G., & Cai, Z. (2011). Whole body hypothermia broadens the therapeutic window of intranasally administered IGF-1 in a neonatal rat model of cerebral hypoxia-ischemia. *Brain Research, 1385*, 246–256.

Lin, S., Fan, L. W., Rhodes, P. G., Cai, Z. (2009). Intranasal administration of IGF-1 attenuates hypoxic-ischemic brain injury in neonatal rats. *Experimental Neurology, 217*(2), 361–370.

Lamballe, F., Klein, R., & Barbacid, M. (1991). trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. *Cell, 66*(5), 967–979.

Lang, U. E., Hellweg, R., Bajbouj, M., Gaus, V., Sander, T., & Gallinat, J. (2008). Gender-dependent association of a functional NGF polymorphism with anxiety-related personality traits. *Pharmacopsychiatry, 41*(5), 196–199.

Larkfors, L., Ebendal, T., Whittemore, S. R., Persson, H., Hoffer, B., & Olson, L. (1987). Decreased level of nerve growth factor (NGF) and its messenger RNA in the aged rat brain. *Brain Research, 427*(1), 55–60.

Larkfors, L., Lindsay, R. M., & Alderson, R. F. (1996). Characterization of the responses of Purkinje cells to neurotrophin treatment. *Journal of Neurochemistry, 66*(4), 1362–1373.

Lasek, A. W., Gesch, J., Giorgetti, F., Kharazia, V., & Heberlein, U. (2011a). Alk is a transcriptional target of LMO4 and ERα that promotes cocaine sensitization and reward. *The Journal of Neuroscience, 31*(40), 14134–14141.

Lasek, A. W., Lim, J., Kliethermes, C. L., Berger, K. H., Joslyn, G., Brush, G., Xue, L., Robertson, M., Moore, M. S., Vranizan, K., Morris, S. W., Schuckit, M. A., White, R. L., & Heberlein, U. (2011b). An evolutionary conserved role for anaplastic lymphoma kinase in behavioral responses to ethanol. *PLoS One, 6*(7), e22636.

Lauterborn, J. C., Isackson, P. J., & Gall, C. M. (1994). Cellular localization of NGF and NT-3 mRNAs in postnatal rat forebrain. *Molecular and Cellular Neurosciences, 5*(1), 46–62.

Lauterborn, J. C., Bizon, J. L., Tran, T. M., & Gall, C. M. (1995). NGF mRNA is expressed by GABAergic but not cholinergic neurons in rat basal forebrain. *The Journal of Comparative Neurology, 360*(3), 454–462.

Ledda, F. (2007). Ligand-induced cell adhesion as a new mechanism to promote synapse formation. *Cell Adhesion & Migration, 3*, 137–139.

Lee, B. C., Choi, I. G., Kim, Y. K., Ham, B. J., Yang, B. H., Roh, S., Choi, J., Lee, J. S., Oh, D. Y., & Chai, Y. G. (2009). Relation between plasma brain-derived neurotrophic factor and nerve growth factor in the male patients with alcohol dependence. *Alcohol, 43*(4), 265–269.

Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H., & Barde, Y. A. (1989). Molecular cloning and expression of brain-derived neurotrophic factor. *Nature, 341*(6238), 149–152.

Lein, E. S., Hohn, A., & Shatz, C. J. (2000). Dynamic regulation of BDNF and NT-3 expression during visual system development. *The Journal of Comparative Neurology, 420*(1), 1–18.

Levi-Montalcini, R., & Cohen, S. (1960). Effects of the extract of the mouse submaxillary salivary glands on the sympathetic system of mammals. *Annals of the New York Academy of Sciences, 29*(85), 324–341.

Li, Z., Ding, M., Thiele, C. J., & Luo, J. (2004). Ethanol inhibits brain-derived neurotrophic factor-mediated intracellular signaling and activator protein-1 activation in cerebellar granule neurons. *Neuroscience, 126*, 149–162.

Li, Y., Yui, D., Luikart, B. W., McKay, R. M., Li, Y., Rubenstein, J. L., & Parada, L. F. (2012). Conditional ablation of brain-derived neurotrophic factor-TrkB signaling impairs striatal neuron development. *Proceedings of the National Academy of Sciences of the United States of America, 109*(38), 15491–15496.

Liebl, D. J., Tessarollo, L., Palko, M. E., & Parada, L. F. (1997). Absence of sensory neurons before target innervation in brain-derived neurotrophic factor-, neurotrophin 3-, and TrkC-deficient embryonic mice. *The Journal of Neuroscience, 17*(23), 9113–9121.

Light, K. E., Ge, Y., & Belcher, S. M. (2001). Early postnatal ethanol exposure selectively decreases BDNF and truncated TrkB-T2 receptor mRNA expression in the rat cerebellum. *Brain Research. Molecular Brain Research, 93*, 46–55.

Light, K. E., Brown, D. P., Newton, B. W., Belcher, S. M., & Kane, C. J. (2002). Ethanol-induced alterations of neurotrophin receptor expression on Purkinje cells in the neonatal rat cerebellum. *Brain Research, 924*, 71–81.

Lindsley, T. A., Shah, S. N., & Ruggiero, E. A. (2011). Ethanol alters BDNF-induced Rho GTPase activation in axonal growth cones. *Alcoholism, Clinical and Experimental Research, 35*(7), 1321–1330.

Ling, M., Trollér, U., Zeidman, R., Lundberg, C., & Larsson, C. (2004). Induction of neurites by the regulatory domains of PKCdelta and epsilon is counteracted by PKC catalytic activity and by the RhoA pathway. *Experimental Cell Research, 292*(1), 135–150.

Logrip, M. L., Janak, P. H., & Ron, D. (2008). Dynorphin is a downstream effector of striatal BDNF regulation of ethanol intake. *The FASEB Journal, 22*(7), 2393–2404.

Logrip, M. L., Janak, P. H., & Ron, D. (2009). Escalating ethanol intake is associated with altered corticostriatal BDNF expression. *Journal of Neurochemistry, 109*(5), 1459–1468.

Lommatzsch, M., Zingler, D., Schuhbaeck, K., Schloetcke, K., Zingler, C., Schuff-Werner, P., & Virchow, J. C. (2005). The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiology of Aging, 26*(1), 115–123.

Luberg, K., Wong, J., Weickert, C. S., & Timmusk, T. (2010). Human TrkB gene: Novel alternative transcripts, protein isoforms and expression pattern in the prefrontal cerebral cortex during postnatal development. *Journal of Neurochemistry, 113*(4), 952–964.

Lukoyanov, N. V., Pereira, P. A., Paula-Barbosa, M. M., & Cadete-Leite, A. (2003). Nerve growth factor improves spatial learning and restores hippocampal cholinergic fibers in rats withdrawn from chronic treatment with ethanol. *Experimental Brain Research, 148*(1), 88–94.

Luo, J., West, J. R., & Pantazis, N. J. (1997). Nerve growth factor and basic fibroblast growth factor protect rat cerebellar granule cells in culture against ethanol-induced cell death. *Alcoholism, Clinical and Experimental Research, 21*(6), 1108–1120.

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., et al. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 15239–15244.

Ma, L., Harada, T., Harada, C., Romero, M., Hebert, J. M., McConnell, S. K., & Parada, L. F. (2002). Neurotrophin-3 is required for appropriate establishment of thalamocortical connections. *Neuron, 36*(4), 623–634.

Macdonald, D. S., Weerapura, M., Beazely, M. A., Martin, L., Czerwinski, W., Roder, J. C., Orser, B. A., & MacDonald, J. F. (2005). Modulation of NMDA receptors by pituitary adenylate cyclase activating peptide in CA1 neurons requires G alpha q, protein kinase C, and activation of Src. *The Journal of Neuroscience, 25*(49), 11374–11384.

MacLennan, A. J., Lee, N., & Walker, D. W. (1995). Chronic ethanol administration decreases brain-derived neurotrophic factor gene expression in the rat hippocampus. *Neuroscience Letters, 197*, 105–108.

Madhav, T. R., Pei, Q., & Zetterstrom, T. S. (2001). Serotonergic cells of the rat raphe nuclei express mRNA of tyrosine kinase B (trkB), the high-affinity receptor for brain derived neurotrophic factor (BDNF). *Brain Research. Molecular Brain Research, 93*, 56–63.

Maisonpierre, P. C., Belluscio, L., Friedman, B., Alderson, R. F., Wiegand, S. J., Furth, M. E., Lindsay, R. M., & Yancopoulos, G. D. (1990a). NT-3, BDNF, and NGF in the developing rat nervous system: Parallel as well as reciprocal patterns of expression. *Neuron, 5*(4), 501–509.

Maisonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M., & Yancopoulos, G. D. (1990b). Neurotrophin-3: A neurotrophic factor related to NGF and BDNF. *Science, 247*(4949 Pt 1), 1446–1451.

Mardy, S., Miura, Y., Endo, F., Matsuda, I., & Indo, Y. (2001). Congenital insensitivity to pain with anhidrosis (CIPA): Effect of TRKA (NTRK1) missense mutations on autophosphorylation of the receptor tyrosine kinase for nerve growth factor. *Human Molecular Genetics, 10*(3), 179–188.

Marini, A. M., Rabin, S. J., Lipsky, R. H., & Mocchetti, I. (1998). Activity-dependent release of brain-derived neurotrophic factor underlies the neuroprotective effect of *N*-methyl-D-aspartate. *The Journal of Biological Chemistry, 273*, 29394–29399.

Mason, I. (2000). The RET, receptor tyrosine kinase: Activation, signaling and significance in neural development and disease. *Pharmaceutica Acta Helvetiae, 74*(2–3), 261–264.

Massa, S. M., Yang, T., Xie, Y., Shi, J., Bilgen, M., Joyce, J. N., Nehama, D., Rajadas, J., & Longo, F. M. (2010). Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. *The Journal of Clinical Investigation, 120*(5), 1774–1785.

Masure, S., Cik, M., Pangalos, M. N., Bonaventure, P., Verhasselt, P., Lesage, A. S., Leysen, J. E., & Gordon, R. D. (1998). Molecular cloning, expression and tissue distribution of glial-cell-line-derived neurotrophic factor family receptor alpha-3 (GFRalpha-3). *European Journal of Biochemistry, 251*(3), 622–630.

Matsushita, S., Kimura, M., Miyakawa, T., Yoshino, A., Murayama, M., Masaki, T., & Higuchi, S. (2004). Association study of brain-derived neurotrophic factor gene polymorphism and alcoholism. *Alcoholism, Clinical and Experimental Research, 28*(11), 1609–1612.

May, P. A., Gossage, J. P., Kalberg, W. O., Robinson, L. K., Buckley, D., Manning, M., & Hoyme, H. E. (2009). Prevalence and epidemiologic characteristics of FASD from various research methods with an emphasis on recent in-school studies. *Developmental Disabilities Research Reviews, 15*(3), 176–192.

McAlhany, R. E., Jr., West, J. R., & Miranda, R. C. (1997). Glial-derived neurotrophic factor rescues calbindin-D28k-immunoreactive neurons in alcohol-treated cerebellar explant cultures. *Journal of Neurobiology, 33*(6), 835–847.

McAlhany, R. E., Jr., Miranda, R. C., Finnell, R. H., & West, J. R. (1999). Ethanol decreases Glial-Derived Neurotrophic Factor (GDNF) protein release but not mRNA expression and increases GDNF-stimulated Shc phosphorylation in the developing cerebellum. *Alcoholism, Clinical and Experimental Research, 23*(10), 1691–1697.

McAlhany, R. E., Jr., West, J. R., & Miranda, R. C. (2000). Glial-derived neurotrophic factor (GDNF) prevents ethanol-induced apoptosis and JUN kinase phosphorylation. *Brain Research. Developmental Brain Research, 119*(2), 209–216.

McGough, N. N., He, D. Y., Logrip, M. L., Jeanblanc, J., Phamluong, K., Luong, K., et al. (2004). RACK1 and brain-derived neurotrophic factor: A homeostatic pathway that regulates alcohol addiction. *The Journal of Neuroscience, 24*, 10542–10552.

Melendez, R. I., McGinty, J. F., Kalivas, P. W., & Becker, H. C. (2012). Brain region-specific gene expression changes after chronic intermittent ethanol exposure and early withdrawal in C57BL/6 J mice. *Addiction Biology, 17*(2), 351–364.

Meng, D., Wu, T., Rao, U., North, C. S., Xiao, H., Javors, M. A., & Adinoff, B. (2011). Serum NPY and BNDF response to a behavioral stressor in alcohol-dependent and healthy control participants. *Psychopharmacology, 218*(1), 59–67.

Messing, R. O., Henteleff, M., & Park, J. J. (1991). Ethanol enhances growth factor-induced neurite formation in PC12 cells. *Brain Research, 565*(2), 301–311.

Miller, M. W. (2004). Repeated episodic exposure to ethanol affects neurotrophin content in the forebrain of the mature rat. *Experimental Neurology, 189*, 173–181.

Miller, M. W., & Mooney, S. M. (2004). Chronic exposure to ethanol alters neurotrophin content in the basal forebrain-cortex system in the mature rat: Effects on autocrine-paracrine mechanisms. *Journal of Neurobiology, 60*, 490–498.

Miller, T. M., Tansey, M. G., Johnson, E. M., & Creedon, D. J. (1997). Inhibition of phosphatidylinositol 3-kinase activity blocks depolarization- and insulin-like growth factor I-mediated survival of cerebellar granule cells. *The Journal of Biological Chemistry, 272*, 9847–9853.

Miller, R., King, M. A., Heaton, M. B., & Walker, D. W. (2002). The effects of chronic ethanol consumption on neurotrophins and their receptors in the rat hippocampus and basal forebrain. *Brain Research, 950*, 137–147.

Miller, M. W., Mooney, S. M., & Middleton, F. A. (2006). Transforming growth factor beta1 and ethanol affect transcription and translation of genes and proteins for cell adhesion molecules in B104 neuroblastoma cells. *Journal of Neurochemistry, 97*(4), 1182–1190.

Mitchell, J. J., Paiva, M., Walker, D. W., & Heaton, M. B. (1999). BDNF and NGF afford in vitro neuroprotection against ethanol combined with acute ischemia and chronic hypoglycemia. *Developmental Neuroscience, 21*(1), 68–75.

Mitschelen, M., Yan, H., Farley, J. A., Warrington, J. P., Han, S., Hereñú, C. B., Csiszar, A., Ungvari, Z., Bailey-Downs, L. C., Bass, C. E., & Sonntag, W. E. (2011). Long-term deficiency of circulating and hippocampal insulin-like growth factor I induces depressive behavior in adult mice: A potential model of geriatric depression. *Neuroscience, 185*, 50–60.

Mobley, W. C., Rutkowski, J. L., Tennekoon, G. I., Gemski, J., Buchanan, K., & Johnston, M. V. (1986). Nerve growth factor increases choline acetyltransferase activity in developing basal forebrain neurons. *Brain Research, 387*(1), 53–62.

Moises, H. C., Womble, M. D., Washburn, M. S., & Williams, L. R. (1995). Nerve growth factor facilitates cholinergic neurotransmission between nucleus basalis and the amygdala in rat: An electrophysiological analysis. *The Journal of Neuroscience, 15*(12), 8131–8142.

Monk, B. R., Leslie, F. M., & Thomas, J. D. (2012). The effects of perinatal choline supplementation on hippocampal cholinergic development in rats exposed to alcohol during the brain growth spurt. *Hippocampus, 22*(8), 1750–1757.

Mooney, S. M., & Miller, M. W. (2007). Nerve growth factor neuroprotection of ethanol-induced neuronal death in rat cerebral cortex is age dependent. *Neuroscience, 149*(2), 372–381.

Moore, D. B., Madorsky, I., Paiva, M., & Barrow Heaton, M. (2004a). Ethanol exposure alters neurotrophin receptor expression in the rat central nervous system: Effects of prenatal exposure. *Journal of Neurobiology, 60*, 101–113.

Moore, D. B., Madorsky, I., Paiva, M., & Barrow Heaton, M. (2004b). Ethanol exposure alters neurotrophin receptor expression in the rat central nervous system: Effects of neonatal exposure. *Journal of Neurobiology, 60*, 114–126.

Mounien, L., Do Rego, J. C., Bizet, P., Boutelet, I., Gourcerol, G., Fournier, A., Brabet, P., Costentin, J., Vaudry, H., & Jegou, S. (2009). Pituitary adenylate cyclase-activating polypeptide inhibits food intake in mice through activation of the hypothalamic melanocortin system. *Neuropsychopharmacology, 34*(2), 424–435.

Mount, H. T., Dreyfus, C. F., & Black, I. B. (1994). Neurotrophin-3 selectively increases cultured Purkinje cell survival. *Neuroreport, 5*(18), 2497–2500.

Mullikin-Kilpatrick, D., & Treistman, S. N. (1995). Inhibition of dihydropyridine-sensitive Ca++ channels by ethanol in undifferentiated and nerve growth factor-treated PC12 cells: Interaction with the inactivated state. *The Journal of Pharmacology and Experimental Therapeutics, 272*(2), 489–497.

Muschler, M. A., Heberlein, A., Frieling, H., Vogel, N., Becker, C. M., Kornhuber, J., Bleich, S., & Hillemacher, T. (2011). Brain-derived neurotrophic factor, Val66Met single nucleotide polymorphism is not associated with alcohol dependence. *Psychiatric Genetics, 21*(1), 53–54.

McClure, K. D., French, R. L., & Heberlein, U. (2011). A *Drosophila* model for fetal alcohol syndrome disorders: Role for the insulin pathway. *Disease Models & Mechanisms, 4*(3), 335–346.

Miki, T., Kuma, H., Yokoyama, T., Sumitani, K., Matsumoto, Y., Kusaka, T., Warita, K., Wang, Z. Y., Hosomi, N., Imagawa, T., S Bedi, K., Itoh, S., Nakamura, Y., & Takeuchi, Y. (2008). Early postnatal ethanol exposure induces fluctuation in the expression of BDNF mRNA in the developing rat hippocampus. *Acta Neurobiologiae Expimentalis (Wars), 68*(4), 484–493.

Mitchell, J. J., Paiva, M., Moore, D. B., Walker, D. W., & Heaton, M. B. (1998). A comparative study of ethanol, hypoglycemia, hypoxia and neurotrophic factor interactions with fetal rat hippocampal neurons: A multi-factor in vitro model developmental ethanol effects. *Brain Research: Developmental Brain Research, 105*(2), 241–250.

Mobley, W. C., Woo, J. E., Edwards, R. H., Riopelle, R. J., Longo, F. M., Weskamp, G., Otten, U., Valletta, J. S., & Johnston, M. V. (1989). Developmental regulation of nerve growth factor and its receptor in the rat caudate-putamen. *Neuron, 3*(5), 655–664.

Mon, A., Durazzo, T. C., Gazdzinski, S., Hutchison, K. E., Pennington, D., & Meyerhoff, D. J. (2013). Brain-derived neurotrophic factor genotype is associated with brain gray and white matter tissue volumes recovery in abstinent alcohol-dependent individuals. *Genes Brain and Behavior, 12*(1), 98–107.

Montag, C., Basten, U., Stelzel, C., Fiebach, C. J., & Reuter, M. (2010). The BDNF Val66Met polymorphism and anxiety: Support for animal knock-in studies from a genetic association study in humans. *Psychiatry Research, 179*(1), 86–90.

Moonat, S., Sakharkar, A. J., Zhang, H., & Pandey, S. C. (2011). The role of amygdaloid brain-derived neurotrophic factor, activity-regulated cytoskeleton-associated protein and dendritic spines in anxiety and alcoholism. *Addiction Biology, 16*(2), 238–250.

Moore, M. S., DeZazzo, J., Luk, A. Y., Tully, T., Singh, C. M., & Heberlein, U. (1998). Ethanol intoxication in *Drosophila*: Genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell, 93*(6), 997–1007.

Nagata, T., Shinagawa, S., Nukariya, K., Nakayama, R., Nakayama, K., & Yamada, H. (2011). Association between nerve growth factor gene polymorphism and executive dysfunction in Japanese patients with early-stage Alzheimer's disease and amnestic mild cognitive impairment. *Dementia and Geriatric Cognitive Disorders, 32*(6), 379–386.

Nakano, T., Fujimoto, T., Shimooki, S., Fukudome, T., Uchida, T., Tsuji, T., Mitsuyama, Y., Akimoto, H., & Furukawa, S. (1996). Transient elevation of nerve growth factor content in the rat hippocampus and frontal cortex by chronic ethanol treatment. *Psychiatry and Clinical Neurosciences, 50*(3), 157–160.

Nakata, M., Kohno, D., Shintani, N., Nemoto, Y., Hashimoto, H., Baba, A., & Yada, T. (2004). PACAP deficient mice display reduced carbohydrate intake and PACAP activates NPY-containing neurons in the rat hypothalamic arcuate nucleus. *Neuroscience Letters, 370*(2–3), 252–256.

Neasta, J., Kiely, P. A., He, D. Y., Adams, D. R., O'Connor, R., & Ron, D. (2012). Direct interaction between scaffolding proteins RACK1 and 14-3-3Œ∂ regulates brain-derived neurotrophic factor (BDNF) transcription. *The Journal of Biological Chemistry, 287*(1), 322–336.

Nishijima, T., Piriz, J., Duflot, S., Fernandez, A. M., Gaitan, G., Gomez-Pinedo, U., Verdugo, J. M., Leroy, F., Soya, H., Nuñez, A., & Torres-Aleman, I. (2010). Neuronal activity drives localized blood–brain-barrier transport of serum insulin-like growth factor-I into the CNS. *Neuron, 67*(5), 834–846.

Nonomura, T., Kubo, T., Oka, T., Shimoke, K., Yamada, M., Enokido, Y., & Hatanaka, H. (1996). Signaling pathways and survival effects of BDNF and NT-3 on cultured cerebellar granule cells. *Brain Research. Developmental Brain Research, 97*(1), 42–50.

Numan, S., & Seroogy, K. B. (1999). Expression of trkB and trkC mRNAs by adult midbrain dopamine neurons: A double-label in situ hybridization study. *The Journal of Comparative Neurology, 403*, 295–308.

Nakahashi, T., Fujimura, H., Altar, C. A., Li, J., Kambayashi, J., Tandon, N. N., & Sun, B. (2000). Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. *FEBS Letters, 470*(2), 113–117.

Okragly, A. J., & Haak-Frendscho, M. (1997) An acid-treatment method for the enhanced detection of GDNF in biological samples. *Experimental Neurology, 145*(2 Pt. 1), 592–596.

Ozan, E., Okur, H., Eker, C., Eker, O. D., Gönül, A. S., & Akarsu, N. (2010). The effect of depression, BDNF gene val66met polymorphism and gender on serum BDNF levels. *Brain Research Bulletin, 81*(1), 61–65.

Oberdoerster, J., & Rabin, R. A. (1999). NGF-differentiated and undifferentiated PC12 cells vary in induction of apoptosis by ethanol. *Life Sciences, 64*(23), PL 267–PL 272.

Ohrtman, J. D., Stancik, E. K., Lovinger, D. M., & Davis, M. I. (2006). Ethanol inhibits brain-derived neurotrophic factor stimulation of extracellular signal-regulated/mitogen-activated protein kinase in cerebellar granule cells. *Alcohol, 39*, 29–37.

Okamoto, H., Miki, T., Lee, K. Y., Yokoyama, T., Kuma, H., Gu, H., et al. (2006). Effects of chronic ethanol administration on the expression levels of neurotrophic factors in the rat hippocampus. *Okajimas Folia Anatomica Japonica, 83*, 1–6.

Oliva, A. A., Jr., Atkins, C. M., Copenagle, L., & Banker, G. A. (2006). Activated c-Jun N-terminal kinase is required for axon formation. *The Journal of Neuroscience, 26*, 9462–9470.

Pan, W., & Kastin, A. J. (2000). Interactions of IGF-1 with the blood–brain barrier in vivo and in situ. *Neuroendocrinology, 72*(3), 171–178.

Pandey, S. C., Zhang, H., Roy, A., & Misra, K. (2005). Central and medial amygdaloid brain-derived neurotrophic factor signaling plays a critical role in alcohol-drinking and anxiety-like behaviors. *The Journal of Neuroscience, 26*, 8320–8331.

Parks, E. A., McMechan, A. P., Hannigan, J. H., & Berman, R. F. (2008). Environmental enrichment alters neurotrophin levels after fetal alcohol exposure in rats. *Alcoholism, Clinical and Experimental Research, 32*(10), 1741–1751.

Pascual, M., Rocamora, N., Acsády, L., Freund, T. F., & Soriano, E. (1998). Expression of nerve growth factor and neurotrophin-3 mRNAs in hippocampal interneurons: Morphological characterization, levels of expression, and colocalization of nerve growth factor and neurotrophin-3. *The Journal of Comparative Neurology, 395*(1), 73–90.

Passilta, M., Kervinen, K., & Kesäniemi, Y. A. (1999). Glucose metabolism, insulin-like growth factor-I, and insulin-like growth factor-binding protein-1 after alcohol withdrawal. *Alcoholism, Clinical and Experimental Research, 23*(3), 471–475.

Patten, A. R., Brocardo, P. S., & Christie, B. R. (2012). Omega-3 supplementation can restore glutathione levels and prevent oxidative damage caused by prenatal ethanol exposure. *Journal of Nutrition Biochemistry, 24*(5), 760–769. doi:10.1016/j.jnutbio.2012.04.003.

Paula-Barbosa, M. M., Pereira, P. A., Cadete-Leite, A., & Dulce Madeira, M. (2003). NGF and NT-3 exert differential effects on the expression of neuropeptides in the suprachiasmatic nucleus of rats withdrawn from ethanol treatment. *Brain Research, 983*(1–2), 64–73.

Pitts, A. F., & Miller, M. W. (1995). Expression of nerve growth factor, p75, and trk in the somatosensory and motor cortices of mature rats: Evidence for local trophic support circuits. *Somatosensory & Motor Research, 12*(3–4), 329–342.

Pitts, A. F., & Miller, M. W. (2000). Expression of nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 in the somatosensory cortex of the mature rat: Coexpression with high-affinity neurotrophin receptors. *The Journal of Comparative Neurology, 418*(3), 241–254.

Pochon, N. A., Menoud, A., Tseng, J. L., Zurn, A. D., & Aebischer, P. (1997). Neuronal GDNF expression in the adult rat nervous system identified by in situ hybridization. *The European Journal of Neuroscience, 9*(3), 463–471.

Prado de Carvalho, L., & Izquierdo, I. (1977). Changes in the frequency of electroencephalographic rhythms of the rat caused by single, intraperitoneal injections of ethanol. *Archives Internationales de Pharmacodynamie et de Thérapie, 229*(1), 157–162.

Pruunsild, P., Kazantseva, A., Aid, T., Palm, K., & Timmusk, T. (2007). Dissecting the human BDNF locus: Bidirectional transcription, complex splicing, and multiple promoters. *Genomics, 90*, 397–406.

Pascual, A., Hidalgo-Figueroa, M., Gómez-Díaz, R., & López-Barneo, J. (2011). GDNF and protection of adult central catecholaminergic neurons. *Journal of Molecular Endocrinology, 46*(3), R83–R92.

Petryshen, T. L., Sabeti, P. C., Aldinger, K. A., Fry, B., Fan, J. B., Schaffner, S. F., Waggoner, S. G., Tahl, A. R., & Sklar, P. (2010). Population genetic study of the brain-derived neurotrophic factor (BDNF) gene. *Molecular Psychiatry, 15*(8), 810–815.

Powrozek, T. A., & Olson, E. C. (2012). Ethanol-induced disruption of Golgi apparatus morphology, primary neurite number and cellular orientation in developing cortical neurons. *Alcohol, 46*(7), 619–627.

Prakash, A., Zhang, H., & Pandey, S. C. (2008). Innate differences in the expression of brain-derived neurotrophic factor in the regions within the extended amygdala between alcohol preferring and nonpreferring rats. *Alcohol Clinical and Experimental Research, 32*(6), 909–920.

Rahman, H., Kentroti, S., & Vernadakis, A. (1993). Early in ovo exposure of chick embryos toethanol prevents the neuronotrophic effects of intracerebral NGF administration on cholinergic phenotypic expression. *Brain Research. Developmental Brain Research, 76*(2), 256–259.

Raivio, N., Tiraboschi, E., Saarikoski, S. T., Castren, E., & Kiianmaa, K. (2012). Brain-derived neurotrophic factor expression after acute administration of ethanol. *European Journal of Pharmacology, 687*(1–3), 9–13.

Rajagopal, R., Chen, Z. Y., Lee, F. S., & Chao, M. V. (2004). Transactivation of Trk neurotrophin receptors by G-protein-coupled receptor ligands occurs on intracellular membranes. *The Journal of Neuroscience, 24*(30), 6650–6658.

Reichardt, L. F. (2006). Neurotrophin-regulated signaling pathways. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 361*, 1545–1564.

Reiter-Funk, C. K., & Dohrman, D. P. (2005). Chronic ethanol exposure increases microtubule content in PC12 cells. *BMC Neuroscience, 6*, 16.

Resnicoff, M., Cui, S., Coppola, D., Hoek, J. B., & Rubin, R. (1996). Ethanol-induced inhibition of cell proliferation is modulated by insulin-like growth factor-I receptor levels. *Alcoholism, Clinical and Experimental Research, 20*(5), 961–966.

Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., et al. (2001). Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Molecular Endocrinology, 15*, 1748–1757.

Roberto, M., Nelson, T. E., Ur, C. L., Brunelli, M., Sanna, P. P., & Gruol, D. L. (2003). The transient depression of hippocampal CA1 LTP induced by chronic intermittent ethanol exposure is associated with an inhibition of the MAP kinase pathway. *The European Journal of Neuroscience, 17*, 1646–1654.

Rocamora, N., Pascual, M., Acsàdy, L., de Lecea, L., Freund, T. F., & Soriano, E. (1996). Expression of NGF and NT3 mRNAs in hippocampal interneurons innervated by the GABAergic septohippocampal pathway. *The Journal of Neuroscience, 16*(12), 3991–4004.

Roivainen, R., McMahon, T., & Messing, R. O. (1993). Protein kinase C isozymes that mediate enhancement of neurite outgrowth by ethanol and phorbol esters in PC12 cells. *Brain Research, 624*(1–2), 85–93.

Roivainen, R., Hundle, B., & Messing, R. O. (1995). Ethanol enhances growth factor activation of mitogen-activated protein kinases by a protein kinase C-dependent mechanism. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 1891–1895.

Rojdmark, S., & Brismar, K. (2001). Decreased IGF-I bioavailability after ethanol abuse in alcoholics: Partial restitution after short-term abstinence. *Journal of Endocrinological Investigation, 24*(7), 476–482.

Rojdmark, S., Rydvald, Y., Aquilonius, A., & Brismar, K. (2000). Insulin-like growth factor (IGF)-1 and IGF-binding protein-1 concentrations in serum of normal subjects after alcohol

ingestion: Evidence for decreased IGF-1 bioavailability. *Clinical Endocrinology, 52*(3), 313–318.

Ron, D., & Messing, R. O. (2013). Signaling pathways mediating alcohol effects. *Current Topics in Behavioral Neurosciences, 13*, 87–126.

Rossignol, D. A., & Frye, R. E. (2012). A review of research trends in physiological abnormalities in autism spectrum disorders: Immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. *Molecular Psychiatry, 17*(4), 389–401.

Rueda, A. V., Teixeira, A. M., Yonamine, M., & Camarini, R. (2012). Environmental enrichment blocks ethanol-induced locomotor sensitization and decreases BDNF levels in the prefrontal cortex in mice. *Addiction Biology, 17*(4), 736–745.

Ryan, S. H., Williams, J. K., & Thomas, J. D. (2008). Choline supplementation attenuates learning deficits associated with neonatal alcohol exposure in the rat: Effects of varying the timing of choline administration. *Brain Research, 27*(1237), 91–100.

Sakata-Haga, H., Sawada, K., Hisano, S., & Fukui, Y. (2001). Abnormalities of cerebellar foliation in rats prenatally exposed to ethanol. *Acta Neuropathologica, 102*(1), 36–40.

Schmitt, M., Gleiter, C. H., Nichol, J. L., Pralle, L., Hasselblatt, M., Poser, W., & Ehrenreich, H. (1999). Haematological abnormalities in early abstinent alcoholics are closely associated with alterations in thrombopoietin and erythropoietin serum profiles. *Thrombosis and Haemostasis, 82*(5), 1422–1427.

Sivaswamy, S., Neafsey, E. J., & Collins, M. A. (2010). Neuroprotective preconditioning of rat brain cultures with ethanol: Potential transduction by PKC isoforms and focal adhesion kinase upstream of increases in effector heat shock proteins. *European Journal of Neuroscience, 32* (11), 1800–1812.

Söderström, S., & Ebendal, T. (1995). The levels of neurotrophin-3 protein in the rat brain determined by enzyme immunoassay show a pattern distinct from nerve growth factor. *Neuroscience Letters, 189*(1), 5–8.

Stornetta, R. L., & Zhu, J. J. (2011). Ras and Rap signaling in synaptic plasticity and mental disorders. *Neuroscientist, 17*(1), 54–78.

Sajdel-Sulkowska, E. M., Xu, M., McGinnis, W., & Koibuchi, N. (2011). Brain region-specific changes in oxidative stress and neurotrophin levels in autism spectrum disorders (ASD). *Cerebellum, 10*(1), 43–48.

Sanchez, M. P., Silos-Santiago, I., Frison, J., He, B., Lira, S. A., & Barbacid, M. (1996). Renalagenesis and the absence of enteric neurons in mice lacking GDNF. *Nature, 382*(6586), 70–73.

Sanchez-Ortiz, E., Yui, D., Song, D., Li, Y., Rubenstein, J. L., Reichardt, L. F., & Parada, L. F. (2012). TrkA gene ablation in basal forebrain results in dysfunction of the cholinergic circuitry. *The Journal of Neuroscience, 32*(12), 4065–4079.

Sariola, H., & Saarma, M. (2003). Novel functions and signalling pathways for GDNF. *Journal of Cell Science, 116*(Pt 19), 3855–3862.

Scarpi, D., Cirelli, D., Matrone, C., Castronovo, G., Rosini, P., Occhiato, E. G., Romano, F., Bartali, L., Clemente, A. M., Bottegoni, G., Cavalli, A., De Chiara, G., Bonini, P., Calissano, P., Palamara, A. T., Garaci, E., Torcia, M. G., Guarna, A., & Cozzolino, F. (2012). Low molecular weight, non-peptidic agonists of TrkA receptor with NGF-mimetic activity. *Cell Death and Disease, 3*, e389.

Scaruffi, P., Cusano, R., Dagnino, M., & Tonini, G. P. (1999). Detection of DNA polymorphisms and point mutations of high-affinity nerve growth factor receptor (TrkA) in human neuroblastoma. *International Journal of Oncology, 14*(5), 935–938.

Schecterson, L. C., & Bothwell, M. (2010). Neurotrophin receptors: Old friends with new partners. *Developmental Neurobiology, 70*(5), 332–338.

Seabold, G. K., Luo, J., & Miller, M. W. (1998). Effect of ethanol on neurotrophin-mediated cell survival and receptor expression in cultures of cortical neurons. *Brain Research. Developmental Brain Research, 108*, 139–145.

Segal, R. A., Takahashi, H., & McKay, R. D. (1992). Changes in neurotrophin responsiveness during the development of cerebellar granule neurons. *Neuron, 9*, 1041–1152.

Seiler, M., & Schwab, M. E. (1984). Specific retrograde transport of nerve growth factor (NGF) from neocortex to nucleus basalis in the rat. *Brain Research, 300*(1), 33–39.

Shelton, D. L., & Reichardt, L. F. (1986). Studies on the expression of the beta nerve growth factor (NGF) gene in the central nervous system: Level and regional distribution of NGF mRNA suggest that NGF functions as a trophic factor for several distinct populations of neurons. *Proceedings of the National Academy of Sciences of the United States of America, 83*(8), 2714–2718.

Shi, G. X., Jin, L., & Andres, D. A. (2010). Src-dependent TrkA transactivation is required for pituitary adenylate cyclase-activating polypeptide 38-mediated Rit activation and neuronal differentiation. *Molecular Biology of the Cell, 21*(9), 1597–1608.

Shimoke, K., Yamagishi, S., Yamada, M., Ikeuchi, T., & Hatanaka, H. (1999). Inhibition of phosphatidylinositol 3-kinase activity elevates c-Jun N-terminal kinase activity in apoptosis of cultured cerebellar granule neurons. *Brain Research. Developmental Brain Research, 112*(2), 245–253.

Shin, S., Stewart, R., Ferri, C. P., Kim, J. M., Shin, I. S., Kim, S. W., Yang, S. J., & Yoon, J. S. (2010). An investigation of associations between alcohol use disorder and polymorphisms on ALDH2, BDNF, 5-HTTLPR, and MTHFR genes in older Korean men. *International Journal of Geriatric Psychiatry, 25*(5), 441–448.

Smeyne, R. J., Klein, R., Schnapp, A., Long, L. K., Bryant, S., Lewin, A., Lira, S. A., & Barbacid, M. (1994). Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. *Nature, 368*(6468), 246–249.

Sobreviela, T., Clary, D. O., Reichardt, L. F., Brandabur, M. M., Kordower, J. H., & Mufson, E. J. (1994). TrkA-immunoreactive profiles in the central nervous system: Colocalization with neurons containing p75 nerve growth factor receptor, choline acetyltransferase, and serotonin. *The Journal of Comparative Neurology, 350*(4), 587–611.

Soliman, F., Glatt, C. E., Bath, K. G., Levita, L., Jones, R. M., Pattwell, S. S., Jing, D., Tottenham, N., Amso, D., Somerville, L. H., Voss, H. U., Glover, G., Ballon, D. J., Liston, C., Teslovich, T., Van Kempen, T., Lee, F. S., & Casey, B. J. (2010). A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science, 327*(5967), 863–866.

Sotelo, C. (2004). Cellular and genetic regulation of the development of the cerebellar system. *Progress in Neurobiology, 72*, 295–339.

Spengler, D., Waeber, C., Pantaloni, C., Holsboer, F., Bockaert, J., Seeburg, P. H., & Journot, L. (1993). Differential signal transduction by five splice variants of the PACAP receptor. *Nature, 365*(6442), 170–175.

Strand, A. D., Baquet, Z. C., Aragaki, A. K., Holmans, P., Yang, L., Cleren, C., et al. (2007). Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. *The Journal of Neuroscience, 27*, 11758–11768.

Studer, L., Spenger, C., Luthman, J., & Seiler, R. W. (1994). NGF increases neuritic complexity of cholinergic interneurons in organotypic cultures of neonatal rat striatum. *The Journal of Comparative Neurology, 340*(2), 281–296.

Su, N., Zhang, L., Fei, F., Hu, H., Wang, K., Hui, H., Jiang, X. F., Li, X., Zhen, H. N., Li, J., Cao, B. P., Dang, W., Qu, Y., & Zhou, F. (2011). The brain-derived neurotrophic factor is associated with alcohol dependence-related depression and antidepressant response. *Brain Research, 1415*, 119–126.

Swanson, D. J., King, M. A., Walker, D. W., & Heaton, M. B. (1995). Chronic prenatal ethanol exposure alters the normal ontogeny of choline acetyltransferase activity in the rat septohippocampal system. *Alcoholism, Clinical and Experimental Research, 19*(5), 1252–1260.

Swanson, D. J., Tonjes, L., King, M. A., Walker, D. W., & Heaton, M. B. (1996). Influence of chronic prenatal ethanol on cholinergic neurons of the septohippocampal system. *The Journal of Comparative Neurology, 364*(1), 104–112.

Szabò, G., & Hoffman, P. L. (1995). Brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4/5 maintain functional tolerance to ethanol. *European Journal of Pharmacology, 287*, 35–41.

Tanaka, K., Hashimoto, H., Shintani, N., Yamamoto, A., & Baba, A. (2004). Reduced hypothermic and hypnotic responses to ethanol in PACAP-deficient mice. *Regulatory Peptides, 123*(1–3), 95–98.

Tapia-Arancibia, L., Rage, F., Givalois, L., Dingeon, P., Arancibia, S., & Beauge, F. (2001). Effects of alcohol on brain-derived neurotrophic factor mRNA expression in discrete regions of the rat hippocampus and hypothalamus. *Journal of Neuroscience Research, 63*, 200–208.

Tararuk, T., Ostman, N., Li, W., Bjorkblom, B., Padzik, A., Zdrojewska, J., Hongisto, V., Herdegen, T., Konopka, W., Courtney, M. J., & Coffey, E. T. (2006). JNK1 phosphorylation of SCG10 determines microtubule dynamics and axodendritic length. *The Journal of Cell Biology, 173*(2), 265–277.

Tateno, M., Ukai, W., Ozawa, H., Yamamoto, M., Toki, S., Ikeda, H., & Saito, T. (2004). Ethanol inhibition of neural stem cell differentiation is reduced by neurotrophic factors. *Alcoholism, Clinical and Experimental Research, 28*(8 Suppl Proceedings), 134S–138S.

Tessarollo, L., Tsoulfas, P., Martin-Zanca, D., Gilbert, D. J., Jenkins, N. A., Copeland, N. G., & Parada, L. F. (1993). trkC, a receptor for neurotrophin-3, is widely expressed in the developing nervous system and in non-neuronal tissues. *Development, 118*(2), 463–475.

Timmusk, T., Palm, K., Metsis, M., Reintam, T., Paalme, V., Saarma, M., et al. (1993). Multiple promoters direct tissue-specific expression of the rat BDNF gene. *Neuron, 10*, 475–489.

Tongiorgi, E., Domenici, L., & Simonato, M. (2006). What is the biological significance of BDNF mRNA targeting in the dendrites? Clues from epilepsy and cortical development. *Molecular Neurobiology, 33*, 17–32.

Trupp, M., Ryden, M., Jörnvall, H., Funakoshi, H., Timmusk, T., Arenas, E., & Ibanez, C. F. (1995). Peripheral expression and biological activities of GDNF, a new neurotrophic factor for avian and mammalian peripheral neurons. *The Journal of Cell Biology, 130*(1), 137–148.

Tsai, S. J., Liao, D. L., Yu, Y. W., Chen, T. J., Wu, H. C., Lin, C. H., Cheng, C. Y., & Hong, C. J. (2005). A study of the association of (Val66Met) polymorphism in the brain-derived neurotrophic factor gene with alcohol dependence and extreme violence in Chinese males. *Neuroscience Letters, 381*(3), 340–343.

Tsuji, R., Guizzetti, M., & Costa, L. G. (2003). In vivo ethanol decreases phosphorylated MAPK and p70S6 kinase in the developing rat brain. *Neuroreport, 14*, 1395–1399.

Tsuji, R., Fattori, V., Abe, S., Costa, L. G., & Kobayashi, K. (2008). Effects of postnatal ethanol exposure at different developmental phases on neurotrophic factors and phosphorylated proteins on signal transductions in rat brain. *Neurotoxicology and Teratology, 30*(3), 228–236.

Tang, N., He, M., O'Riordan, M. A., Farkas, C., Buck, K., Lemmon, V., Bearer, C. F. (2006). Ethanol inhibits L1 cell adhesion molecule activation of mitogen-activated protein kinases. *Journal of Neurochemistry, 96*(5), 1480–1490.

Terracciano, A., Piras, M. G., Lobina, M., Mulas, A., Meirelles, O., Sutin, A. R., Chan, W., Sanna, S., Uda, M., Crisponi, L., & Schlessinger, D. (2011). Genetics of serum BDNF: Meta-analysis of the Val66Met and genome-wide association study. *World Journal of Biological Psychiatry*.

Trajkovska, V., Marcussen, A. B., Vinberg, M., Hartvig, P., Aznar, S., & Knudsen, G. M. (2007). Measurements of brain-derived neurotrophic factor: Methodological aspects and demographical data. *Brain Research Bulletin, 73*(1–3), 143–149.

Uhl, G. R., Liu, Q. R., Walther, D., Hess, J., & Naiman, D. (2001). Polysubstance abuse-vulnerability genes: Genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. *American Journal of Human Genetics, 69*, 1290–1300.

Umene-Nakano, W., Yoshimura, R., Ikenouchi-Sugita, A., Hori, H., Hayashi, K., Ueda, N., & Nakamura, J. (2009). Serum levels of brain-derived neurotrophic factor in comorbidity of depression and alcohol dependence. *Human Psychopharmacology, 24*(5), 409–413.

Vangipuram, S. D., Grever, W. E., Parker, G. C., & Lyman, W. D. (2008). Ethanol increases fetal human neurosphere size and alters adhesion molecule gene expression. *Alcoholism, Clinical and Experimental Research, 32*(2), 339–347.

Vaudry, D., Hamelink, C., Damadzic, R., Eskay, R. L., Gonzalez, B., & Eiden, L. E. (2005). Endogenous PACAP acts as a stress response peptide to protect cerebellar neurons from ethanol or oxidative insult. *Peptides, 26*(12), 2518–2524.

Ventimiglia, R., Mather, P. E., Jones, B. E., & Lindsay, R. M. (1995). The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. *The European Journal of Neuroscience, 7*(2), 213–222.

Vig, P. J., Subramony, S. H., D'Souza, D. R., Wei, J., & Lopez, M. E. (2006). Intranasal administration of IGF-I improves behavior and Purkinje cell pathology in SCA1 mice. *Brain Research Bulletin, 69*(5), 573–579.

Vigers, A. J., Baquet, Z. C., & Jones, K. R. (2000). Expression of neurotrophin-3 in the mouse forebrain: Insights from a targeted LacZ reporter. *The Journal of Comparative Neurology, 416*(3), 398–415.

Villegas, S. N., Njaine, B., Linden, R., & Carri, N. G. (2006). Glial-derived neurotrophic factor (GDNF) prevents ethanol (EtOH) induced B92 glial cell death by both PI3K/AKT and MEK/ERK signaling pathways. *Brain Research Bulletin, 71*(1–3), 116–126.

Van Vulpen, E. H., & Van Der Kooy, D. (1999). NGF facilitates the developmental maturation of the previously committed cholinergic interneurons in the striatal matrix. *Journal of Comparative Neurology, 411*(1), 87–96.

Wang, M. C., Bohmann, D., & Jasper, H. (2003). JNK signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*. *Developmental Cell, 5*(5), 811–816.

Wang, Z. Y., Miki, T., Lee, K. Y., Yokoyama, T., Kusaka, T., Sumitani, K., Warita, K., Matsumoto, Y., Yakura, T., Hosomi, N., Ameno, K., Bedi, K. S., & Takeuchi, Y. (2010). Short-term exposure to ethanol causes a differential response between nerve growth factor and brain-derived neurotrophic factor ligand/receptor systems in the mouse cerebellum. *Neuroscience, 165*(2), 485–491.

Welch, K. A. (2011). Neurological complications of alcohol and misuse of drugs. *Practical Neurology, 11*(4), 206–219.

Wentzel, P., & Eriksson, U. J. (2009). Altered gene expression in neural crest cells exposed to ethanol in vitro. *Brain Research, 1305*(Suppl), S50–S60.

West, J. R. (1993). Acute and long-term changes in the cerebellum following developmental exposure to ethanol. *Alcohol and Alcoholism. Supplement, 2*, 199–202.

Weston, C. R., & Davis, R. J. (2007). The JNK signal transduction pathway. *Current Opinion in Cell Biology, 19*, 142–149.

Whittemore, S. R., Ebendal, T., Lärkfors, L., Olson, L., Seiger, A., Strömberg, I., & Persson, H. (1986). Development and regional expression of beta nerve growth factor messenger RNA and protein in the rat central nervous system. *Proceedings of the National Academy of Sciences of the United States of America, 83*(3), 817–821.

Wojnar, M., Brower, K. J., Strobbe, S., Ilgen, M., Matsumoto, H., Nowosad, I., Sliwerska, E., & Burmeister, M. (2009). Association between Val66Met brain-derived neurotrophic factor (BDNF) gene polymorphism and post-treatment relapse in alcohol dependence. *Alcoholism, Clinical and Experimental Research, 33*(4), 693–702.

Woo, N. H., Teng, H. K., Siao, C. J., Chiaruttini, C., Pang, P. T., Milner, T. A., et al. (2005). Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nature Neuroscience, 8*, 1069–1077.

Wooten, M. W., & Ewald, S. J. (1991). Alcohols synergize with NGF to induce early differentiation of PC12 cells. *Brain Research, 550*(2), 333–339.

Xu, K., Anderson, T. R., Neyer, K. M., Lamparella, N., Jenkins, G., Zhou, Z., et al. (2007). Nucleotide sequence variation within the human tyrosine kinase B neurotrophin receptor gene: Association with antisocial alcohol dependence. *The Pharmacogenomics Journal, 7*, 368–379.

Xu, S., Chan, T., Shah, V., Zhang, S., Pletcher, S. D., & Roman, G. (2012). The propensity for consuming ethanol in *Drosophila* requires rutabaga adenylyl cyclase expression within mushroom body neurons. *Genes, Brain, and Behavior, 11*(6), 727–739.

Yaka, R., He, D. Y., Phamluong, K., & Ron, D. (2003a). Pituitary adenylate cyclase-activating polypeptide (PACAP(1–38)) enhances *N*-methyl-D-aspartate receptor function and brain-derived neurotrophic factor expression via RACK1. *The Journal of Biological Chemistry, 278*(11), 9630–9638.

Yaka, R., Phamluong, K., & Ron, D. (2003b). Scaffolding of Fyn kinase to the NMDA receptor determines brain region sensitivity to ethanol. *The Journal of Neuroscience, 23*(9), 3623–3632.

Yaka, R., Tang, K. C., Camarini, R., Janak, P. H., & Ron, D. (2003c). Fyn kinase and NR2B-containing NMDA receptors regulate acute ethanol sensitivity but not ethanol intake or conditioned reward. *Alcoholism, Clinical and Experimental Research, 27*(11), 1736–1742.

Yan, Q. S., Feng, M. J., & Yan, S. E. (2005). Different expression of brain-derived neurotrophic factor in the nucleus accumbens of alcohol-preferring (P) and -nonpreferring (NP) rats. *Brain Research, 1035*, 215–218.

Yanagisawa, H., Komuta, Y., Kawano, H., Toyoda, M., & Sango, K. (2010). Pleiotrophin induces neurite outgrowth and up-regulates growth-associated protein (GAP)-43 mRNA through the ALK/GSK3beta/beta-catenin signaling in developing mouse neurons. *Neuroscience Research, 66*(1), 111–116.

Yeiser, E. C., Rutkoski, N. J., Naito, A., Inoue, J., & Carter, B. D. (2004). Neurotrophin signaling through the p75 receptor is deficient in traf6−/− mice. *The Journal of Neuroscience, 24*(46), 10521–10529.

Yeo, G. S., Connie Hung, C. C., Rochford, J., Keogh, J., Gray, J., Sivaramakrishnan, S., et al. (2004). A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. *Nature Neuroscience, 7*, 1187–1189.

Yoon, S. J., Roh, S., Lee, H., Lee, J. Y., Lee, B. H., Kim, Y. K., & Kim, D. J. (2006). Possible role of nerve growth factor in the pathogenesis of alcohol dependence. *Alcoholism, Clinical and Experimental Research, 30*(6), 1060–1065.

Young, C., Straiko, M. M., Johnson, S. A., Creeley, C., & Olney, J. W. (2008). Ethanol causes and lithium prevents neuroapoptosis and suppression of pERK in the infant mouse brain. *Neurobiology of Disease, 31*(3), 355–360.

Yeaney, N. K., He, M., Tang, N., Malouf, A. T., O'Riordan, M. A., Lemmon, V., & Bearer, C. F. (2009). Ethanol inhibits L1 cell adhesion molecule tyrosine phosphorylation and dephosphorylation and activation of pp60(src). *Journal of Neurochemistry, 110*(3), 779–790.

Yoshimura, R,, Kishi, T., Suzuki, A., Umene-Nakano, W., Ikenouchi-Sugita, A., Hori, H., Otani, K., Iwata, N., & Nakamura, J. (2011). The brain-derived neurotrophic factor (BDNF) polymorphism Val66Met is associated with neither serum BDNF level nor response to selective serotonin reuptake inhibitors in depressed Japanese patients. *Progress in Neuropsychopharmacology and Biological Psychiatry, 35*(4), 1022–1025.

Zhang, H., Ozbay, F., Lappalainen, J., Kranzler, H. R., van Dyck, C. H., Charney, D. S., Price, L. H., Southwick, S., Yang, B. Z., Rasmussen, A., & Gelernter, J. (2006). Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. *American Journal of Medical Genetics B Neuropsychiatric Genetics, 141B*(4), 387–393.

Zhang, S., Zettler, C., Cupler, E. J., Hurtado, P., Wong, K., & Rush, R. A. (2000). Neurotrophin 4/5 immunoassay: Identification of sources of errors for the quantification of neurotrophins. *Journal of Neuroscience Methods, 99*(1–2), 119–127.

Zahr, N. M., Kaufman, K. L., & Harper, C. G. (2011). Clinical and pathological features of alcohol-related brain damage. *Nature Reviews. Neurology, 7*(5), 284–294.

Zanardini, R., Fontana, A., Pagano, R., Mazzaro, E., Bergamasco, F., Romagnosi, G., Gennarelli, M., & Bocchio-Chiavetto, L. (2011). Alterations of brain-derived neurotrophic factor serum levels in patients with alcohol dependence. *Alcoholism, Clinical and Experimental Research, 8*, 1529–1533.

Zeidman, R., Löfgren, B., Påhlman, S., & Larsson, C. (1999). PKCepsilon, via its regulatory domain and independently of its catalytic domain, induces neurite-like processes in neuroblastoma cells. *The Journal of Cell Biology, 145*(4), 713–726.

Zemlyak, I., Sapolsky, R., & Gozes, I. (2009). NAP protects against cytochrome c release: Inhibition of the initiation of apoptosis. *European Journal of Pharmacology, 618*(1–3), 9–14.

Zhou, X. F., & Rush, R. A. (1994). Localization of neurotrophin-3-like immunoreactivity in the rat central nervous system. *Brain Research, 643*(1–2), 162–172.

Zhou, F. C., Fang, Y., & Goodlett, C. (2008). Peptidergic agonists of activity-dependent neurotrophic factor protect against prenatal alcohol-induced neural tube defects and serotonin neuron loss. *Alcoholism, Clinical and Experimental Research, 32*(8), 1361–1371.

Zorner, B., Wolfer, D. P., Brandis, D., Kretz, O., Zacher, C., Madani, R., et al. (2003). Forebrain-specific trkB-receptor knockout mice: Behaviorally more hyperactive than "depressive". *Biological Psychiatry, 54*, 972–982.

Zucca, S., & Valenzuela, C. F. (2010). Low concentrations of alcohol inhibit BDNF-dependent GABAergic plasticity via L-type Ca^{2+} channel inhibition in developing CA3 hippocampal pyramidal neurons. *The Journal of Neuroscience, 30*(19), 6776–6781.

Neurotrophic Factors and NeuroAIDS: A Lesson from Brain-Derived Neurotrophic Factor

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Contents

Abstract

Human immunodeficiency virus type 1 (HIV) causes mild or severe neurological problems, termed HIV-associated neurocognitive disorder (HAND), even when HIV patients receive antiretroviral therapy. Thus, novel adjunctive therapies are necessary to reduce or abolish the neurotoxic effect of HIV. However, new therapies require a better understanding of the molecular and cellular mechanisms of HIV-induced neurotoxicity. HAND subjects are characterized by being profoundly depressed, and they experience deficits in memory, learning, and movements. These deficits resemble those occurring in premature brain aging.

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Thus, it appears that HIV diminishes neuronal survival, along with reduced neuronal connections. These two phenomena should not occur in the adult brain when synaptic plasticity is promoted by neurotrophic factors. In particular, synaptic plasticity is enhanced by brain-derived neurotrophic factor (BDNF), a potent neurotrophic factor that is present in abundance in the adult synapses. This chapter will outline experimental evidence as well as present emerging concepts for the use of BDNF as an adjunct therapy to prevent HIV-mediated neuronal degeneration and restore the loss of synaptic connections.

Keywords

Acquired immunodeficiency syndrome • Adeno associated viruses • Apoptosis • Basal ganglia • BDNF synthesis • Brain-derived neurotrophic factor • Caspase-3 • CCR5 • CXCR4 • Dendrite branching • Dendritic • Excitotoxicity • Frontal cortex • gp120 • HIV-associated dementia • HIV-associated neurocognitive disorders • HIV encephalitis • Human immunodeficiency virus type-1 • Microglia • Nerve growth factor • Neurotrophic factors • Neurotrophins • Nigrostriatal dopamine neurons • p75NTR • Proneurotrophins • Simplification • Synaptodendritic pathology • TrkB • Viral protein

1 Introduction

Human immunodeficiency virus type 1 (HIV) which depletes the immune system causing *acquired immunodeficiency syndrome* (AIDS) also infects the central nervous system (CNS) and promotes neurological problems in more than 50 % of patients not receiving any form of antiretroviral therapy. Symptoms include profound motor and behavioral/psychosocial abnormalities that disrupt work or other activities of daily living. Abnormalities that can be manifested as attention deficit can also be seen in children.

The introduction of combined antiretroviral therapy (cART) has improved immune recovery and decreased the gravity of neurological signs and the more severe form of cognitive impairment (Sacktor et al. 1999) or *HIV-associated dementia* (HAD). By controlling the viral load, cART allows an individual with HIV to typically live longer with milder medical symptoms. Indeed, recent estimates of AIDS subjects after the advent of cART place HAD in the 1–2 % of the HIV cohort (Woods et al. 2009). Yet, cART has not eliminated mild neurocognitive deficits and asymptomatic neurocognitive impairments (McArthur et al. 2003). These deficits are referred to as *HIV-associated neurocognitive disorders* (HAND). Current estimates find that nearly 50 % of HIV patients in the United States demonstrate neuropsychological testing performance that is below expectations compared to age-, education-, gender-, and ethnicity-matched reference groups. The reason why HAND persists among treated individuals is still under debate. Some of the drugs used in cART may not reduce HIV infection in the CNS because they poorly penetrate the blood–brain barrier (BBB) or because the virus develops drug resistance. It is noteworthy that neurologically impaired individuals have a higher viral load in the cerebrospinal fluid

(CSF) than in plasma. Indeed, clinical evidence has shown that the CNS of these individuals is an HIV sanctuary site (Masliah et al. 2000) unless they initiate a highly CNS-penetrating antiretroviral regimen (Letendre et al. 2004).

HIV does not infect neurons; nevertheless, postmortem brains of HAD subjects have shown neuronal loss accompanied by synaptic simplifications (Everall et al. 2005). Thus, our great hope lies in our ability to develop alternative adjunct therapies that can provide a more efficient treatment of HIV-mediated neuronal degeneration. The search for pharmacological compounds that prevent HIV neurotoxicity must rely on the general concept of the so-called biological therapies, which are defined by two principal features: they require a better understanding of the molecular and cellular mechanisms of HIV neurotoxicity, and, ideally, they use physiological compounds utilized by the CNS to prevent neuronal injury. These compounds include *neurotrophic factors*, which are naturally occurring diffusible polypeptides that promote survival of a variety of CNS cells and are equally essential for inducing differentiation of surviving neurons into their mature phenotypes (Reichardt 2001). This chapter will introduce new emerging concepts and principles in the use of neurotrophic factors as an adjunct therapy to prevent HIV-mediated neuronal degeneration and restore the loss of synaptic connections.

2 Brain-Derived Neurotrophic Factor: The Most Abundant Neurotrophin in the Adult Brain

2.1 Neurotrophins

HAND pathology includes loss of both synaptic connections and neuronal differentiation (Ellis et al. 2007). In addition, HIV promotes neuronal apoptosis, especially in children (Gelbard and Epstein 1995). Thus, this disease is an excellent target for a neurotrophic factors-based therapy. In fact, neurotrophic factors have been shown to reduce synaptic and axonal degeneration mediated by a number of neurotoxins and to interfere with the fundamental mechanism of apoptotic cell death in numerous conditions (Reichardt 2001). One of the most potent neurotrophic factors is *brain-derived neurotrophic factor* (BDNF) (Barde et al. 1982), the most abundant member of the neurotrophin family of trophic factors in the adult CNS that also includes *nerve growth factor* (NGF) (Levi-Montalcini 1987), neurotrophin-3, and neurotrophin-4 (Maisonpierre et al. 1990).

BDNF exerts multiple neurotrophic activities during development as well as in the adult CNS. After its initial discovery by Barde et al. (1982), interest in BDNF grew due to its potent effect on cortical (Cabelli et al. 1995) and subcortical plasticity (Lyons et al. 1999; Mamounas et al. 1995). Neurotrophic effects include modulation of dendritic branching and spines in the cortex and long-term potentiation in the hippocampus (Patterson et al. 1996; Zakharenko et al. 2003). Through these properties, BDNF plays a critical role in learning and memory and preservation of cortical circuits. Conversely, a reduction of BDNF secretion/activity is

responsible for the loss of cortical and hippocampal synapses and fear learning. This has been demonstrated not only in animals but also in humans. In fact, impaired learning and memory have been observed in both human subjects (Egan et al. 2003) and mice (Soliman et al. 2010) in which the regulated BDNF secretion is reduced due to a single nucleotide polymorphism in the BDNF gene that encodes a valine (Val) to methionine (Met) substitution at codon 66 (Val66Met). In addition, correlative studies of human chronic neurodegenerative diseases characterized by reduced BDNF levels/activity have confirmed the role of BDNF in adult plasticity. For instance, a deficiency in BDNF synthesis has been described in postmortem brains (Phillips et al. 1991) or cerebrospinal fluid (Laske et al. 2007) of patients with Alzheimer's disease (AD), which is categorized as a disease with reduced number of neurons and connections in the cortex and hippocampus as well as impaired short-term memory (Scheff and Price 2006).

BDNF is also decreased in *nigrostriatal dopamine neurons* in Parkinson's disease (PD) (Howells et al. 2000; Nagatsu et al. 2000), cortical neurons in Huntington's disease (HD) (Zuccato et al. 2001), and schizophrenia (Durany and Thome 2004). Nevertheless, results obtained from these studies may reflect secondary effects of the main pathological process rather than primary pathological events. This limitation is especially problematic for the *neurotrophins* whose expression and availability is regulated by synaptic activity (Ghosh et al. 1994). However, BDNF treatment has the potential to reduce or abolish neuronal death in several brain areas. Indeed, early experimental studies in animal models have shown a remarkable neuroprotective effect of BDNF against various neurotoxins that mimic human chronic neurodegenerative diseases such as PD and HD (Alberch et al. 2002; Frim et al. 1994; Hyman et al. 1991). Similar neuroprotection has been observed in rodents and nonhuman primates after the lesion of the perforant path, an animal model of AD (Nagahara et al. 2009). These and other experimental evidence have prompted the suggestion that BDNF could be a potential therapeutic agent for the treatment of these diseases. Most importantly, these observations suggest that an environment characterized by lower BDNF levels or activity (and other neurotrophic factors) could be a common risk factor for the development of neurological diseases. This issue rarely receives the attention it deserves, a problem that may have a negative impact on the success of future clinical trials.

2.2 Neurotrophin Receptors: Dual Mechanism of Action

The biological activity of the neurotrophins begins when they bind to a receptor complex composed of two different receptors. The first neurotrophin receptor that was characterized was a member of the tumor necrosis factor receptor family that was named p75 NGF (Johnson et al. 1986; Radeke et al. 1987); however, p75 binds to all neurotrophins with a similar affinity (Rodriguez-Tebar et al. 1990) and was therefore named *p75NTR*. The other component of the neurotrophin receptor complex is the proto-oncogene Trk. This is a receptor tyrosine kinase which, like other tyrosine kinase receptors, is activated by ligand-induced formation of non-covalently

associated receptor dimers (Kaplan et al. 1991). There are three structurally related Trks and each neurotrophin binds selectively to Trk: BDNF binds to *TrkB*, NGF to TrkA, and NT-3 to TrkC (Chao and Hempstead 1995); however, at high concentrations BDNF can also bind to TrkC (Klein et al. 1991). Both Trk and p75NTR are necessary to confer high-affinity binding to the neurotrophins and to influence their biological activities (Brennan et al. 1999; Hempstead et al. 1991). When activation of p75NTR occurs without a concomitant activation of Trk, p75NTR promotes death of oligodendrocytes (Gu et al. 1999) as well as axonal degeneration in the peripheral (Kenchappa et al. 2006) and CNS (Park et al. 2010). In addition to Trk, p75NTR can also form a complex with the so-called truncated Trk (or Trk.T1), an isoform of Trk generated from an alternative splicing which does not contain the tyrosine kinase domain. In certain neuronal populations, TrkB.T1 or TrkC.T1 can function as inhibitors of full-length Trk through a dominant-negative mechanism. Nevertheless, Trk isoforms and p75NTR also exhibit some neurotrophic properties such as neuronal crest proliferation and differentiation (Hapner et al. 1998) and regulation of neuronal branching as well as BDNF signaling (Carim-Todd et al. 2009).

p75NTR can also bind the larger precursor protein for the neurotrophins or *proneurotrophins* including proBDNF (Hempstead 2002). Proneurotrophins are abundant in the mature brain (Fahnestock et al. 2001) and can be released from neurons (Yang et al. 2009). Proneurotrophins can be cleaved in the endoplasmic reticulum by the proconvertase furin (Seidah et al. 1996) or extracellularly by proteases such as plasmin and matrix metalloproteases to be converted to mature neurotrophins (Mowla et al. 1999). Conversion of proneurotrophin to mature neurotrophin is an important process for synaptic plasticity. In fact, when processing is impaired, proNGF or proBDNF can accumulate outside neurons and induce neuronal loss by binding to p75NTR (Harrington et al. 2004; Teng et al. 2005). The mechanisms of p75NTR-mediated cell death include several "toxic" signaling pathways that are unrelated to tyrosine kinase activation but that produce ceramide (Dobrowsky et al. 1994) and activate nuclear factor kB and Jun kinase (Bhakar et al. 2003; Carter et al. 1996). Given the fact that at high concentrations neurotrophins can indiscriminately activate p75NTR, caution must be exerted in delivering neurotrophins into the mature injured brain where there is a possibility of an upregulation of p75NTR without a concomitant increase in Trk (Beattie et al. 2002; Brunello et al. 1990; Volosin et al. 2006).

3 BDNF and HIV Dementia: Basic Tenets

Subjects with the most severe form of HAND or HAD exhibit depression and mania (Grant 1990; Pumpradit et al. 2010), combined with all degrees of cognitive and motor impairments. In fact, the clinical manifestations of HAD include tremor, gait ataxia, loss of fine motor movement, mental slowing, forgetfulness, poor concentration, and behavioral abnormalities (McArthur et al. 2010). Neuroimaging studies of HAD patients have revealed generalized white matter reduction, with additional grey matter loss particularly in the basal ganglia and posterior cortex

(Aylward et al. 1995; Dal Pan et al. 1992). Neuronal loss has been confirmed in postmortem brains in the *basal ganglia* and other regions of the brain including the hippocampus and *frontal cortex* (Davies et al. 1998; Everall et al. 1993). Pathological alterations of the basal ganglia in HAD include neuronal loss in the putamen (Everall et al. 2005) and the globus pallidus (Fox et al. 1997), degeneration of nigrostriatal dopamine (DA) neurons (Itoh et al. 2000; Reyes et al. 1991), and dysfunctional DAergic transport (Wang et al. 2004). Thus, it is not surprising to note clinical features in HAD resembling those found in PD, such as the postural instability, involuntary movements, bradykinesia, and impairment in fine motor skills (Berger and Nath 1997; Koutsilieri et al. 2002; McArthur 2004).

HAD also exhibits a number of striking similarities with AD in terms of clinical manifestations and neuropathology. Indeed, cognitive impairments (moderate to severe) in HAD are similar to those observed in AD. Higher cognitive functions depend on a highly complex synaptodendritic network in the frontal cortex, and damage to this network results in abnormal output, measured as deficiencies in cognitive skills and behavior. Granted that memory and attention alterations could be also due to the high prevalence of CNS comorbidities in HIV populations (e.g., drug abuse), it is important to keep in mind that these cognitive alterations correlate with the loss of synaptodendritic networks in the cortex and hippocampus. Moreover, neuroimaging studies have revealed microstructural abnormalities in the cerebral white matter, supporting the theory of *synaptodendritic pathology* as the main cause of neuronal loss.

The notion that BDNF limits the neuronal pathology of brain diseases such as AD, PD, depression, and schizophrenia has been extensively established (Zuccato and Cattaneo 2009). Yet, very little is known about a possible role of this neurotrophin in HAD, the most severe form of HAND. As presented above, AD (Kunugi et al. 2001; Phillips et al. 1991), PD (Howells et al. 2000; Nagatsu et al. 2000), depression, and other psychiatric disorders (Duman 2004; Post 2007; Toyooka et al. 2002) have in common a decrease in *BDNF synthesis*. Thus, while BDNF treatment could be a beneficial therapeutic approach in these diseases (Nagahara and Tuszynski 2011), at the same time, it is appealing to suggest that HIV may promote neuronal degeneration by lowering BDNF levels. This hypothesis has recently been tested in animal models of HAD as well as in HIV-positive subjects. It was found that HIV reduces BDNF both in rodents (Nosheny et al. 2004) and humans (Avdoshina et al. 2011). These data pose the question as to whether HIV promotes synaptic simplification by reducing BDNF or other neurotrophic factors whose physiological role is to support and maintain synapses (Fig. 1). This theory was first developed for sensory and sympathetic neurons in which the neurotrophins regulate the density of their innervation to a target cell. Consequently, the extent of neuronal loss is regulated by interactions with postsynaptic targets producing NGF (Johnson et al. 1980; Levi-Montalcini 1987). Nevertheless, such concept can also be applied to the CNS. Indeed, low levels of BDNF have been shown to impair the innervation of the cortex by serotonergic neurons (Lyons et al. 1999).

BDNF can avidly be transported in axons and released upon activation of neuronal activity (Conner et al. 1997; Marini et al. 1998). When such transport is interrupted, the release diminishes, and, consequently, neurons do degenerate as

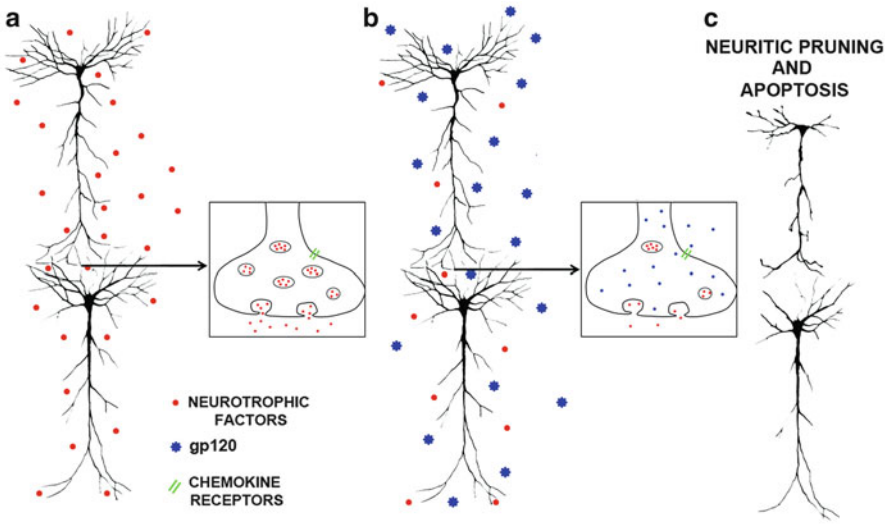

Fig. 1 *Model illustrating how HIV induces neuronal degeneration.* (**a**) Physiological environment. BDNF, released in an activity-dependent manner, promotes maintenance of neuronal architecture. BDNF can also signal back to the cell body to keep it from undergoing apoptosis. (**b**) Infected CNS. HIV infects the brain and sheds gp120. The viral protein binds to chemokine receptors and reduce BDNF trafficking and release. (**c**) Pathological brain. The lack of trophic support evokes short processes and elimination or pruning of processes that eventually culminate in apoptosis

BDNF profoundly affects neuronal homeostasis (Fig. 1) and the ability of neurons to counteract naturally occurring neuroinflammatory responses. This is applicable to different neuronal populations that express TrkB (Chao 2003), such as striatal (Zuccato et al. 2001), hippocampal (Erickson et al. 2011), and cortical (Mamounas et al. 1995; Xie et al. 2010) neurons. Conversely, BDNF inhibits neuronal *apoptosis* and cell loss in animal models of HAD both in vitro (Bachis et al. 2003) and in vivo (Nosheny et al. 2007). The neuroprotective property of BDNF raises the question of how this neurotrophin interacts with membrane receptors to avoid the destructive process of HIV. The next section reviews some experimental evidence that illustrates a unique molecular mechanism of BDNF action that might allow for the suppression of the main neurotoxic pathway of HIV. In order to fully comprehend this mechanism, a brief overview of how HIV infects cells is necessary.

4 HIV in the Brain

4.1 HIV and Chemokine Receptors

The most likely route for brain invasion by HIV is across the blood–brain barrier (BBB) (Price et al. 1988) although the choroid plexus and CSF pathway may also be

implicated (Petito et al. 1999). The primary CNS cell types that are vulnerable to HIV infection are the parenchymal microglia and the perivascular microglia/macrophages (Kramer-Hammerle et al. 2005). These monocyte lineage cells express the primary HIV receptor, CD4, a member of the immunoglobulin superfamily essential for activating helper cells, and chemokine co-receptors *CCR*5 or CXCR4 (Clapham and McKnight 2001). Chemokines represent a subset of cytokines with chemoattractant properties that are particularly important in HIV-related pathology. Indeed, both *CXCR*4 and CCR5 mediate entry of different strains of HIV into macrophages and T lymphocytes, respectively (Berger et al. 1999). Resident macrophages and microglia are the only indigenous CNS cell types to express both CD4 and CXCR4/CCR5, while neurons and astrocytes only express chemokine receptors (Albright et al. 1999). Consequently, macrophages and microglia are the primary target cells that are infected by a chemokine receptor-mediated mechanism (He et al. 1997). Infection of microglia by HIV has two potential effects: firstly, production of new virions and viral proteins and, secondly, induction of aberrant cytokine expression within the CNS (Anderson et al. 2002). Apart from microglia, astrocytes are also a possible target but are thought to be capable of only a restricted form of HIV infection in vivo (Brack-Werner 1999). The mechanism by which astrocyte infection occurs is unclear, as these cells do not express CD4, although, as stated above, they do express the chemokine receptors CCR5 and CXCR4. Recent reports have suggested a CD4-independent viral entry into astrocytes which is mediated either by mannose receptors (Liu et al. 2004) or through a gap junction mechanism (Eugenin et al. 2011). Regardless of whether astrocytes are infected or not, it is likely that these cells play a key role in the neuropathogenesis of HIV infection if their crucial functions in supporting neurons and maintaining BBB integrity are disrupted. These functions include the production of neurotrophic cytokines and buffering of substances such as glutamate which are neurotoxic at high concentrations.

There have also been occasional reports of neurons being infected with HIV, though neurons, like astrocytes, do not express CD4 but do express the chemokine receptors CCR5 and CXCR4 (Lavi et al. 1997; van der Meer et al. 2000). Given the lack of evidence for significant neuronal infection, it is thought that neuronal damage and death in AIDS is an indirect result of HIV infection of other cells in the brain. However, the mechanism(s) by which HIV infection of the CNS leads to neurocognitive disorders is not well understood and it is, therefore, the subject of intense research. The next chapters will briefly review some of the theories about HIV neurotoxicity that were obtained mostly from animal studies. The caveat is that simple animal models of AIDS/HAD needed to be carefully reevaluated in terms of relevance to the condition observed in humans.

4.2 Viral Proteins

HIV may promote neuronal injury by distinct series of events. HIV infection causes *HIV encephalitis* (Everall et al. 2005) which is characterized by neuroinflammation, astrogliosis, and microgliosis and results in an overall production and release of

pro-apoptotic chemokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα), and overexpression of CXCR4 (Mocchetti et al. 2008) and its ligand CXCL12 (Langford et al. 2002). These findings have led to the notion that activated microglia play a role in the pathogenesis of HIV encephalitis and suggested that neurodegeneration is triggered by host factors generated from the infection (Kaul et al. 2001). However, this theory does not take in account that activated (proliferating) microglia and released chemokines could be beneficial to recruit other cells in order to prevent any further damage to the infected brain (Cardona et al. 2006). An alternative mechanism of HIV-mediated neurodegeneration involves toxic proteins derived directly from the virus. The HIV genome codes for nine proteins that, apart from their roles in the viral life cycle, can damage neuronal cells and/or interfere with CNS function. One *viral protein* that can cause neuronal injury is the transactivator of transcription, or Tat, which has been shown to be released from HIV-infected cells (Ensoli et al. 1993). Tat can reduce neuronal survival by several indirect and direct mechanisms, among others the production of inflammatory cytokines such as tumor necrosis factor-α (Chen et al. 1997), impairment of mitochondrial function (Norman et al. 2007), activation of NMDA receptors (Haughey et al. 2001), and apoptosis (Kruman et al. 1998). These effects occur at concentrations lower than those needed to support viral replication. Additional viral proteins that evoke neuronal cell death include the accessory proteins Nef and Vpr (Li et al. 2005). These proteins are not released (Tornatore et al. 1994) but can cause neurotoxicity by a number of mechanisms including activation of potassium and calcium channels (Piller et al. 1996; Zegarra-Moran et al. 1999). Thus, a new theory of neurotoxicity has been suggested that calls for a direct interaction of these proteins with membrane-associated receptors and activation of a signaling pathway that promotes cell death.

One important viral protein that has been linked to the pathogenesis of HAD is the envelope protein *gp*120. This protein is necessary for infectivity as it allows the virus to change conformation after binding to chemokine co-receptors CXCR4 and CCR5 (Scarlatti et al. 1997). During viral entry gp120 can be shed from the virus and bind to CCR5 and CXCR4. This binding activates a cytotoxic pathway in both T cells and neurons (Grivel and Margolis 1999; Kaul et al. 2007). Indeed, activation of neuronal CXCR4 and CCR5 has been shown to promote neuronal loss/atrophy that is relevant to explain the neuropathology of HAD (Hesselgesser et al. 1998; Meucci et al. 1998; Zheng et al. 1999). Proof of this theory has also been obtained in transgenic mice expressing gp120 or in rats infused intracerebroventricularly with gp120. In fact, transgenic mice exhibit neuronal loss and *dendritic simplification* (Toggas et al. 1994), pathological features seen in postmortem brains of HAD subjects. Infusion of gp120 into rat brains causes apoptosis and neuronal loss (Acquas et al. 2004; Bagetta et al. 1995). Moreover, gp120 has also been shown to cause axonal degeneration (Melli et al. 2006) and dendritic injury (Everall et al. 2002; Iskander et al. 2004), two key pathological events that may account for the synaptodendritic atrophy observed in HAD (Masliah et al. 1997). These data are significant from the standpoint of experimental therapeutics because they allow the speculation that gp120 signal transduction could be a valid target for an

adjunct therapy to prevent HIV neurotoxicity. Most importantly, they indicate that experimenters can recreate an animal model of HIV neurotoxicity without the need to deal with HIV infection.

5 Chemokine Receptors and Neurotrophins: From Experiments to Therapy

5.1 Chemokine Receptors and Synaptic Injury

As described above, CXCR4 and CCR5 are crucial for gp120-induced neurotoxicity. Therefore, inhibitors of the expression/signaling of these receptors may have an important therapeutic property against HIV-mediated neurological impairment caused by CXCR4/CCR5 activation. These receptors are abundant in neurons and areas of the CNS that also express TrkB, such as the cortex, hippocampus, and striatum (Ahmed et al. 2008). CXCR4 and CCR5 are G-protein-coupled receptors that can be desensitized by tyrosine kinase intracellular signal crosstalk (Daub et al. 1996). Thus, TrkB activation may downregulate CXCR4 and CCR5. Indeed, experimental evidence has shown that BDNF and the other neurotrophins modulate the expression of CXCR4 and CCR5. This evidence includes the ability of BDNF to reduce CXCR4 in vitro (Bachis et al. 2003) and in vivo (Nosheny et al. 2007). Conversely, BDNF heterozygous animals exhibit increased levels of CXCR4 and CCR5 mRNA in the cortex, hippocampus, and striatum when compared to wild type (Ahmed et al. 2008). Moreover, in BDNF heterozygous mice, increased CXCR4 correlates with a more robust neurotoxic effect of gp120 (Nosheny et al. 2004), supporting the role of CXCR4 in gp120-mediated neuronal degeneration. Thus, from a pharmacological point of view, BDNF is particularly important as a neuroprotective compound against HIV or gp120 neurotoxicity that occurs through CXCR4 or CCR5 receptors.

The neuroprotective effect of BDNF against gp120 relies on TrkB-mediated activation of the extracellular-signal-regulated kinase (ERK) pathway (Mocchetti and Bachis 2004). Conversely, BDNF does not prevent gp120 toxicity in neurons that do not express TrkB (Ahmed et al. 2008), suggesting that BDNF may not be suitable to reduce CXCR4 and HIV toxicity in all brain cells. However, from a therapeutic perspective, this is not an issue, as trophic factors can promote plasticity on overlapping as well as dissimilar neuronal populations/compartments. For example, cholinergic neurons of the basal forebrain express TrkB as well as TrkA. These neurons are more responsive to NGF than BDNF (Koliatsos et al. 1994). Thus, NGF could be used to modulate the plasticity of cholinergic neurons and control their function in learning and memory in HAD. Moreover, basic fibroblast growth factor, another neurotrophic factor that binds to tyrosine kinase receptors and has been shown to downregulate CXCR4 (Sanders et al. 2000), could also be used to rescue Trk-negative neurons and therefore overcome the toxic effects of HIV in neurons that do not respond to the neurotrophins. Based on all the experimental data collected so far, it is clear that specific neurotrophins may be necessary to stimulate growth in a defined population of CNS neurons. The functional significance of the ability of the

neurotrophins to modulate CXCR4 remains to be established as well as whether neurotrophins act on more than one chemokine receptor.

The survival of neurons exposed to HIV is greatly enhanced by CXCR4 or CCR5 receptor antagonists (Bachis et al. 2009). Moreover, as discussed before, several groups have demonstrated that gp120 binding to chemokine receptors evokes *caspase*-3-mediated apoptosis. This is relevant for HAD because cortical neurons of HIV subjects are caspase-3 positive (Garden et al. 2002). Thus, the ability of BDNF to prevent gp120 activation of caspase-3 (Bachis et al. 2003) suggests that this neurotrophic factor will also be neuroprotective against HIV. However, post-mortem studies of HAD have also indicated that neuronal apoptosis does not correlate with dementia and that loss of dendritic spines and synaptic densities may be the primary cause leading to apoptosis (Adle-Biassette et al. 1999). Consistent with this hypothesis, gp120 reduces dendritic arborization and dendritic volume in human neurons in vitro (Iskander et al. 2004). However, such pathological changes can also be reproduced in rodent neurons in vitro by HIV. In fact, a dramatic alteration of axonal processes, characterized by fragmentation and varicosities, can be seen as quickly as 6–8 h after exposure to HIV (Fig. 2). Most importantly, the axonal disruption is preventable by BDNF. Thus, BDNF can also help neurons to survive by inhibiting the disruption of axons caused by HIV.

5.2 Delivery of BDNF

Of particular theoretical importance is the use of BDNF in HIV-positive patients as an adjunct therapy combined with cART. As discussed above, the physiological role of BDNF in promoting the survival and plasticity of adult neurons adds credence to the experimental data supporting such therapy. Moreover, in vitro models of HIV neuropathogenesis have indicated that HIV infection of the brain triggers the release of glutamate (Bezzi et al. 2001). Glutamate, through excitotoxicity, may help propagate the initial "injury" caused by inflammation to adjacent neurons. BDNF is a strong inhibitor of *excitotoxicity* whether it is induced by neurotoxins (Marini et al. 1998; Martinez-Serrano and Bjorklund 1996) or ischemia (Han and Holtzman 2000). Thus, BDNF may also be a potential therapeutic agent in preventing HIV-mediated excitotoxicity, especially considering the side effects of glutamate receptor antagonists.

A crucial question to be considered is what constitutes a safe way of administering BDNF into the CNS. A major concern is the degree of penetration of BDNF into the CNS. BDNF is a charged protein that does not efficiently cross the BBB. When delivered by direct infusion into the ventricles, its parenchymal diffusion is limited; thus, the delivery must be constant. More recently, viral vectors have been designed to deliver the gene encoding for BDNF into the CNS (Klein et al. 1998). These vectors, such as *adeno-associated viruses* (AAVs), use viral backbones to transduce host cells in a brain area of interest. AAVs are safe and capable of sustaining long-term BDNF expression in neurons (Klein et al. 1998). Using an AAV-expressing BDNF, it was shown that BDNF prevents gp120-mediated

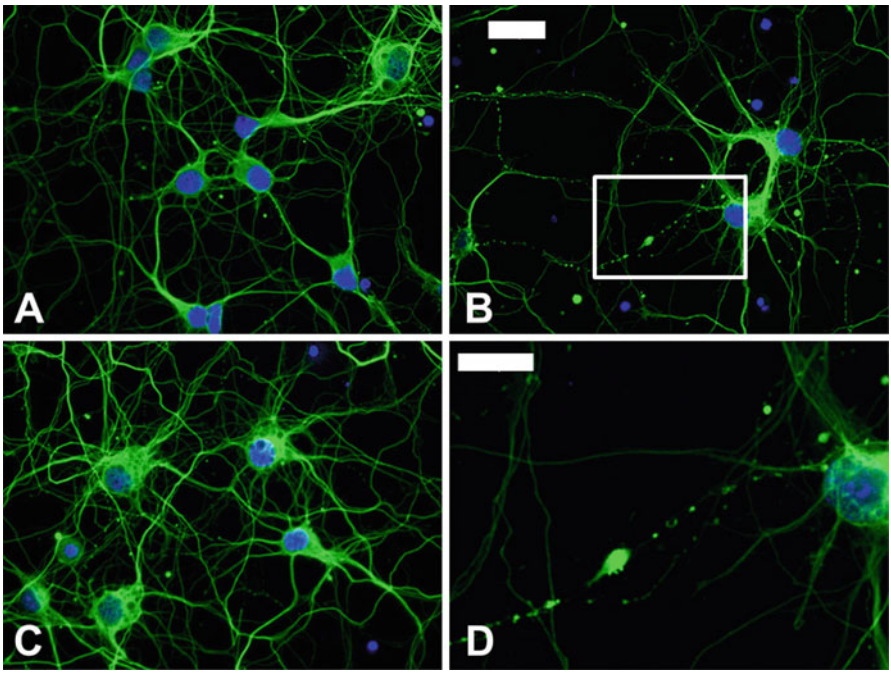

Fig. 2 *BDNF prevents HIV-mediated axonal damage*. Examples of rat cortical neurons grown for 7 days in vitro and then exposed for 6 h to (**A**) control medium (0.1 % BSA); (**B**) medium containing HIV; (**C**) medium containing BDNF and HIV; and (**D**) higher magnification of the *square* shown in (**B**) to show typical axonal varicosities. *Blue* DAPI to visualize cells, *green* βIII-tubulin to visualize neuronal processes. *Bar*: A, B, and C = 20 μm and D = 10 μm. Immunofluorescence was analyzed using a FV300 laser confocal scanning system attached to an Olympus IX70 upright microscope. Please note the lack of axonal swelling and varicosities, the hallmarks of diffuse axonal injury (Tang-Schomer et al. 2010), in BDNF-treated cultures

neuronal apoptosis in vivo (Nosheny et al. 2007), raising the possibility of using BDNF in clinical trials in the near future. However, caution must be exerted as clinical effects could be accompanied by undesired effects. For instance, BDNF also promotes sprouting of dendrites of dopaminergic neurons in gp120-treated rats (Nosheny et al. 2007). Although, in general, this is a trophic effect of the neurotrophins on neuronal processes, it may be a negative side effect of overdosing. Indeed, according to the classical view of trophic molecules, a trophic factor must be delivered in small amounts and only to specific populations of neurons to avoid such side effects.

5.3 BDNF and Immune Responses

There are other effects of BDNF that can be beneficial for HIV-positive subjects. For instance, in the immune system, BDNF decreases apoptosis of T cells

(De Santi et al. 2009; Maroder et al. 1996). These cells are depleted in AIDS. Thus, one may envision the use of BDNF in conjunction with cART to maintain the appropriate number of immune cells and delay AIDS. This effect also has a broader implication for the CNS function. In fact, as progressive neurological deficits occur after the onset of severe immunodeficiency (McArthur et al. 2010), keeping healthy immune cells will reduce "inflammation" of the immune system that can amplify nervous system damage via inflammatory cytokines. These may enter the CNS either from the periphery or produced locally by *microglia*. For example, in a state of chronic inflammation induced in a nonhuman primate by infection with simian immunodeficiency virus, the brain was positive for peripheral circulating monocytes trafficking from bone marrow. This event correlates with the severity of encephalitis (Burdo et al. 2010). Abnormal activation of cytokine and chemokine receptors in the context of HIV infection results in dendritic beading and loss of dendritic spines. These changes are accompanied by failure of long-term potentiation (LTP), which might underlie impaired learning and memory. Given the well-known property of BDNF in promoting dendritic branching and spine morphology (Horch and Katz 2002; Tanaka et al. 2008) which are key for the BDNF-mediated LTP (Figurov et al. 1996; Gartner and Staiger 2002), BDNF could be used to prevent atrophy of *dendrite branching* and memory loss. Of course such treatment will require an early diagnosis of cognitive impairments.

6 Conclusion

The dividing line between viral- and host-mediated neurotoxicities in HIV infection is neither precise nor rigid. An effective therapy aimed at treating HIV-induced CNS diseases will require both cART and adjunctive therapies that include neuroprotective and neuro-regenerative agents. Neurotrophic factors are large hydrophilic molecules that do not cross the BBB. Therefore, these molecules need to be delivered within the CNS, which lowers the enthusiasm for a chronic instrumentation in a nonclinical setting. In addition, when infused into the CNS, BDNF has limited diffusion. The delivery problems may not be severe for AIDS patients with neurodegeneration of peripheral neurons (i.e., peripheral neuropathy) as these neurons are located outside the CNS and are capable of retrogradely transporting BDNF to the soma. However, the efficiency of terminal uptake and axonal transport of trophic molecules is uncertain as there is evidence of impaired axonal transport in AIDS. One strategy, still under experimentation in humans, is the delivery of BDNF via viral vectors. A major concern about a clinical approach to increase BDNF in the CNS by this method is that an abnormal overproduction of BDNF may cause side effects that may be intolerable over a long period of time. Such effects may be seen locally (fiber spouting around the site of BDNF production) or systemically (weight loss). Thus, additional strategies must be applied to circumvent these problems. Ongoing research has demonstrated more safe and reliable methods to increase endogenous BDNF and consequently influence neuronal survival. This could replace the need for delivering BDNF by invasive approaches. For instance, physical exercise has been reported to increase BDNF

levels in the hippocampus (Cotman and Berchtold 2002) which, in turn, promotes hippocampus-based learning, synaptic plasticity, and neurogenesis (Vaynman et al. 2004). Antidepressant drugs, which are safely used in humans, also increase BDNF synthesis (Hashimoto et al. 2002; Nibuya et al. 1996). Thus, there are alternatives to minimize the use of chronic instrumentation.

Besides its traditional role as a molecule promoting survival and regeneration, BDNF is seen as a potent mediator of synaptic plasticity in the adult CNS. These effects could play a role in limiting AIDS-mediated neuronal degeneration and stimulating neuronal function. Although still tested only in animal models of HAD, the powerful properties of BDNF provide incentive for further research and raise hope for the therapeutic possibilities in the near future.

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References

Acquas, E., Bachis, A., Nosheny, R. L., Cernak, I., & Mocchetti, I. (2004). Human immunodeficiency virus type 1 protein gp120 causes neuronal cell death in the rat brain by activating caspases. *Neurotoxicity Research, 5*, 605–615.

Adle-Biassette, H., Chretien, F., Wingertsmann, L., Hery, C., Ereau, T., Scaravilli, F., Tardieu, M., & Gray, F. (1999). Neuronal apoptosis does not correlate with dementia in HIV infection but is related to microglial activation and axonal damage. *Neuropathology and Applied Neurobiology, 25*, 123–133.

Ahmed, F., Tessarollo, L., Thiele, C., & Mocchetti, I. (2008). Brain-derived neurotrophic factor modulates expression of chemokine receptors in the brain. *Brain Research, 1227*, 1–11.

Alberch, J., Perez-Navarro, E., & Canals, J. M. (2002). Neuroprotection by neurotrophins and GDNF family members in the excitotoxic model of Huntington's disease. *Brain Research Bulletin, 57*, 817–822.

Albright, A. V., Shieh, J. T., Itoh, T., Lee, B., Pleasure, D., O'Connor, M. J., Doms, R. W., & Gonzalez-Scarano, F. (1999). Microglia express CCR5, CXCR4, and CCR3, but of these, CCR5 is the principal coreceptor for human immunodeficiency virus type 1 dementia isolates. *Journal of Virology, 73*, 205–213.

Anderson, E., Zink, W., Xiong, H., & Gendelman, H. E. (2002). HIV-1-associated dementia: A metabolic encephalopathy perpetrated by virus-infected and immune-competent mononuclear phagocytes. *Journal of Acquired Immune Deficiency Syndromes, 31*(Suppl 2), S43–S54.

Avdoshina, V., Garzino-Demo, A., Bachis, A., Monaco, M. C., Maki, P. M., Tractenberg, R. E., Liu, C., Young, M. A., & Mocchetti, I. (2011). HIV-1 decreases the levels of neurotrophins in human lymphocytes. *AIDS, 25*, 1126–1128.

Aylward, E. H., Brettschneider, P. D., McArthur, J. C., Harris, G. J., Schlaepfer, T. E., Henderer, J. D., Barta, P. E., Tien, A. Y., & Pearlson, G. D. (1995). Magnetic resonance imaging measurement of gray matter volume reductions in HIV dementia. *The American Journal of Psychiatry, 152*, 987–994.

Bachis, A., Major, E. O., & Mocchetti, I. (2003). Brain-derived neurotrophic factor inhibits human immunodeficiency virus-1/gp120-mediated cerebellar granule cell death by preventing gp120 internalization. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 23*, 5715–5722.

Bachis, A., Biggio, F., Major, E. O., & Mocchetti, I. (2009). M- and T-tropic HIVs promote apoptosis in rat neurons. *Journal of Neuroimmune Pharmacology, 4*, 150–160.

Bagetta, G., Corasaniti, M. T., Berliocchi, L., Navarra, M., Finazzi-Agro, A., & Nistico, G. (1995). HIV-1 gp120 produces DNA fragmentation in the cerebral cortex of rat. *Biochemical and Biophysical Research Communications, 211*, 130–136.

Barde, Y. A., Edgar, D., & Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *The EMBO Journal, 1*, 549–553.

Beattie, M. S., Harrington, A. W., Lee, R., Kim, J. Y., Boyce, S. L., Longo, F. M., Bresnahan, J. C., Hempstead, B. L., & Yoon, S. O. (2002). ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron, 36*, 375–386.

Berger, J. R., & Nath, A. (1997). HIV dementia and the basal ganglia. *Intervirology, 40*, 122–131.

Berger, E. A., Murphy, P. M., & Farber, J. M. (1999). Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annual Review of Immunology, 17*, 657–700.

Bezzi, P., Domercq, M., Brambilla, L., Galli, R., Schols, D., De Clercq, E., Vescovi, A., Bagetta, G., Kollias, G., Meldolesi, J., & Volterra, A. (2001). CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. *Nature Neuroscience, 4*, 702–710.

Bhakar, A. L., Howell, J. L., Paul, C. E., Salehi, A. H., Becker, E. B., Said, F., Bonni, A., & Barker, P. A. (2003). Apoptosis induced by p75NTR overexpression requires Jun kinase-dependent phosphorylation of Bad. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 23*, 11373–11381.

Brack-Werner, R. (1999). Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS, 13*, 1–22.

Brennan, C., Rivas-Plata, K., & Landis, S. C. (1999). The p75 neurotrophin receptor influences NT-3 responsiveness of sympathetic neurons in vivo. *Nature Neuroscience, 2*, 699–705.

Brunello, N., Reynolds, M., Wrathall, J. R., & Mocchetti, I. (1990). Increased nerve growth factor receptor mRNA in contused rat spinal cord. *Neuroscience Letters, 118*, 238–240.

Burdo, T. H., Soulas, C., Orzechowski, K., Button, J., Krishnan, A., Sugimoto, C., Alvarez, X., Kuroda, M. J., & Williams, K. C. (2010). Increased monocyte turnover from bone marrow correlates with severity of SIV encephalitis and CD163 levels in plasma. *PLoS Pathogens, 6*, e1000842.

Cabelli, R. J., Hohn, A., & Shatz, C. J. (1995). Inhibition of ocular dominance column formation by infusion of NT-4/5 or BDNF. *Science, 267*, 1662–1666.

Cardona, A. E., Pioro, E. P., Sasse, M. E., Kostenko, V., Cardona, S. M., Dijkstra, I. M., Huang, D., Kidd, G., Dombrowski, S., Dutta, R., Lee, J.-C., Cook, D. N., Jung, S., Lira, S. A., Littman, D. R., & Ransohoff, R. M. (2006). Control of microglial neurotoxicity by the fractalkine receptor. *Nature Neuroscience, 9*, 917–924.

Carim-Todd, L., Bath, K. G., Fulgenzi, G., Yanpallewar, S., Jing, D., Barrick, C. A., Becker, J., Buckley, H., Dorsey, S. G., Lee, F. S., & Tessarollo, L. (2009). Endogenous truncated TrkB.T1 receptor regulates neuronal complexity and TrkB kinase receptor function in vivo. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 29*, 678–685.

Carter, B. D., Kaltschmidt, C., Kaltschmidt, B., Offenhauser, N., Bohm-Matthaei, R., Baeuerle, P. A., & Barde, Y. A. (1996). Selective activation of NF-kappa B by nerve growth factor through the neurotrophin receptor p75. *Science, 272*, 542–545.

Chao, M. V. (2003). Neurotrophins and their receptors: A convergence point for many signalling pathways. *Nature Reviews. Neuroscience, 4*, 299–309.

Chao, M. V., & Hempstead, B. L. (1995). p75 and Trk: a two-receptor system. *Trends in Neurosciences, 18*, 321–326.

Chen, P., Mayne, M., Power, C., & Nath, A. (1997). The Tat protein of HIV-1 induces tumor necrosis factor-alpha production. Implications for HIV-1-associated neurological diseases. *The Journal of Biological Chemistry, 272*, 22385–22388.

Clapham, P. R., & McKnight, A. (2001). HIV-1 receptors and cell tropism. *British Medical Bulletin, 58*, 43–59.

Conner, J. M., Lauterborn, J. C., Yan, Q., Gall, C. M., & Varon, S. (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence

for anterograde axonal transport. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 17*, 2295–2313.

Cotman, C. W., & Berchtold, N. C. (2002). Exercise: A behavioral intervention to enhance brain health and plasticity. *Trends in Neurosciences, 25*, 295–301.

Dal Pan, G. J., McArthur, J. H., Aylward, E., Selnes, O. A., Nance-Sproson, T. E., Kumar, A. J., Mellits, E. D., & McArthur, J. C. (1992). Patterns of cerebral atrophy in HIV-1-infected individuals: results of a quantitative MRI analysis. *Neurology, 42*, 2125–2130.

Daub, H., Weiss, F. U., Wallasch, C., & Ullrich, A. (1996). Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature, 379*, 557–560.

Davies, J., Everall, I. P., Weich, S., Glass, J., Sharer, L. R., Cho, E. S., Bell, J. E., Majteny, C., Gray, F., Scaravilli, F., & Lantos, P. L. (1998). HIV-associated brain pathology: A comparative international study. *Neuropathology and Applied Neurobiology, 24*, 118–124.

De Santi, L., Cantalupo, L., Tassi, M., Raspadori, D., Cioni, C., & Annunziata, P. (2009). Higher expression of BDNF receptor gp145trkB is associated with lower apoptosis intensity in T cell lines in multiple sclerosis. *Journal of the Neurological Sciences, 277*, 65–70.

Dobrowsky, R., Werner, M., Castellino, A., Chao, M., & Hannun, Y. (1994). Activation of the sphingomyelin cycle through the low affinity neurotrophin receptor. *Science, 265*, 1596–1599.

Duman, R. S. (2004). Role of neurotrophic factors in the etiology and treatment of mood disorders. *Neuromolecular Medicine, 5*, 11–25.

Durany, N., & Thome, J. (2004). Neurotrophic factors and the pathophysiology of schizophrenic psychoses. *European Psychiatry, 19*, 326–337.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Weinberger, D. R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257–269.

Ellis, R., Langford, D., & Masliah, E. (2007). HIV and antiretroviral therapy in the brain: Neuronal injury and repair. *Nature Reviews. Neuroscience, 8*, 33–44.

Ensoli, B., Buonaguro, L., Barillari, G., Fiorelli, V., Gendelman, R., Morgan, R. A., Wingfield, P., & Gallo, R. C. (1993). Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *Journal of Virology, 67*, 277–287.

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., Kim, J. S., Heo, S., Alves, H., White, S. M., Wojcicki, T. R., Mailey, E., Vieira, V. J., Martin, S. A., Pence, B. D., Woods, J. A., McAuley, E., & Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 3017–3022.

Eugenin, E. A., Clements, J. E., Zink, M. C., & Berman, J. W. (2011). Human immunodeficiency virus infection of human astrocytes disrupts blood–brain barrier integrity by a gap junction-dependent mechanism. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 31*, 9456–9465.

Everall, I., Luthert, P., & Lantos, P. (1993). A review of neuronal damage in human immunodeficiency virus infection: Its assessment, possible mechanism and relationship to dementia. *Journal of Neuropathology and Experimental Neurology, 52*, 561–566.

Everall, I. P., Bell, C., Mallory, M., Langford, D., Adame, A., Rockestein, E., & Masliah, E. (2002). Lithium ameliorates HIV-gp120-mediated neurotoxicity. *Molecular and Cellular Neurosciences, 21*, 493–501.

Everall, I. P., Hansen, L. A., & Masliah, E. (2005). The shifting patterns of HIV encephalitis neuropathology. *Neurotoxicity Research, 8*, 51–61.

Fahnestock, M., Michalski, B., Xu, B., & Coughlin, M. D. (2001). The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Molecular and Cellular Neurosciences, 18*, 210–220.

Figurov, A., Pozzo-Miller, L. D., Olafsson, P., Wang, T., & Lu, B. (1996). Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature, 381*, 706–709.

Fox, L., Alford, M., Achim, C., Mallory, M., & Masliah, E. (1997). Neurodegeneration of somatostatin-immunoreactive neurons in HIV encephalitis. *Journal of Neuropathology and Experimental Neurology, 56*, 360–368.

Frim, D. M., Uhler, T. A., Galpern, W. R., Beal, M. F., Breakefield, X. O., & Isacson, O. (1994). Implanted fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevent 1-methyl-4-phenylpyridinium toxicity to dopaminergic neurons in the rat. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 5104–5108.

Garden, G. A., Budd, S. L., Tsai, E., Hanson, L., Kaul, M., D'Emilia, D. M., Friedlander, R. M., Yuan, J., Masliah, E., & Lipton, S. A. (2002). Caspase cascades in human immunodeficiency virus-associated neurodegeneration. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 22*, 4015–4024.

Gartner, A., & Staiger, V. (2002). Neurotrophin secretion from hippocampal neurons evoked by long-term-potentiation-inducing electrical stimulation patterns. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 6386–6391.

Gelbard, H. A., & Epstein, L. G. (1995). HIV-1 encephalopathy in children. *Current Opinion in Pediatrics, 7*, 655–662.

Ghosh, A., Carnahan, J., & Greenberg, M. E. (1994). Requirement for BDNF in activity-dependent survival of cortical neurons. *Science, 263*, 1618–1623.

Grant, I. (1990). The neuropsychiatry of human immunodeficiency virus. *Seminars in Neurology, 10*, 267–275.

Grivel, J. C., & Margolis, L. B. (1999). CCR5- and CXCR4-tropic HIV-1 are equally cytopathic for their T-cell targets in human lymphoid tissue. *Nature Medicine, 5*, 344–346.

Gu, C., Casaccia-Bonnefil, P., Srinivasan, A., & Chao, M. V. (1999). Oligodendrocyte apoptosis mediated by caspase activation. *The Journal of Neuroscience, 19*, 3043–3049.

Han, B. H., & Holtzman, D. M. (2000). BDNF protects the neonatal brain from hypoxic-ischemic injury in vivo via the ERK pathway. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 20*, 5775–5781.

Hapner, S. J., Boeshore, K. L., Large, T. H., & Lefcort, F. (1998). Neural differentiation promoted by truncated trkC receptors in collaboration with p75(NTR). *Developmental Biology, 201*, 90–100.

Harrington, A. W., Leiner, B., Blechschmitt, C., Arevalo, J. C., Lee, R., Morl, K., Meyer, M., Hempstead, B. L., Yoon, S. O., & Giehl, K. M. (2004). Secreted proNGF is a pathophysiological death-inducing ligand after adult CNS injury. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 6226–6230.

Hashimoto, R., Takei, N., Shimazu, K., Christ, L., Lu, B., & Chuang, D.-M. (2002). Lithium induces brain-derived neurotrophic factor and activates TrkB in rodent cortical neurons: An essential step for neuroprotection against glutamate excitotoxicity. *Neuropharmacology, 43*, 1173.

Haughey, N. J., Nath, A., Mattson, M. P., Slevin, J. T., & Geiger, J. D. (2001). HIV-1 Tat through phosphorylation of NMDA receptors potentiates glutamate excitotoxicity. *Journal of Neurochemistry, 78*, 457–467.

He, J., Chen, Y., Farzan, M., Choe, H., Ohagen, A., Gartner, S., Busciglio, J., Yang, X., Hofmann, W., Newman, W., Mackay, C. R., Sodroski, J., & Gabuzda, D. (1997). CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature, 385*, 645–649.

Hempstead, B. L. (2002). The many faces of p75NTR. *Current Opinion in Neurobiology, 12*, 260–267.

Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R., Parada, L. F., & Chao, M. V. (1991). High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. *Nature, 350*, 678–683.

Hesselgesser, J., Taub, D., Baskar, P., Greenberg, M., Hoxie, J., Kolson, D. L., & Horuk, R. (1998). Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1 alpha is mediated by the chemokine receptor CXCR4. *Current Biology, 8*, 595–598.

Horch, H. W., & Katz, L. C. (2002). BDNF release from single cells elicits local dendritic growth in nearby neurons. *Nature Neuroscience, 5*, 1177–1184.

Howells, D. W., Porritt, M. J., Wong, J. Y. F., Batchelor, P. E., Kalnins, R., Hughes, A. J., & Donnan, G. A. (2000). Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. *Experimental Neurology, 166*, 127–135.

Hyman, C., Hofer, M., Barde, Y. A., Juhasz, M., Yancopoulos, G. D., Squinto, S. P., & Lindsay, R. M. (1991). BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature, 350*, 230–232.

Iskander, S., Walsh, K. A., & Hammond, R. R. (2004). Human CNS cultures exposed to HIV-1 gp120 reproduce dendritic injuries of HIV-1-associated dementia. *Journal of Neuroinflammation, 1*, 7.

Itoh, K., Mehraein, P., & Weis, S. (2000). Neuronal damage of the substantia nigra in HIV-1 infected brains. *Acta Neuropathol (Berlin), 99*, 376–384.

Johnson, E. M., Jr., Gorin, P. D., Brandeis, L. D., & Pearson, J. (1980). Dorsal root ganglion neurons are destroyed by exposure in utero to maternal antibody to nerve growth factor. *Science, 210*, 916–918.

Johnson, D., Lanahan, A., Buck, C. R., Sehgal, A., Morgan, C., Mercer, E., Bothwell, M., & Chao, M. (1986). Expression and structure of the human NGF receptor. *Cell, 47*, 545–554.

Kaplan, D. R., Hempstead, B. L., Martin-Zanca, D., Chao, M. V., & Parada, L. F. (1991). The trk proto-oncogene product: A signal transducing receptor for nerve growth factor. *Science, 252*, 554–558.

Kaul, M., Garden, G. A., & Lipton, S. A. (2001). Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature, 410*, 988–994.

Kaul, M., Ma, Q., Medders, K. E., Desai, M. K., & Lipton, S. A. (2007). HIV-1 coreceptors CCR5 and CXCR4 both mediate neuronal cell death but CCR5 paradoxically can also contribute to protection. *Cell Death and Differentiation, 14*, 296–305.

Kenchappa, R. S., Zampieri, N., Chao, M. V., Barker, P. A., Teng, H. K., Hempstead, B. L., & Carter, B. D. (2006). Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. *Neuron, 50*, 219–232.

Klein, R., Nanduri, V., Jing, S. A., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F., & Barbacid, M. (1991). The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell, 66*, 395–403.

Klein, R. L., Meyer, E. M., Peel, A. L., Zolotukhin, S., Meyers, C., Muzyczka, N., & King, M. A. (1998). Neuron-specific transduction in the rat septohippocampal or nigrostriatal pathway by recombinant adeno-associated virus vectors. *Experimental Neurology, 150*, 183–194.

Koliatsos, V. E., Price, D. L., Gouras, G. K., Cayouette, M. H., Burton, L. E., & Winslow, J. W. (1994). Highly selective effects of nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 on intact and injured basal forebrain magnocellular neurons. *The Journal of Comparative Neurology, 343*, 247–262.

Koutsilieri, E., Sopper, S., Scheller, C., ter Meulen, V., & Riederer, P. (2002). Parkinsonism in HIV dementia. *Journal of Neural Transmission, 109*, 767–775.

Kramer-Hammerle, S., Rothenaigner, I., Wolff, H., Bell, J. E., & Brack-Werner, R. (2005). Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. *Virus Research, 111*, 194–213.

Kruman, I. I., Nath, A., & Mattson, M. P. (1998). HIV-1 protein Tat induces apoptosis of hippocampal neurons by a mechanism involving caspase activation, calcium overload, and oxidative stress. *Experimental Neurology, 154*, 276–288.

Kunugi, H., Ueki, A., Otsuka, M., Isse, K., Hirasawa, H., Kato, N., Nabika, T., Kobayashi, S., & Nanko, S. (2001). A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. *Molecular Psychiatry, 6*, 83–86.

Langford, D., Sanders, V. J., Mallory, M., Kaul, M., & Masliah, E. (2002). Expression of stromal cell-derived factor 1alpha protein in HIV encephalitis. *Journal of Neuroimmunology, 127*, 115–126.

Laske, C., Stransky, E., Leyhe, T., Eschweiler, G. W., Maetzler, W., Wittorf, A., Soekadar, S., Richartz, E., Koehler, N., Bartels, M., Buchkremer, G., & Schott, K. (2007). BDNF serum and

CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls. *Journal of Psychiatric Research, 41*, 387–394.

Lavi, E., Strizki, J. M., Ulrich, A. M., Zhang, W., Fu, L., Wang, Q., O'Connor, M., Hoxie, J. A., & Gonzalez-Scarano, F. (1997). CXCR-4 (Fusin), a co-receptor for the type 1 human immunodeficiency virus (HIV-1), is expressed in the human brain in a variety of cell types, including microglia and neurons. *The American Journal of Pathology, 151*, 1035–1042.

Letendre, S. L., McCutchan, J. A., Childers, M. E., Woods, S. P., Lazzaretto, D., Heaton, R. K., Grant, I., & Ellis, R. J. (2004). Enhancing antiretroviral therapy for human immunodeficiency virus cognitive disorders. *Annals of Neurology, 56*, 416–423.

Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. *Science, 237*, 1154–1162.

Li, W., Galey, D., Mattson, M. P., & Nath, A. (2005). Molecular and cellular mechanisms of neuronal cell death in HIV dementia. *Neurotoxicity Research, 8*, 119–134.

Liu, Y., Liu, H., Kim, B. O., Gattone, V. H., Li, J., Nath, A., Blum, J., & He, J. J. (2004). CD4-independent infection of astrocytes by human immunodeficiency virus type 1: Requirement for the human mannose receptor. *Journal of Virology, 78*, 4120–4133.

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., Wihler, C., Koliatsos, V. E., & Tessarollo, L. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 15239–15244.

Maisonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M., & Yancopoulos, G. D. (1990). Neurotrophin-3: A neurotrophic factor related to NGF and BDNF. *Science, 247*, 1446–1451.

Mamounas, L. A., Blue, M. E., Siuciak, J. A., & Altar, C. A. (1995). Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 15*, 7929–7939.

Marini, A. M., Rabin, S. J., Lipsky, R. H., & Mocchetti, I. (1998). Activity-dependent release of brain-derived neurotrophic factor underlies the neuroprotective effect of N-methyl-D-aspartate. *The Journal of Biological Chemistry, 273*, 29394–29399.

Maroder, M., Bellavia, D., Meco, D., Napolitano, M., Stigliano, A., Alesse, E., Vacca, A., Giannini, G., Frati, L., Gulino, A., & Screpanti, I. (1996). Expression of trKB neurotrophin receptor during T cell development. Role of brain derived neurotrophic factor in immature thymocyte survival. *Journal of Immunology, 157*, 2864–2872.

Martinez-Serrano, A., & Bjorklund, A. (1996). Protection of the neostriatum against excitotoxic damage by neurotrophin-producing, genetically modified neural stem cells. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 16*, 4604–4616.

Masliah, E., Heaton, R. K., Marcotte, T. D., Ellis, R. J., Wiley, C. A., Mallory, M., Achim, C. L., McCutchan, J. A., Nelson, J. A., Atkinson, J. H., & Grant, I. (1997). Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. HNRC Group. The HIV Neurobehavioral Research Center. *Annals of Neurology, 42*, 963–972.

Masliah, E., DeTeresa, R. M., Mallory, M. E., & Hansen, L. A. (2000). Changes in pathological findings at autopsy in AIDS cases for the last 15 years. *AIDS, 14*, 69–74.

McArthur, J. C. (2004). HIV dementia: An evolving disease. *Journal of Neuroimmunology, 157*, 3–10.

McArthur, J. C., Haughey, N., Gartner, S., Conant, K., Pardo, C., Nath, A., & Sacktor, N. (2003). Human immunodeficiency virus-associated dementia: An evolving disease. *Journal of Neurovirology, 9*, 205–221.

McArthur, J. C., Steiner, J. P., Sacktor, N., & Nath, A. (2010). Human immunodeficiency virus-associated neurocognitive disorders: Mind the gap. *Annals of Neurology, 67*, 699–714.

Melli, G., Keswani, S. C., Fischer, A., Chen, W., & Hoke, A. (2006). Spatially distinct and functionally independent mechanisms of axonal degeneration in a model of HIV-associated sensory neuropathy. *Brain, 129*, 1330–1338.

Meucci, O., Fatatis, A., Simen, A. A., Bushell, T. J., Gray, P. W., & Miller, R. J. (1998). Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 14500–14505.

Mocchetti, I., & Bachis, A. (2004). Brain-derived neurotrophic factor activation of TrkB protects neurons from HIV-1/gp120-induced cell death. *Critical Reviews in Neurobiology, 16*, 51–57.

Mocchetti, I., Bachis, A., & Masliah, E. (2008). Chemokine receptors and neurotrophic factors: Potential therapy against aids dementia? *Journal of Neuroscience Research, 86*, 243–255.

Mowla, S. J., Pareek, S., Farhadi, H. F., Petrecca, K., Fawcett, J. P., Seidah, N. G., Morris, S. J., Sossin, W. S., & Murphy, R. A. (1999). Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 19*, 2069–2080.

Nagahara, A. H., & Tuszynski, M. H. (2011). Potential therapeutic uses of BDNF in neurological and psychiatric disorders. *Nature Reviews. Drug Discovery, 10*, 209–219.

Nagahara, A. H., Merrill, D. A., Coppola, G., Tsukada, S., Schroeder, B. E., Shaked, G. M., Wang, L., Blesch, A., Kim, A., Conner, J. M., Rockenstein, E., Chao, M. V., Koo, E. H., Geschwind, D., Masliah, E., Chiba, A. A., & Tuszynski, M. H. (2009). Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nature Medicine, 15*, 331–337.

Nagatsu, T., Mogi, M., Ichinose, H., & Togari, A. (2000). Changes in cytokines and neurotrophins in Parkinson's disease. *Journal of Neural Transmission. Supplementum, 60*, 277–290.

Nibuya, M., Nestler, E. J., & Duman, R. S. (1996). Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 16*, 2365–2372.

Norman, J. P., Perry, S. W., Kasischke, K. A., Volsky, D. J., & Gelbard, H. A. (2007). HIV-1 trans activator of transcription protein elicits mitochondrial hyperpolarization and respiratory deficit, with dysregulation of complex IV and nicotinamide adenine dinucleotide homeostasis in cortical neurons. *Journal of Immunology, 178*, 869–876.

Nosheny, R. L., Bachis, A., Acquas, E., & Mocchetti, I. (2004). Human immunodeficiency virus type 1 glycoprotein gp120 reduces the levels of brain-derived neurotrophic factor in vivo: Potential implication for neuronal cell death. *European Journal of Neuroscience, 20*, 2857–2864.

Nosheny, R. L., Amhed, F., Yakovlev, A. G., Meyer, E. M., Ren, K., Tessarollo, L., & Mocchetti, I. (2007). Brain-derived neurotrophic factor prevents the nigrostriatal degeneration induced by human immunodeficiency virus-1 glycoprotein 120 in vivo. *European Journal of Neuroscience, 25*, 2275–2284.

Park, K. J., Grosso, C. A., Aubert, I., Kaplan, D. R., & Miller, F. D. (2010). p75NTR-dependent, myelin-mediated axonal degeneration regulates neural connectivity in the adult brain. *Nature Neuroscience, 13*, 559–566.

Patterson, S. L., Abel, T., Deuel, T. A., Martin, K. C., Rose, J. C., & Kandel, E. R. (1996). Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron, 16*, 1137–1145.

Petito, C. K., Kerza-Kwiatecki, A. P., Gendelman, H. E., McCarthy, M., Nath, A., Podack, E. R., Shapshak, P., & Wiley, C. A. (1999). Review: Neuronal injury in HIV infection. *Journal of Neurovirology, 5*, 327–341.

Phillips, H. S., Hains, J. M., Armanini, M., Laramee, G. R., Johnson, S. A., & Winslow, J. W. (1991). BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron, 7*, 695–702.

Piller, S. C., Ewart, G. D., Premkumar, A., Cox, G. B., & Gage, P. W. (1996). Vpr protein of human immunodeficiency virus type 1 forms cation-selective channels in planar lipid bilayers. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 111–115.

Post, R. M. (2007). Role of BDNF in bipolar and unipolar disorder: Clinical and theoretical implications. *Journal of Psychiatric Research, 41*, 979–990.

Price, R. W., Brew, B., Sidtis, J., Rosenblum, M., Scheck, A. C., & Cleary, P. (1988). The brain in AIDS: Central nervous system HIV-1 infection and AIDS dementia complex. *Science, 239*, 586–592.

Pumpradit, W., Ananworanich, J., Lolak, S., Shikuma, C., Paul, R., Siangphoe, U., Chaoniti, N., Kaew-On, P., Paris, R., Ruxrungtham, K., & Valcour, V. (2010). Neurocognitive impairment

and psychiatric comorbidity in well-controlled human immunodeficiency virus-infected Thais from the 2NN Cohort Study. *Journal of Neurovirology, 16*, 76–82.

Radeke, M. J., Misko, T. P., Hsu, C., Herzenberg, L. A., & Shooter, E. M. (1987). Gene transfer and molecular cloning of the rat nerve growth factor receptor. *Nature, 325*, 593–597.

Reichardt, L. F. (2001). Neurotrophic factors: A window into the nervous system. In I. Mocchetti (Ed.), *Neurobiology of the neurotrophins* (pp. 3–28). Johnson City, TN: FP Graham Publishing.

Reyes, M. G., Faraldi, F., Senseng, C. S., Flowers, C., & Fariello, R. (1991). Nigral degeneration in acquired immune deficiency syndrome (AIDS). *Acta Neuropathol (Berlin), 82*, 39–44.

Rodriguez-Tebar, A., Dechant, G., & Barder, Y. (1990). Binding of brain derived neurotrophic factor to the nerve growth factor receptor. *Neuron, 4*, 487–492.

Sacktor, N. C., Lyles, R. H., Skolasky, R. L., Anderson, D. E., McArthur, J. C., McFarlane, G., Selnes, O. A., Becker, J. T., Cohen, B., Wesch, J., & Miller, E. N. (1999). Combination antiretroviral therapy improves psychomotor speed performance in HIV-seropositive homosexual men. Multicenter AIDS Cohort Study (MACS). *Neurology, 52*, 1640–1647.

Sanders, V. J., Everall, I. P., Johnson, R. W., & Masliah, E. (2000). Fibroblast growth factor modulates HIV coreceptor CXCR4 expression by neural cells. HNRC Group. *Journal of Neuroscience Research, 59*, 671–679.

Scarlatti, G., Tresoldi, E., Bjorndal, A., Fredriksson, R., Colognesi, C., Deng, H. K., Malnati, M. S., Plebani, A., Siccardi, A. G., Littman, D. R., Fenyo, E. M., & Lusso, P. (1997). In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine-mediated suppression. *Nature Medicine, 3*, 1259–1265.

Scheff, S. W., & Price, D. A. (2006). Alzheimer's disease-related alterations in synaptic density: Neocortex and hippocampus. *Journal of Alzheimer's Disease, 9*, 101–115.

Seidah, N. G., Benjannet, S., Pareek, S., Chretien, M., & Murphy, R. A. (1996). Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. *FEBS Letters, 379*, 247–250.

Soliman, F., Glatt, C. E., Bath, K. G., Levita, L., Jones, R. M., Pattwell, S. S., Jing, D., Tottenham, N., Amso, D., Somerville, L. H., Voss, H. U., Glover, G., Ballon, D. J., Liston, C., Teslovich, T., Van Kempen, T., Lee, F. S., & Casey, B. J. (2010). A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science, 327*, 863–866.

Tanaka, J., Horiike, Y., Matsuzaki, M., Miyazaki, T., Ellis-Davies, G. C., & Kasai, H. (2008). Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. *Science, 319*, 1683–1687.

Tang-Schomer, M. D., Patel, A. R., Baas, P. W., & Smith, D. H. (2010). Mechanical breaking of microtubules in axons during dynamic stretch injury underlies delayed elasticity, microtubule disassembly, and axon degeneration. *FASEB Journal, 24*, 1401–1410.

Teng, H. K., Teng, K. K., Lee, R., Wright, S., Tevar, S., Almeida, R. D., Kermani, P., Torkin, R., Chen, Z. Y., Lee, F. S., Kraemer, R. T., Nykjaer, A., & Hempstead, B. L. (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 25*, 5455–5463.

Toggas, S. M., Masliah, E., Rockenstein, E. M., Rall, G. F., Abraham, C. R., & Mucke, L. (1994). Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. *Nature, 367*, 188–193.

Tornatore, C., Meyers, K., Atwood, W., Conant, K., & Major, E. (1994). Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes. *Journal of Virology, 68*, 93–102.

Toyooka, K., Asama, K., Watanabe, Y., Muratake, T., Takahashi, M., Someya, T., & Nawa, H. (2002). Decreased levels of brain-derived neurotrophic factor in serum of chronic schizophrenic patients. *Psychiatry Research, 110*, 249–257.

van der Meer, P., Ulrich, A. M., Gonzalez-Scarano, F., & Lavi, E. (2000). Immunohistochemical analysis of CCR2, CCR3, CCR5, and CXCR4 in the human brain: Potential mechanisms for HIV dementia. *Experimental and Molecular Pathology, 69*, 192–201.

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *European Journal of Neuroscience, 20*, 2580–2590.

Volosin, M., Song, W., Almeida, R. D., Kaplan, D. R., Hempstead, B. L., & Friedman, W. J. (2006). Interaction of survival and death signaling in basal forebrain neurons: roles of neurotrophins and proneurotrophins. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 26*, 7756–7766.

Wang, G.-J., Chang, L., Volkow, N. D., Telang, F., Logan, J., Ernst, T., & Fowler, J. S. (2004). Decreased brain dopaminergic transporters in HIV-associated dementia patients. *Brain, 127*, 2452–2458.

Woods, S. P., Moore, D. J., Weber, E., & Grant, I. (2009). Cognitive neuropsychology of HIV-associated neurocognitive disorders. *Neuropsychology Review, 19*, 152–168.

Xie, Y., Hayden, M. R., & Xu, B. (2010). BDNF overexpression in the forebrain rescues Huntington's disease phenotypes in YAC128 mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 30*, 14708–14718.

Yang, J., Siao, C. J., Nagappan, G., Marinic, T., Jing, D., McGrath, K., Chen, Z. Y., Mark, W., Tessarollo, L., Lee, F. S., Lu, B., & Hempstead, B. L. (2009). Neuronal release of proBDNF. *Nature Neuroscience, 12*, 113–115.

Zakharenko, S. S., Patterson, S. L., Dragatsis, I., Zeitlin, S. O., Siegelbaum, S. A., Kandel, E. R., & Morozov, A. (2003). Presynaptic BDNF required for a presynaptic but not postsynaptic component of LTP at hippocampal CA1-CA3 synapses. *Neuron, 39*, 975–990.

Zegarra-Moran, O., Rasola, A., Rugolo, M., Porcelli, A. M., Rossi, B., & Galietta, L. J. (1999). HIV-1 nef expression inhibits the activity of a Ca^{2+}-dependent K^{+}channel involved in the control of the resting potential in CEM lymphocytes. *Journal of Immunology, 162*, 5359–5366.

Zheng, J., Thylin, M. R., Ghorpade, A., Xiong, H., Persidsky, Y., Cotter, R., Niemann, D., Che, M., Zeng, Y. C., Gelbard, H. A., Shepard, R. B., Swartz, J. M., & Gendelman, H. E. (1999). Intracellular CXCR4 signaling, neuronal apoptosis and neuropathogenic mechanisms of HIV-1-associated dementia. *Journal of Neuroimmunology, 98*, 185–200.

Zuccato, C., & Cattaneo, E. (2009). Brain-derived neurotrophic factor in neurodegenerative diseases. *Nature Reviews. Neurology, 5*, 311–322.

Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B. R., Goffredo, D., Conti, L., MacDonald, M. E., Friedlander, R. M., Silani, V., Hayden, M. R., Timmusk, T., Sipione, S., & Cattaneo, E. (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science, 293*, 493–498.

Neurotrophic Therapy for ALS/MND

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Contents

Abstract

Neurotrophic factors have a large range of activities in the nervous system that consist of functions in development, plasticity, neurogenesis, disease, and injury. In the context of amyotrophic lateral sclerosis (ALS), it has long been hypothesized that *lack* of neurotrophic growth factors *is one of the neurotoxic* contributors to the

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disease that results in death of motor neurons. This has led to a considerable number of clinical trials undertaken involving neurotrophic therapy for ALS, although none have shown benefit. This chapter will review the cause and pathology of ALS and how neurotrophic factors relate to neurotoxicity in this disease. The treatments targeted at neurotoxicity and results of trials will be discussed, in particular neurotrophic factors. This will include glial cell-derived neurotrophic factor (GDNF), brain-derived growth factor (BDNF), neurotrophin-3 (NT-3), ciliary neurotrophic factor (CNTF), insulin-like growth factor (IGF), vascular endothelial cell growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF). Also highlighted is the potential for reexamining neurotrophic factors as treatments for ALS, including new delivery methods.

Keywords
ALS • BDNF • CNTF • FGF • GDNF • IGF • NGF • NT-3 • NT-4 • NT-4 • VEGF

List of Abbreviations

ALS	Amyotrophic lateral sclerosis
BBB	Blood–brain barrier
BDNF	Brain-derived growth factor
CNTF	Ciliary neurotrophic factor
CNS	Central nervous system
FGF	Fibroblast growth factor
GDNF	Glial cell-derived neurotrophic factor
HGF	Hepatocyte growth factor
IGF	Insulin-like growth factor
NGF	Nerve growth factor
NMJ	Neuromuscular junction
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4
NT-5	Neurotrophin-5
SOD1	Superoxide dismutase 1
SOD1G93A	Mutated SOD1 glycine to alanine position 93
TDP-43	Transactivation response DNA-binding protein of 43 kDa
VEGF	Vascular endothelial cell growth factor

1 Introduction: What Are the Neurotrophic Factors and How Do They Relate to Neurotoxicity in ALS?

The story of neurotrophic growth factors in the nervous system began when Rita Levi-Montalcini, Viktor Hamburger, and Stanley Cohen discovered the actions of nerve growth factor (NGF) in the 1950s (Levi-Montalcini and Hamburger 1951; Levi-Montalcini and Cohen 1960). In 1986, both Levi-Montalcini and Cohen

received the Nobel Prize for the "discovery of growth factors" and at that time were already acknowledged for opening up a large area of research and potential therapeutic approaches for these agents within and outside the nervous system. The list of proteins regarded as neurotrophic growth factors has grown, and a lot has been learned about common and specialized activities of these factors. Some of them, the four-member neurotrophin family that includes NGF, were first described as acting on neuronal cells, whereas other more recently described neurotrophic factors were initially discovered in a nonneuronal context.

Neurotrophic factors have an array of activities in the nervous system that consist of functions in development, plasticity, neurogenesis, disease, and injury. In the context of amyotrophic lateral sclerosis (ALS), it has long been hypothesized that *lack* of neurotrophic growth factors *is one of the neurotoxic* contributors to the disease that results in death of motor neurons. This has led to a considerable number of clinical trials undertaken involving neurotrophic therapy for ALS, although none have shown benefit. In retrospect this may be due to inadequate dosing and/or route of application of the growth factor in question. This chapter will review the general contributors to neurotoxicity in ALS including withdrawal of neurotrophic support and then the therapies trialled which are aimed at correcting neurotoxicity including neurotrophic therapy, highlighting the potential for reexamining neurotrophic factors as treatments.

The first neurotrophic factor to be discovered was NGF (Levi-Montalcini and Hamburger 1951). Subsequent systematic search of mammalian tissues revealed that male mouse salivary glands contained substantial amounts of NGF (Cohen 1960; An odd finding that has never been clearly understood!). Thirty years after the discovery of NGF, another neurotrophin that demonstrated neuronal growth-promoting properties was purified from pig brain, and later called brain-derived neurotrophic factor (BDNF; Barde et al. 1982). A third member of the family called neurotrophin 3 (NT-3) was identified in 1990 (Hohn et al. 1990) and neurotrophin 4 (NT-4) in 1991 (Hallböök et al. 1991), with neurotrophin 5 (NT-5) later identified as identical to NT-4 (Ip et al. 1993). After the discovery of NGF, a number of other, unrelated neurotrophic factors in the nervous system were discovered that have a similar scope of activities, including ciliary neurotrophic factor (CNTF; Barbin et al. 1984; Lin et al. 1989) and the glial cell line-derived neurotrophic factor (GDNF; Lin et al. 1993). Members of the insulin-like growth factor (IGF; Shemer et al. 1987; Rotwein et al. 1988) fibroblast growth factor (FGF; (Emoto et al. 1989)) and vascular endothelial growth factor (VEGF; Alvarez et al. 1992) families are known to have roles in the nervous system additional to those outside the nervous system. In addition, hepatocyte growth factor (HGF), which was found in the liver, has further actions in the nervous system (Jung et al. 1994).

2 Searches for the Cause and Pathophysiology of ALS

In 1869, the French neurologist Jean-Martin Charcot described a fatal and progressive paralysis that he termed amyotrophic lateral sclerosis (ALS) (Rowland 2001). This devastating neurodegenerative disease, also known as Lou Gehrig's disease

in the USA or motor neuron disease in the UK and Australia, has a worldwide incidence of 2 per 100,000 and prevalence of 7 per 100,000 (Ferraiuolo et al. 2011). ALS is characterized by progressive, debilitating paralysis. Patients experience muscle weakness and atrophy, leading to inevitable paralysis, due to the selective and progressive loss of motor neurons of the cerebral cortex, brain stem, and spinal cord that is characteristic of the disease. Most often, patients succumb to the disease because of denervation of muscles involved in respiration (Vande Velde et al. 2011). ALS/MND occurs mainly in adults (45–60 years of age), and only 50 % of patients survive 2–3 years after diagnosis. There is only one therapeutic option for patients, riluzole, which offers a modest 3- to 6-month extension in survival. However, in the last 3 years, real progress has been made in the identification of genetic causes of the disease, such as expanded GGGGCC hexanucleotide repeats in a noncoding region of C9ORF72 (Renton et al. 2011; Zinman and Cudkowicz 2011; Ludolph et al. 2012).

Around 5–10 % of ALS patients have inherited a mutation that can cause familial ALS (Andersen and Al-Chalabi 2011), with the remaining 90 % developing the disease sporadically through environmental and lifestyle risk factors (Sutedja et al. 2009). The highest proportion of familial ALS patients identified is that with expanded GGGGCC hexanucleotide repeats in the noncoding region of C9ORF72 (Renton et al. 2011), followed by patients that carry Cu/Zn superoxide dismutase 1 (SOD1), mutations (Ludolph et al. 2012). Other rarer mutations leading to ALS occur in genes such as alsin, senataxin, dynactin, transactivation response DNA-binding protein of 43 kD (TDP-43), fused in sarcoma/translated in liposarcoma (FUS), optineurin, and ubiquilin 2 (Deng et al. 2011; Andersen and Al-Chalabi 2011; Ludolph et al. 2012). Over 160 SOD1 mutations have been identified (Gurney et al. 1994; Al-Chalabi et al. 2012) in humans.

Transgenic rodents carrying SOD1 mutations have been essential to understanding the mechanisms of ALS in humans (Peviani et al. 2010; Kanning et al. 2010). All mimic human familial and sporadic ALS, with a dominant gain of toxic function resulting in motor symptoms and death, due to the neurotoxicity of the mutant SOD1 protein (Reaume et al. 1996; Turner and Talbot 2008; Peviani et al. 2010; Kanning et al. 2010). Transgenic mice that carry 23 copies of the human mutant SOD1G93A transgene are used for preclinical testing of ALS treatments (Scott et al. 2008; Ludolph et al. 2010). Mice that overexpress normal or human mutant TDP-43 have been produced (Cohen et al. 2011), but they have not yet lived up to their promise as a model for ALS. Several groups have generated TDP-43 knockout mice and in each instance all die during embryonic life, indicating the importance of TDP-43 in multiple biological processes (Wu et al. 2010; Kraemer et al. 2010). Issues with mice expressing mutant TDP-43 have been identified such as inconsistent pathology and disease progression across TDP-43 mouse models and most recently the TDP-43 A315T mutant mice dying from neuropathy-linked bowel obstruction (Igaz et al. 2011; Guo et al. 2012). Conversely, evidence supports the continued relevance of SOD1G93A animal models to sporadic disease. Misfolding of SOD1 into a disease-specific and toxic conformation has been found in both sporadic and familial ALS (Bosco et al. 2010). Moreover, SOD1G93A mice replicate features of human MND including progressive limb

paresis, respiratory failure, and reduced life span (Gurney et al. 1994; Turner and Talbot 2008). Behavioral symptoms appear around 90–110 days, but pathological changes occur much earlier (Peviani et al. 2010; Kanning et al. 2010).

Although we are yet to understand how the disease initiates, there is a large body of data on mechanisms that cause the pathology in motor neurons such as oxidative stress, protein aggregation, excitotoxicity, impairment of axonal transport, mitochondrial dysfunction, altered DNA/RNA processing, endoplasmic reticulum stress, and apoptosis (reviewed in Ferraiuolo et al. 2011). A common feature is ubiquitin-positive inclusions, most of which contain TDP-43 (Geser et al. 2011) that does not appear to be related to TDP-43 mutations. The pathophysiology of the disease includes a reduced secretion of neurotrophic factors, protein aggregations, malfunctioning of the mitochondria, rupture in the axonal passage, destruction in the calcium metabolism, changes in the skeletal proteins, high levels of glutamate, and oxidative damage (Ferraiuolo et al. 2011). Preventing and slowing down motor neuron degeneration and death in ALS are critical goals of future therapies, as are means of enhancing axonal regeneration.

Axonal transport has long been implicated in ALS. Axonal transport uses microtubule-dependent kinesin and cytoplasmic dynein molecular motors, which mediate transport towards the neuromuscular junction (anterograde transport) and towards the cell body (retrograde transport), respectively. Given the extremely long axons of motor neurons and their dependence on axonal transport for the delivery of ATP-producing mitochondria and other essential constituents, including neurotrophic factors, it comes as no surprise that transport deficiencies cause debilitating effects. Tumor necrosis factor elevated in SOD1G93A mice disrupts kinesin function via a mechanism involving p38 mitogen-activated protein kinase (p38 MAPK; De Vos et al. 2000), activation of which has been demonstrated in models of ALS (Holasek et al. 2005; Dewil et al. 2007). Mutant SOD1 also inhibits anterograde fast axonal transport of mitochondria and enhances the retrograde movement resulting in a depletion of axonal mitochondria content (De Vos et al. 2007). A study suggested that alterations in retrograde signalling contribute to neurodegeneration in ALS, by shifting from the transport of survival-promoting to death-promoting signalling cargo molecules (Perlson et al. 2009), suggesting less neurotrophic factors can be transported. Point mutation of the p150 subunit of dynactin has been detected in both sporadic and familial ALS patients (Munch et al. 2004). Mutations in dynactin lead to a reduction in retrograde transport in SOD1G93A mice (Puls et al. 2003).

Peripherin and neurofilament are intermediate filament (IF) proteins that can be abnormally found in inclusion bodies of the perikaryon and axon of motor neurons in ALS. Rare mutations in peripherin have been identified in a small number of people with ALS (Gros-Louis et al. 2004; Corrado et al. 2011). In addition to being found in inclusion bodies, neurofilaments are transported by axons and disruption of neurofilament transport occurs in ALS. Neurofilaments comprise three major proteins, neurofilament light, middle, and heavy chains (NFL, NFM, and NFH), that are synthesized within cell bodies and then usually transported into and through axons by “axonal transport.” Decreased amounts of normal NFH in axons of

SOD1G93A mice are correlated to shrinkage of axons in the ventral root and precede symptoms in SOD1G93A mice (Zhang et al. 1997). Abnormal phosphorylation of the side-arm domain of NFH by the stress-activated kinase p38alpha has been reported in sporadic and familial ALS and SOD1G93A mice (Ackerley et al. 2000, 2004). This work indicated the speed of neurofilament transport through axons is altered (Ackerley et al. 2004) with a slowing of transport proportional to the increase in phosphorylation (Ackerley et al. 2000). Excitotoxic damage by glutamate is thought to contribute to motor neuron injury in ALS. In cultured neurons, glutamate reduces axonal transport of neurofilaments via activation of protein kinases that phosphorylate NFM such as p38 (Ackerley et al. 2000), and riluzole protects against glutamate-induced slowing of neurofilament axonal transport (Stevenson et al. 2009). An association of neurofilaments with ALS is also represented by rare mutations in the neurofilament gene in ALS patients (Al-Chalabi et al. 1999). There is a significant increase in NFM in CSF and serum of humans with ALS and SOD1G93A mice (Brettschneider et al. 2006; Ganesalingam et al. 2011), especially in the late stage of disease (Lu et al. 2012). Thus, axonal transport defects are an important feature of ALS that may precede motor neuron degeneration and clinical symptoms.

At the tissue level, it is clear that cells beyond motor neurons are involved in ALS pathophysiology (Ilieva et al. 2009). Cells such as glia and immune cells are proposed to be involved in non-cell-autonomous toxicity. For example, astrocytes are reactive and apparently toxic in ALS (Ince et al. 2011). Also, reactive microglia and T-cell infiltration have been noted as players in non-cell-autonomous toxicity (Ilieva et al. 2009). Most of this data has come from studies in which human mtSOD1 is expressed in rodents. There is evidence that the onset of ALS in mutant SOD1 mice results from mutant SOD1 action in the motor neurons, whereas progression of the disease is a result of the action of SOD1 mutant protein in glia (Ilieva et al. 2009). Selective reduction of mutant SOD1 from astrocytes or microglia using cre-lox system slowed disease progression in SOD1G37R mice (Yamanaka et al. 2008; Boillee et al. 2006). A complimentary approach replacing microglia/macrophage via bone marrow transplantation demonstrated that wild-type microglia/macrophages slow disease progression in SOD1G93A mice although inflammation may be a consequence, not an initiator of disease (Kano et al. 2012).

Advances in identification of upstream mechanisms that might contribute to the death of motor neurons in ALS and compounds directed at these potential targets are being developed. Ongoing research is addressing the question of where the motor neuron disease process is initiated, candidates being the neuromuscular junction (NMJ), the axon, mitochondria, proteasome, or the cell nucleus (Dupuis and Loeffler 2009; Bilsland et al. 2010; Shi et al. 2010; Dupuis et al. 2004; Baron et al. 2007; Daoud and Rouleau 2011; Keller et al. 2012). Progress in understanding this etiology has led to therapies being trialled to target the various candidates that are causative in the disease. In addition, although ALS is a motor neuron disease, interventions directed towards improving muscle function such as muscle troponin activators, myostatin inhibitors, and reticulon 4 (Nogo-A) inhibitors are also proposed as therapies (Zinman and Cudkowicz 2011).

3 Treatments for ALS Targeted at Neurotoxicity

3.1 Treatments Targeted at Downregulation of SOD1 Mutations

For the approximately 20 % of familial patients with SOD1 mutations leading to familial ALS, a phase 1 human trial of safety, tolerability, and pharmacokinetics of antisense SOD1 oligonucleotides administered intrathecally to patients with SOD1 familial ALS is underway (ISIS 333611/clinicaltrials.gov/NCT01041222). The trial enrolled 21 patients with SOD1 mutations who were randomized into either three ascending dose cohorts or a placebo group. Some patients elected to enroll a second time. The phase 1 trial was completed in Jan 2012 with no significant adverse effects (Robinson 2012). However, some concerns were raised that the antisense oligonucleotide used in the trial targeted the mRNA of the non-mutated portion of SOD1 and that there may be a risk of inflammation in long-term, high-dose treatments. Isis the pharmaceutical company involved in this trial has recommended further animal trials with a redesigned antisense SOD1 oligonucleotide (Robinson 2012). The development of vaccine or passive infusion delivery of immunoglobulin to remove misfolded protein in ALS is a novel therapeutic strategy under investigation. Bosco et al. (2010) found misfolding of SOD1 into a disease-specific and toxic conformation underlies both sporadic and familial MND. Subsequently, vaccination with SOD1 mutant protein or antigenic peptide directed to monomer/misfolded SOD in the ALS SOD1 transgenic mouse model delayed disease onset and significantly extended survival (Takeuchi et al. 2010; Liu et al. 2012).

3.2 Treatments Targeted at Excitotoxicity

Glutamate-mediated excitotoxic effects have long been postulated to have an important role in motor neuron degeneration, with riluzole an example of a glutamate inhibitor used to treat ALS patients (Bensimon et al. 1994; Lacomblez et al. 1996). In an ALS SOD1 mouse model of ALS, lithium showed reduced aggregates of alpha-synuclein, ubiquitin, and SOD1 in motor neurons, and in addition to its known effects on protein clearance, lithium was found to increase the number of mitochondria and suppressed reactive astrogliosis (Fornai et al. 2008). However, lithium by itself failed in human clinical trials (Verstraete et al. 2012), and more recently in combination with riluzole, although well tolerated had no benefit (Aggarwal et al. 2010). Arimoclomol is an oral compound that amplifies the heat-shock response leading to induction of endogenous cellular cytoprotective mechanisms (Kieran et al. 2004) that might prevent motor neuron degeneration under conditions of stress. Arimoclomol delayed disease progression and extended survival by 22 % in the SOD1G93A transgenic ALS mice (Kieran et al. 2004; Kalmar et al. 2008; Lu et al. 2012). Efficacy studies in patients with familial ALS are underway with promising early results indicating this drug is safe in human beings, and it crosses the blood–brain barrier (BBB) following oral administration (Cudkowicz et al. 2008).

3.3 Treatments Targeted at Mitochondria

Results from ongoing trials using dexpramipexole, a drug that improves mitochondrial health, showed initial promise in ALS trials. Dexpramipexole is an optical enantiomer of pramipexole, a Parkinson's disease drug that maintains prolonged survival of ALS SOD1 transgenic mice (Danzeisen et al. 2006). A large, Phase 3 international study of treatment of ALS patients with dexpramipexole (EMPOWER) has been completed with disappointing results. There was initial promise with the first reports from a carefully controlled Phase 2 study indicating subjects show a slower rate of decline in measures of motor function and a lower level of mortality over the course of the study (Bozik et al. 2010; Cudkowicz et al. 2011; Rudnicki et al. 2013). However, in January 2013, the Phase 3 trial was suspended due to lack of effect (alsa.org/news/archive/biogen-discontinues.html). Some compounds proposed to improve mitochondrial function, such as minocycline and creatine, have had beneficial effects in the SOD1 transgenic mouse model (Zhu et al. 2002) but have yet to prove beneficial in human trials (Klopstock et al. 2011; Gordon et al. 2007). A new trial of creatine in combination with tamoxifen is underway (www.clinicaltrials.gov: NCT01257581b). Other agents targeting mitochondrial function have been identified. Olesoxime (previously TRO19622) is a mitochondrial pore modulator that improves SOD1G93A mice survival and attenuates activation of surrounding glial cells. A phase 2/3 study of olesoxime is underway in Europe (NCT00868166).

4 Neurotrophic Factor Withdrawals and Neurotrophic Factors as Treatments

4.1 Rationale

ALS is a complex disease and cannot be explained by the occurrence of a single event or the disturbance of a single gene or protein. The disease is likely to be the result of a multistep process, ultimately leading to motor neuron degeneration and clinical symptoms. Currently there are two hypotheses to explain the etiology of ALS. The first is the "dying-forward" hypothesis which proposes that ALS is mainly a disorder of cortico-motor neurons, which connect monosynaptically with anterior horn cells, mediating anterograde degeneration of anterior horn cells via glutamate excitotoxicity (Kiernan et al. 2011). The second is the "dying-back" hypothesis where pathological changes in motor axons and nerve terminals appear to precede motor neuron degeneration and clinical symptoms. Moreover, this second hypothesis raises the possibility that the disease process may start distally at the nerve terminal or at the neuromuscular junction (NMJ) and progress towards the cell body (Dadon-Nachum et al. 2011). The main therapeutic strategy for ALS directed to the dying-back hypothesis is the use of neurotrophic factors.

No genetic defects have been identified to date that interfere with any of the growth factor genes in ALS. The lack of trophic support appears to be a downstream

consequence of axonal transport of neurotrophic factors in ALS rather than expression. A deficiency of growth factor support could provoke motor neuron death in patients with ALS. In early stages of the disease, nerve terminals and neuromuscular junctions are partially degraded, while cell bodies in the spinal cord are mostly intact. This is clearly shown by Fischer and colleagues (Fischer et al. 2004) who demonstrated, in autopsy material from an ALS patient who died from an unrelated cause only 6 months after symptom onset, that denervation and reinnervation were pronounced, while the spinal motor neurons were not yet affected. The concept is further supported by recent work showing progressive motor axonal changes play a significant role in the course of disease including clinical impairment (Cheah et al. 2012). Studies in animal models of spinal muscular atrophy (SMA), a pure lower motor neuron disease, have shown that the first event to be observed is withdrawal of the axon terminals from the adjacent muscle fibers (Simon et al. 2010). In SOD1G93A mice axonal degeneration also precedes onset of symptoms as well as spinal motor neuron loss. Disease progression in these animals follows the dying-back principle characterized by an ascending neuronal degeneration from the terminals of the motor axons to the motor neuron cell body (Gould et al. 2006). Retrograde transport of neurotrophic factors such as BDNF and GDNF is impaired, whereas death-promoting signalling molecules are enhanced in axons of motor neurons of presymptomatic SOD1G93A mice (Perlson et al. 2009). Both retrograde transport and anterograde axonal transport are impaired in human ALS and in mouse models. This is supportive of the dying-back hypothesis that leads to loss of muscle function and the inability of neurotrophic factors, such as glial cell-derived neurotrophic factor (GDNF), to be transported to the motor neuron cell body.

4.2 Glial Cell-Derived Neurotrophic Factor

GDNF can be efficiently transported by motor neurons both retrogradely (Leitner et al. 1999) and anterogradely (Rind and von Bartheld 2002). In a study where stem cell-derived motor neurons were transplanted into paralyzed rat spinal cord and GDNF-secreting cells implanted into the sciatic nerve, axonal outgrowth and attachment to the muscle could be achieved. This thus supports the notion that combining protection of motor neuron cell body and support of nerve terminals in the muscle by GDNF may be beneficial in ALS (Deshpande et al. 2006). A study examining the overexpression of GDNF either centrally (astrocytes) or peripherally (muscle) showed that overexpression of GDNF in skeletal muscle, but not in the central nervous system (CNS), improved survival in the SOD1G93A model of ALS (Li et al. 2007). When stem cell-derived GDNF was expressed in astrocytes at disease onset, there was no improvement in survival in SOD1G93A mice although there was some improvement in health of motor neurons (Park et al. 2009). The outcomes of studies in SOD1G93A mice argued for a benefit of GDNF treatment. GDNF-treated motor neurons of SOD1 animals are clearly protected (Mohajeri et al. 1999; Acsadi et al. 2002; Suzuki et al. 2007). Some studies report a benefit for disease progression and/or survival (Acsadi et al. 2002; Li et al. 2007). Others

found GDNF has no effect on disease progression and integrity of neuromuscular junctions (Suzuki et al. 2007; Park et al. 2009), although Park et al. (2009) were the only ones to initiate treatment at the onset of disease.

Although GDNF has been used in clinical trial for Parkinson's disease, the factor has not yet been tested clinically for the treatment of ALS patients. Clinical observations demonstrated that the level of GDNF is increased in the CSF of ALS patients (Grundstrom et al. 2000) and that its receptor is expressed in the spinal cord, even at late stages of the disease (Mitsuma et al. 1999). Due to its in vivo neuroprotective effects on motor neurons, GDNF remains a potential candidate for a clinical trial in ALS, despite controversial efficacy data on survival. However, as there is evidence that GDNF does not cross the blood–brain barrier (Kastin et al. 2003), a future challenge remains in developing a protocol to resolve this issue, such as using an intrathecal delivery device for GDNF. Intraventricularly, GDNF has been trialled as a treatment for Parkinson's disease, but a clinical trial was halted because of adverse effects. Subsequent trials involving continuous intraputamenal infusion had no adverse effects but are inconclusive (Yasuda and Mochizuki 2010). Alternate routes for GDNF delivery include coupling to proteins that do cross the BBB. For example, intravenous, nonviral GDNF gene therapy in a rat model of Parkinson's disease was highly effective because the nonviral agent contained antibodies to the transferrin receptor that allowed entry across the BB (Zhang and Pardridge 2009). In another example, delivery of plasmid GDNF in neonatal rats was achieved using a nonviral complex containing antibodies to p75NTR receptor that enabled retrograde entry to motor neuron cell bodies from terminals in muscles (Barati et al 2006). GDNF gene therapy via direct delivery into the brain using a viral vector (AAV2-GDNF) is underway (clinicaltrials.gov).

4.3 Brain-Derived Neurotrophic Factor (BDNF)

BDNF was originally isolated as a trophic factor for neurons from brain extract (Barde et al. 1982). Expression of BDNF (Henderson et al. 1993) and the high-affinity BDNF receptor tropomyosin-related kinase B receptor (TrkB; Yan et al. 1993) is observed in skeletal muscle and motor neurons during motor neuron-programmed cell death. BDNF/TrkB kinase signalling is required for maintenance of the cholinergic phenotype of adult motor neurons and prevents embryonic and postnatal motor neuron cell death in a variety of experimental paradigms. For example, BDNF rescues motor neurons from embryonic-programmed cell death and neonatal axotomy-induced death (Oppenheim et al. 1992; Yan et al. 1992; Koliatsos et al. 1993). Research has shown BDNF and NT-3 slow motor neuron degeneration and increase lifespan in two models of inherited motor neuron disease, the wobbler and progressive motor neuropathy (pmn) mice (Ikeda et al. 1995; Haase et al. 1997). BDNF can also protect neurons from in vivo excitotoxicity (Bemelmans et al. 2006), a situation relevant to ALS.

In several clinical trials BDNF has failed to show any beneficial effect for ALS. A trial where 1,136 patients were given 25–100 μg/kg/day subcutaneously

for 9 months was reported in 1999 (The BDNF Study Group 1999). In this trial, the tolerability of BDNF was shown to be acceptable, despite some adverse effects such as diarrhea. While the results failed to show an effect on survival after treatment with BDNF, it appeared that a subgroup of patients responded positively to the treatment and demonstrated a higher survival rate. This subgroup consisted of patients having the lowest vital capacity and receiving the highest dose of BDNF, suggesting that BDNF could be beneficial for patients in advanced stages of the disease. The investigators suggested use of higher doses or intrathecal delivery to deliver BDNF to the diseased spinal cord. 1 year later, a phase I trial was published, showing the feasibility of intrathecal delivery of BDNF. Indeed, delivering BDNF with this method showed that BDNF leads to a detectable level of BDNF in the CSF, especially at the lumbar level (Ochs et al. 2000). The method being feasible, two clinical trials were conducted in 2003 and 2005. In 2003, 11 ALS patients were enrolled in a trial to receive an intrathecal delivery of BDNF. Another trial with intrathecal delivery was conducted in 2005 with 13 patients (Kalra et al. 2003; Beck et al. 2005). Both trials were too small to detect any meaningful efficacy signal.

What have we learned? Failure of BDNF therapy has been attributed to factors such as short half-life, inadequate dosage, and limited bioavailability. The first issue is the short half-life; BDNF has a half-life of 0.92 min in the circulation (Poduslo and Curran 1996). Bioavailability is a major issue with new methods of delivery being sought. Since Tetanus toxin C (TTC) is effectively retrogradely transported to motor neurons following intramuscular delivery (Moreno-Igoa et al. 2010) it seemed that fusion proteins containing TTC may improve delivery of neurotrophins including BDNF to motor neurons. However, TTC and BDNF share the same internalization pathways and localize in the same endocytic compartments, suggesting that addition of TTC to the neurotrophins does not improve their bioavailability to motor neurons and may even prevent their entry (Roux et al. 2006). Indeed, when plasmids expressing recombinant fusion proteins containing BDNF and TTC were given to SOD1G93A mice there was no improvement of motor neuron health or survival above that of TTC (Calvo et al. 2011), suggesting that TTC competes more effectively than BDNF for entry into motor neurons. Lessons can also be learned from the signalling that is generated from activation of the TrkB receptor and its truncated form, TrkB.T1 One study showed that TrkB phosphorylation in ALS patients is decreased although the number of receptors is increased (Mutoh et al. 2000) suggesting a mechanism affecting the TrkB response to BDNF. In addition, knocking out the TrkB variant receptor TrkB.T1 receptors in SOD1G93A mice delayed the onset of motor neuron loss and functional symptoms (Yanpallewar et al. 2012) suggesting physiological or pathological imbalances in TrkB receptor isoform levels can also affect motoneuron survival.

4.4 Neurotrophin-3

Neurotrophin-3 (NT-3) is important for maintaining the integrity of neuromuscular junctions. It is well described that NT-3 increases retrograde transport within

degenerating motor neurons (Sagot et al. 1998) and enhances synaptic transmission (Poo 2001), in addition to stimulating neuronal survival. Importantly, muscles of NT-3-knockout mice lose neuromuscular innervation postnatally (Woolley et al. 2005) reflecting the requirement of peripherally derived NT-3 for neuron survival (Meier et al. 1999; Riethmacher et al. 1997) as well as for the maintenance of the neuromuscular junction (Reddy et al. 2003). NT-3 has not been trialled as a potential treatment. Importantly, it should be noted that NT-3, like BDNF, has an exceptionally short half-life in the circulation of only 1.28 min (Poduslo and Curran 1996).

4.5 Ciliary Neurotrophic Factor

The history of CNTF in ALS is similar to that of BDNF. CNTF was one of the earliest neurotrophic factors to be shown to have a direct neuroprotective effect on degenerating motor neurons in stress-induced conditions. Two studies in 1990 demonstrated that CNTF protects motor neurons in cell culture (Arakawa et al. 1990) and in vivo after axotomy-induced apoptosis in the rodent (Sendtner et al. 1990). Those results were strengthened by the beneficial effect of CNTF in a mouse model of neuronopathy (pmn) with motor neuron degeneration (Sendtner et al. 1992) and by increased vulnerability of spinal motor neurons in a knockout mouse line for CNTF (Masu et al. 1993). These results were published in the early 1990s, prior to the development of SOD1G93A mouse model of ALS (Gurney et al. 1994); today the most frequently employed model for ALS. Because of these promising effects on motor neurons, CNTF went directly to the clinic and became the first growth factor investigated clinically for ALS. Two clinical trials were conducted in 1996 and enrolled 570 and 730 patients, respectively (Miller et al. 1996; ALS CNTF Treatment Study Group 1996). The investigators focused mainly on daily living activities outcomes and survival. Unfortunately, both of those trials reported no observable benefit of treatment. Moreover, adverse effects, especially for the doses over 5 μg/kg, were severe, leading to the cessation of treatment in many patients. What could be the reasons to explain the lack of efficiency of CNTF after such promising preclinical findings? Firstly, despite CNTF's ability to cross the BBB (Pan et al. 1999), a pharmacokinetic study in the rodent showed that CNTF has a half-life of only 3 min after intravenous injection (Poduslo and Curran 1996). Due to this low half-life, subcutaneous application of CNTF does not appear appropriate to target the CNS. Secondly, the doses used in animals were about 1 mg/kg, whereas the highest safe dose in the human is 200 times lower (5 μg/kg). This limited dose and short half-life of CNTF could easily account for the lack of observable efficacy of CNTF in patients. No further clinical projects were pursued with CNTF in ALS after these two trials. A recent article from Sendtner's group (Selvaraj et al. 2012) has highlighted the promise of CNTF in stabilizing neuromuscular endplates and improving axon health. The action of a cytoplasmic protein called stathmin that destabilizes microtubules was inhibited by the activated signal transducer and activator of transcription-3 (STAT3). Importantly, STAT-3 was

itself activated specifically by CNTF. This was done with motor neurons isolated from the pmn mouse model of neuronopathy; the same model used to show CNTF improves motor neuron health (Sendtner et al. 1992).

4.6 Insulin-Like Growth Factor-1

The action of IGF-1 in the nervous system (Shemer et al. 1987; Rotwein et al. 1988) was described long after its multitude of actions and targets in the body were known. Neurons in the central nervous system (CNS) express functional receptors of IGF-1 throughout the nervous system (Shemer et al. 1987; Rotwein et al. 1988; Bilak et al. 2001). IGF-1 is able to rescue motor neurons (Caroni 1993; Li et al. 1994) and reduce glutamate excitotoxicity (Nakao et al. 1996) both in vitro and in vivo. Therefore IGF-1 became an important drug candidate for ALS. Several clinical studies with IGF-1 in ALS were conducted early, before testing of IGF-1 in SOD1G93A rodents. In the late 1990s, two investigations were published with different conclusions (Lai et al. 1997; Borasio et al. 1998). Lai et al. enrolled 266 patients and administrated IGF-1 subcutaneously (Lai et al. 1997). The conclusion of the investigators was that IGF-1 is safe, slows progression of motor impairment, and increases quality of life. In a second trial, (Borasio et al. 1998) 183 patients were enrolled with the same mode of administration. The investigators focused on disease progression but found no improvement after treatment. Indeed, IGF-1 treatment appeared to be safe but did not improve quality of life of the patients nor motor functions. Additionally, a pilot study was conducted with intrathecal delivery of IGF-1 (Nagano et al. 2005b). Investigators claimed that the treatment was beneficial for the patients. Two groups of patients were compared, patients with a high and a low dose of IGF-1, and it was observed that the decline in motor function was slowed with the high dose of IGF-1, but not the low dose. However, as this study was not placebo controlled, the results should be considered with caution.

Many years after the first trials of IGF-1 in humans, preclinical reports investigating the effects of IGF-1 in SOD1 animals were published. The majority of the reports argue in favor of a potential protective effect in ALS, with an increase in survival and motor function parameters (Kaspar 2003; Nagano et al. 2005a; Lepore et al. 2007; Dodge et al. 2008; Franz et al. 2009). Also, IGF-1 mediates neuromuscular junction regeneration in a non-ALS-related animal model (Apel et al. 2010), although some contradictory results were published (Messi et al. 2007; Grumbles et al. 2009). For example, Messi et al. (2007) generated a transgenic form of SOD1G93A mice that overexpress IGF-1 in skeletal muscle but found no improvement of survival. The work of Franz et al. (2009) suggests that IGF-1 effects were only observed in male mice. At the same time, a new clinical trial was initiated with subcutaneous administration of IGF-1. This clinical trial enrolled 330 patients and studied the effects of IGF-1 on muscular strength and survival (Sorenson et al. 2008). Unfortunately, IGF-1-treated patients did not improve relative to the control patients.

There are a variety of reasons why IGF-1 failed at clinical trials. IGF-1 is increased at the spinal level of ALS patients (Wilczak et al. 2003) and in the

serum (Corbo et al. 2010). Conversely, muscle expression of IGF-1 and IGF-binding proteins was decreased in ALS patients (Lunetta et al. 2012), suggesting IGF-1 within the muscle tissue could participate in motor unit degeneration. However, as discussed above animal trials where IGF-1 is overexpressed in skeletal muscle in SOD1G93A mice did not improve survival (Messi et al. 2007). This may suggest the lack of success of the human clinical trial could be related to the transport of IGF-1 from muscle to the effected motor neurons and NMJ. In addition, it is hard to relate the preclinical trials involving viral gene delivery of IGF-1 (Kaspar 2003; Lepore et al. 2007; Dodge et al. 2008) or intrathecal administration (Nagano et al. 2005a) to the clinical trials where subcutaneous injections were used (Lai et al. 1997; Borasio et al. 1998; Sorenson et al. 2008). A genetic approach cannot be translated into subcutaneous delivery in patients, due to the high and constant expression of IGF-1 over time. Nor can the intrathecal administration that delivers IGF-1 to the CNS be directly comparable to subcutaneous injections. Indeed, IGF-1 has a half-life of only 4 min in the circulation (Pan and Kastin 2000), and intravenous IGF-1 enters the CNS by a saturable transport system at the BBB, which functions in synchrony with IGF-binding proteins in the periphery to regulate the availability of IGF-1. Therefore subcutaneous IGF-1 would seem unlikely to be an adequate mode of delivery. As Howe et al. (2009) observed it is highly unlikely that meaningful levels of IGF-1 were reached in the CSF and CNS tissue with the route and dose of IGF-1 used in the most recent clinical trial (Sorenson et al. 2008).

4.7 Vascular Endothelial Growth Factor

Discovered in the late 1980s as a growth factor for vascular endothelial cells, vascular endothelial growth factor (VEGF) induces endothelial cell growth and angiogenesis (Connolly et al. 1989). The first evidence for neurotrophic proprieties of VEGF arose with studies on neuronal regeneration (Sondell et al. 2000; Hobson et al. 2000). Shortly after, VEGF was implicated as a growth factor possibly involved in ALS by the discovery that mice with deletion of the HIF-response element in the VEGF promoter developed an ALS-like phenotype (Oosthuyse et al. 2001). Subsequent genetic analysis however failed to produce clear evidence for an effect of VEGF polymorphisms on the risk of developing ALS in humans (Gros-Louis et al. 2003; Lambrechts et al. 2003; Fernandez-Santiago et al. 2006; Chen et al. 2006; Zhang et al. 2006). A combined meta-analysis of American and European data showed the 2578AA VEGF genotype as a candidate for male ALS (Lambrechts et al. 2009). However, independent of the genetic discussion, VEGF promotes survival of cells overexpressing a mutated SOD1G93A protein (Li et al. 2003) and ameliorates the effects of glutamate excitotoxicity (Tolosa et al. 2008; Bogaert et al. 2010). In SOD1G93A rodents, VEGF treatment leads to a delay of disease onset, an improvement of motor functions, protection of motor neurons and neuromuscular junctions, and increase in survival (Zheng et al. 2004; Azzouz et al. 2004; Storkebaum et al. 2005; Wang et al. 2007; Hwang et al. 2009).

The preclinical data supports further clinical investigation of VEGF as a treatment target for ALS. Besides the animal studies, clinical observations show that VEGF is downregulated in the late stage of ALS (Devos et al. 2004), and restoring its level therefore could bring benefit for patients. The potential of VEGF as a therapeutic agent is further strengthened by its ability to cross the BBB (Wang et al. 1996; Dobrogowska et al. 1998; Ay et al. 2008). VEGF has an effective half-life of 10.7 min in the circulation (Wu et al. 2009). There are no published data on VEGF treatments for human ALS so far, but a clinical trial involving intracerebroventricular infusion of VEGF (NCT0080050) with 28 patients is now at phase I/II. Another trial using plasmid-encoding zinc finger protein transcription factor that upregulates VEGF-A expression (NCT00748501) has completed phase II testing involving the treatment of 40 patients, but no outcomes have yet been published. Clearly, the angiogenic side effects of VEGF treatment will have to be monitored closely.

4.8 Fibroblast Growth Factors

The fibroblast growth factors (FGF) proteins are growth factors with homologous structure that promote angiogenesis and the proliferation of fibroblasts. It was demonstrated that both FGF-1 and FGF-2 can protect motor neurons after axotomy-induced apoptosis and that they support neuronal regeneration (Cuevas et al. 1995; Piehl et al. 1995; Grothe et al. 1991; Klimaschewski et al. 1999). No disease-specific alterations in the expression pattern of FGF-2 were found in post-mortem spinal cord of ALS patients (Petri et al. 2009), but increased FGF-2 levels were measured in serum and cerebrospinal fluid of ALS patients (Johansson et al. 2003). Dependent on activation of different receptors, FGF-1 and FGF-2 can either promote cell survival or death (Grothe et al. 2006; Cassina et al. 2005). One report points out that FGF-1 might activate motor neuron apoptosis by astrocytes, thereby possibly contributing to ALS pathophysiology (Cassina et al. 2005). Subcutaneous administration of FGF-2 had no effect on the survival in transgenic SOD1G93A mice (Upton-Rice et al. 1999), but this may reflect the instability of native FGFs without heparin protection. A recent study where SOD1G93A mice were produced lacking endogenous FGF-2 showed increased lifespan of mice and upregulation of GDNF and CNTF (Thau et al. 2012). This supports the idea that endogenous FGF-2 contributes to the pathophysiology of SOD1G93A mice. Clearly, more work is needed to establish if the FGFs can be considered as drug candidates for ALS.

4.9 Hepatocyte Growth Factor

Hepatocyte growth factor (HGF) was discovered as growth factor acting on the liver (Nakamura et al. 1985). This growth factor exerts antiapoptotic activity in the liver after endotoxin-induced hepatic failure (Kosai et al. 1999). Neuronal effects consist of activity in the guidance and survival of neurons in the developing nervous

system (Ebens et al. 1996). Moreover, HGF is protective on motor neurons in culture and in vivo after axotomy (Wong et al. 1997; Okura et al. 1999). In rodent models of ALS, HGF reduces motor neuron degeneration and increases survival (Sun et al. 2002; Ishigaki et al. 2007; Kadoyama et al. 2007).

While HGF appears to be a good candidate for the treatment of ALS, especially since HGF levels in ALS patients appear to be dysregulated (Tsuboi et al. 2002), additional preclinical work is required. Indeed, more insight at the level of the NMJ is needed since it seems that HGF can have an influence on the formation of NMJs (Madhavan and Peng 2006; Tatsumi et al. 2009) but appears to be inefficient on their regeneration unless used in conjunction with other neurotrophic factors (Grumbles et al. 2009; Casella et al. 2010). There is work suggesting this may be because of pool-specific responsiveness of motor neurons to different neurotrophic factors. When the HGF receptor Met is turned off in mid-embryonic life, there is pool-specific loss of motor neurons at the NMJ within the pectoralis muscle from lack of Met (Lamballe et al. 2011). It will also be of interest for the design of a clinical trial to investigate whether a systemic delivery of HGF can reproduce the promising results observed after intrathecal administration in rodents (Ishigaki et al. 2007). HGF prevents the decline of choline acetyltransferase (ChAT) expression after adult hypoglossal nerve axotomy and also reduces motor neuron death in response to excitotoxic stress (Okura et al. 1999). Delayed disease onset and improved lifespan is observed when SOD1G93A mice overexpress transgenic HGF (Sun et al. 2002; Kadoyama et al. 2007). Reduced levels of the HGF receptor, Met, are found in the spinal cord of familial SOD1 patients over the course of disease, indicating disruption of the HGF-Met system contributes to neuronal degeneration in familial ALS (Kato et al. 2003). A recent study showed motor neuron development and maintenance are unaffected in mice with conditional enhancement of neuronal Met, whereas SOD1G93GA mice with the same enhancement of neuronal Met had a delayed disease onset (Genestine et al. 2011). Intrathecal HGF extends the lifespan of SOD1G93A mice, overcoming the HGF-Met deficit (Ishigaki et al. 2007). Another study showed in mice that the half-life of HGF is 10 min in the circulation, with 30 % of HGF crossing the BBB (Pan et al. 2006). A phase 1 clinical trial was intended to assess the safety and pharmacokinetics of single and multiple doses of rhHGF intrathecally in patients with ALS at Tohoku University Hospital (Japan) but was delayed because of the 2011 tsunami.

4.10 The Future of Neurotrophic Therapy for ALS

The demonstration of beneficial effects of neurotrophic factors for various CNS disease conditions raised great enthusiasm about their use as therapeutic agents for ALS. The clinical failure of neurotrophic factors is in no way ascribable to one single reason, especially in ALS.

Because of the failure to find clinical efficacy so far, the value of preclinical data has been questioned. There are two aspects to this issue. First, the models used may not be predictive for the working of a drug in the human system. This is of course an

irrefutable argument, which cannot be proven or disproven in the absence of any clinical positive outcome seen so far. Second, animal models may be predictive but have not been conducted with sufficient rigor and diligence to really tell us the truth about efficacy of a drug candidate in the rodent system. Reasons for this include insufficient blinding procedures, neglect of copy number issues in transgenic models, insufficient number of animals used, inadequate statistical methods, and inadequate independent replication of study results (Scott et al. 2008). An example may have been BDNF, where preclinical studies were not done in SOD1G93A rodent models of ALS as they were not available at the time but were achieved using axotomy models, pmn and wobbler mice. After reports were published on the failure of the large clinical trial of BDNF (The BDNF Study Group 1999), there have been no effective BDNF-based treatments reported for the SOD1G93A ALS rodent models but one failure (Park et al. 2009).

Since there is insufficient proof that any one ALS animal model produced so far can be used to predict the effectiveness of human treatments, a combination of different animal models could be trialled. This could include different animal models that monitor different aspects of ALS disease pathophysiology, for example, two different SOD1-mutant models, possibly mouse and rat models, but with rodents that have adequate checks on copy number and preclinical trials using carefully designed studies. Ideally, a dose-effect relationship should be established. All tests have to be conducted in a rigorously blinded and randomized manner and ideally be performed in at least two independent laboratories. If novel animal models become available, they should be immediately tested using a variety of preclinical approaches.

In all major clinical trials performed so far, it appears the dosing was inadequate. Examples of this include BDNF, CNTF, and IGF-1 (The BDNF Study Group 1999; Ochs et al. 2000; Miller et al. 1996; ALS CNTF Treatment Study Group 1996; Lai et al. 1997; Borasio et al. 1998). For CNTF, the dose showing benefit for motor neurons in vivo was three orders of magnitude lower in the human case. In addition, subcutaneous-injected CNTF has a plasma half-life time of only a few minutes (Poduslo and Curran 1996) and does not reach the target tissues. A similar scenario can be drawn for BDNF, with questionable delivery to the CNS. For IGF-1, CSF levels reached with the chosen subcutaneous dose of 0.05 mg/kg twice daily, more than an order of magnitude below endogenous levels in the CSF, and thus are likely to be therapeutically meaningless (Howe et al. 2009).

The relevance of neurotrophic factor treatment for ALS should be pursued with a thorough understanding of how the neurotrophic factors work and if they address mechanisms relevant to ALS. For example, neurotrophic factors should show some efficacy against cell death either or both apoptotic and excitotoxic cell death demonstrated on motor neurons, other primary neurons, and/or motor neuron lines. Also, effects on the integrity of the NMJ should be ascertained. Only VEGF is known to preserve innervation to the NMJs in ALS (Zheng et al. 2007), although IGF-1 has shown benefit in preserving the NMJ after nerve transection (Apel et al. 2010). Addressing additive or synergistic effects of neurotrophic factors on NMJ as well as axonal and cell body health is an area yet to be adequately

explored as a treatment for ALS. However, there is an indication that some neurotrophic factors in combination improve NMJs; for example, cells transplanted with a combination of IGF-1, HGF, and GDNF appear to improve NMJs after transection, the combinatorial approach being better than individual factors (Grumbles et al. 2009; Casella et al. 2010). A recent article supports the notion of growth factor combinations having positive synergistic effects (Krakora et al. 2013). Mesenchymal stem cells were implanted into three muscle groups of SOD1G93A rats and secreted either GDNF, VEGF, IGF-1, or BDNF and compared to those implanted with cells secreting a combination of GDNF and VEGF (Krakora et al. 2013). The results indicated that rats treated with cells secreting both VEGF and GDNF have improved NMJ health and extended survival compared to rats treated with cells secreting individual neurotrophic factors. This indicates the need to further understand the cross talk between neurotrophic factors to improve their therapeutic effect in ALS.

For a chronic CNS disease like ALS, the application and dose chosen should guarantee a therapeutic level of the drug in the brain and spinal cord. For a motor neuron-supportive principle like neurotrophic factor support, the drug level should likely be held constant over time. Options for this include gene-based delivery of recombinant protein, including viral and nonviral methods, stem cell-based treatments that include secretion of relevant neurotrophic factors, or constant infusion pumps.

There is a good rationale for using other methods of delivery besides administration of the naked molecules, including half-life, getting to the site of action and prolonged neurotrophic factor production. For example, virally delivered genes for VEGF and IGF-1 have all shown promise in SOD1G93A rodents (Azzouz et al. 2004; Kaspar 2003; Lepore et al. 2007; Dodge et al. 2008). There is also a strong rationale for using nonviral delivery methods of targeting neurotrophic factors to specific sites of action such as motor neurons, NMJs, and glia. A nonviral gene delivery treatment capable of generating small quantities of therapeutic protein such as GDNF or HGF over a limited time targeting specific cells could have significant therapeutic value (Rogers and Rush 2012). This has been demonstrated for GDNF in an axotomy model in rats (Barati et al. 2006).

Stem cell-based delivery of neurotrophic factors has been suggested as an alternative method of delivering neurotrophic factors for ALS. Several studies report stem cell production of neurotrophic factors when transplanted into SOD1G93A rodents, including VEGF, IGF-1, GDNF, and BDNF (Hwang et al. 2009; Suzuki et al. 2007; Park et al. 2009). Cortical human neural progenitor cells engineered to secrete GDNF confer motor neuron protection after transplantation into the spinal cords of SOD1G93A transgenic rats (Suzuki et al. 2007). Intramuscular delivery of GDNF or GDNF-/VEGF-producing mesenchymal stem cells in SOD1G93A transgenic rats also increases neuromuscular contacts, motor neuron survival, and lifespan (Suzuki et al. 2008; Krakora et al. 2013). Coadministration of embryonic spinal cord cells with GDNF, HGF, and IGF-1 after sciatic nerve resection resulted in increased number of reinnervated muscle fibers and improved

motor neuron health (Grumbles et al. 2009). Human spinal stem cells (HSSC) that differentiate into both neurons and glia have been transplanted into SOD1G93A rodents and shown to secrete endogenous neurotrophic factors that may improve functional outcome. Following transplantation, HSSC express excitatory amino acid transporters that potentially restore functional glutamate reuptake around vulnerable motor neurons and also release neurotrophic factors such as GDNF and BDNF. Grafted HSSC express several neurotrophic factors, including GDNF and BDNF, and form synaptic contacts with host motor neurons (Xu et al. 2009). Moreover, intraspinal HSSC transplantation delays symptom onset and extends the lifespan in SOD1G93A rodents (Yan et al. 2006; Xu et al. 2006, 2011). Some caution has to be included as a recent study (Koh et al. 2012) has indicated that bone marrow mesenchymal stromal cells (BM-MSC) from ALS patients have a lower capacity to proliferate and secrete neurotrophic factors, indicating using autologous stem cells may be not be an effective in ALS patients.

5 Conclusion

It is nearly 20 years since the first trial of neurotrophic factors was reported for ALS. Since this time we have not only made significant inroads into understanding the pathophysiology and genetic cause of the disease, but we have gained more knowledge on the potential action of neurotrophic factors in ALS and proposed potentially smarter ways to deliver effective therapy. This provides reason for cautious optimism that a truly effective treatment is on the horizon.

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References

Ackerley, S., Grierson, A. J., Brownlees, J., Thornhill, P., Anderton, B. H., Leigh, P. N., Shaw, C. E., & Miller, C. C. (2000). Glutamate slows axonal transport of neurofilaments in transfected neurons. *The Journal of Cell Biology, 150*, 165–176.

Ackerley, S., Grierson, A. J., Banner, S., et al. (2004). p38alpha stress-activated protein kinase phosphorylates neurofilaments and is associated with neurofilament pathology in amyotrophic lateral sclerosis. *Molecular and Cellular Neurosciences, 26*, 354–364.

Acsadi, G., Anguelov, R. A., Yang, H., et al. (2002). Increased survival and function of SOD1 mice after glial cell-derived neurotrophic factor gene therapy. *Human Gene Therapy, 13*, 1047–1059.

Aggarwal, S. P., Zinman, L., Simpson, E., et al. (2010). Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: A randomised, double-blind, placebo-controlled trial. *Lancet Neurology, 9*, 481–488.

Al-Chalabi, A., Andersen, P. M., Nilsson, P., Chioza, B., Andersson, J. L., Russ, C., Shaw, C. E., Powell, J. F., & Leigh, P. N. (1999). Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. *Human Molecular Genetics, 8*, 157–164.

Al-Chalabi, A., Jones, A., Troakes, C., King, A., Al-Sarraj, S., & Van Den Berg, L. H. (2012). The genetics and neuropathology of amyotrophic lateral sclerosis. *Acta Neuropathologica, 124*, 339–352.

AlS CNTF Treatment Study Group. (1996). A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis. ALS CNTF Treatment Study Group. *Neurology, 46*, 1244–1249.

Alvarez, J. A., Baird, A., Tatum, A., Daucher, J., Chorsky, R., Gonzalez, A. M., & Stopa, E. G. (1992). Localization of basic fibroblast growth factor and vascular endothelial growth factor in human glial neoplasms. *Modern Pathology, 5*, 303–307.

Andersen, P. M., & Al-Chalabi, A. (2011). Clinical genetics of amyotrophic lateral sclerosis: What do we really know? *Nature Reviews Neurology, 7*, 603–615.

Apel, P. J., Ma, J., Callahan, M., Northam, C. N., Alton, T. B., Sonntag, W. E., & Li, Z. (2010). Effect of locally delivered IGF-1 on nerve regeneration during aging: An experimental study in rats. *Muscle & Nerve, 41*, 335–341.

Arakawa, Y., Sendtner, M., & Thoenen, H. (1990). Survival effect of ciliary neurotrophic factor (CNTF) on chick embryonic motoneurons in culture: Comparison with other neurotrophic factors and cytokines. *The Journal of Neuroscience, 10*, 3507–3515.

Ay, I., Francis, J. W., & Brown, R. H. (2008). VEGF increases blood–brain barrier permeability to Evans blue dye and tetanus toxin fragment C but not adeno-associated virus in ALS mice. *Brain Research, 1234*, 198–205.

Azzouz, M., Ralph, G. S., Storkebaum, E., Walmsley, L. E., Mitrophanous, K. A., Kingsman, S. M., Carmeliet, P., & Mazarakis, N. D. (2004). VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature, 429*, 413–417.

Barati, S., Hurtado, P. R., Zhang, S. H., Tinsley, R., Ferguson, I. A., & Rush, R. A. (2006). GDNF gene delivery via the p75(NTR) receptor rescues injured motor neurons. *Experimental Neurology, 202*, 179–188.

Barbin, G., Manthorpe, M., & Varon, S. (1984). Purification of the chick eye ciliary neuronotrophic factor. *Journal of Neurochemistry, 43*, 1468–1478.

Barde, Y. A., Edgar, D., & Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *The EMBO Journal, 1*, 549–553.

Baron, M., Kudin, A. P., & Kunz, W. S. (2007). Mitochondrial dysfunction in neurodegenerative disorders. *Biochemical Society Transactions, 35*, 1228–1231.

Beck, M., Flachenecker, P., Magnus, T., Giess, R., Reiners, K., Toyka, K. V., & Naumann, M. (2005). Autonomic dysfunction in ALS: A preliminary study on the effects of intrathecal BDNF. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 6*, 100–103.

Bemelmans, A.-P., Husson, I., Jaquet, M., Mallet, J., Kosofsky, B. E., & Gressens, P. (2006). Lentiviral-mediated gene transfer of brain-derived neurotrophic factor is neuroprotective in a mouse model of neonatal excitotoxic challenge. *Journal of Neuroscience Research, 83*, 50–60.

Bensimon, G., Lacomblez, L., & Meininger, V. (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *The New England Journal of Medicine, 330*, 585–591.

Bilak, M. M., Corse, A. M., & Kuncl, R. W. (2001). Additivity and potentiation of IGF-I and GDNF in the complete rescue of postnatal motor neurons. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 2*, 83–91.

Bilsland, L. G., Sahai, E., Kelly, G., Golding, M., Greensmith, L., & Schiavo, G. (2010). Deficits in axonal transport precede ALS symptoms in vivo. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 20523–20528.

Bogaert, E., Van Damme, P., Poesen, K., Dhondt, J., Hersmus, N., Kiraly, D., Scheveneels, W., Robberecht, W., & Van Den Bosch, L. (2010). VEGF protects motor neurons against excitotoxicity by upregulation of GluR2. *Neurobiology of Aging, 31*, 2185–2191.

Boillee, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., Kollias, G., & Cleveland, D. W. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science, 312*, 1389–1392.

Borasio, G. D., Robberecht, W., Leigh, P. N., et al. (1998). A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. European ALS/IGF-I Study Group. *Neurology, 51*, 583–586.

Bosco, D. A., Morfini, G., Karabacak, N. M., et al. (2010). Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. *Nature Neuroscience, 13*, 1396–1403.

Bozik, M. E., Mather, J. L., Kramer, W. G., Gribkoff, V. K., & Ingersoll, E. W. (2010). Safety, tolerability, and pharmacokinetics of KNS-760704 (dexpramipexole) in healthy adult subjects. *Journal of Clinical Pharmacology, 51*, 1177–1185.

Brettschneider, J., Petzold, A., Süssmuth, S. D., Ludolph, A. C., & Tumani, H. (2006). Axonal damage markers in cerebrospinal fluid are increased in ALS. *Neurology, 66*, 852–856.

Calvo, A. C., Moreno-Igoa, M., Mancuso, R., et al. (2011). Lack of a synergistic effect of a non-viral ALS gene therapy based on BDNF and a TTC fusion molecule. *Orphanet Journal of Rare Diseases, 6*, 10.

Caroni, P. (1993). Activity-sensitive signaling by muscle-derived insulin-like growth factors in the developing and regenerating neuromuscular system. *Annals of the New York Academy of Sciences, 692*, 209–222.

Casella, G. T. B., Almeida, V. W., Grumbles, R. M., Liu, Y., & Thomas, C. K. (2010). Neurotrophic factors improve muscle reinnervation from embryonic neurons. *Muscle and Nerve, 42*, 788–797.

Cassina, P., Pehar, M., Vargas, M. R., Castellanos, R., Barbeito, A. G., Estevez, A. G., Thompson, J. A., Beckman, J. S., & Barbeito, L. (2005). Astrocyte activation by fibroblast growth factor-1 and motor neuron apoptosis: Implications for amyotrophic lateral sclerosis. *Journal of Neurochemistry, 93*, 38–46.

Cheah, B. C., Lin, C. S. Y., Park, S. B., Vucic, S., Krishnan, A. V., & Kiernan, M. C. (2012). Progressive axonal dysfunction and clinical impairment in amyotrophic lateral sclerosis. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology, 123*, 2460.

Chen, W., Saeed, M., Mao, H., et al. (2006). Lack of association of VEGF promoter polymorphisms with sporadic ALS. *Neurology, 67*, 508–510.

Cohen, S. (1960). Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. *Proceedings of the National Academy of Sciences of the United States of America, 46*, 302–311.

Cohen, T. J., Lee, V. M.-Y., & Trojanowski, J. Q. (2011). TDP-43 functions and pathogenic mechanisms implicated in TDP-43 proteinopathies. *Trends in Molecular Medicine, 17*, 659–667.

Connolly, D. T., Heuvelman, D. M., Nelson, R., Olander, J. V., Eppley, B. L., Delfino, J. J., Siegel, N. R., Leimgruber, R. M., & Feder, J. (1989). Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. *Journal of Clinical Investigation, 84*, 1470–1478.

Corbo, M., Lunetta, C., Magni, P., Dozio, E., Ruscica, M., Adobbati, L., & Silani, V. (2010). Free insulin-like growth factor (IGF)-1 and IGF-binding proteins-2 and −3 in serum and cerebrospinal fluid of amyotrophic lateral sclerosis patients. *European Journal of Neurology, 17*, 398–404.

Corrado, L., Carlomagno, Y., Falasco, L., et al. (2011). A novel peripherin gene (PRPH) mutation identified in one sporadic amyotrophic lateral sclerosis patient. *Neurobiology of Aging, 32* (e552), 1–6.

Cudkowicz, M. E., Shefner, J. M., Simpson, E., et al. (2008). Arimoclomol at dosages up to 300 mg/day is well tolerated and safe in amyotrophic lateral sclerosis. *Muscle and Nerve, 38*, 837–844.

Cudkowicz, M., Bozik, M. E., Ingersoll, E. W., et al. (2011). The effects of dexpramipexole (KNS-760704) in individuals with amyotrophic lateral sclerosis. *Nature Medicine, 17*, 1652–1656.

Cuevas, P., Carceller, F., & Gimenez-Gallego, G. (1995). Acidic fibroblast growth factor prevents post-axotomy neuronal death of the newborn rat facial nerve. *Neuroscience Letters, 197*, 183–186.

Dadon-Nachum, M., Melamed, E., & Offen, D. (2011). The "dying-back" phenomenon of motor neurons in ALS. *Journal of Molecular Neuroscience, 43*, 470–477.

Danzeisen, R., Schwalenstoecker, B., Gillardon, F., et al. (2006). Targeted antioxidative and neuroprotective properties of the dopamine agonist pramipexole and its nondopaminergic

enantiomer SND919CL2x [(+)2-amino-4,5,6,7-tetrahydro-6-Lpropylamino-benzathiazole dihydrochloride]. *The Journal of Pharmacology and Experimental Therapeutics, 316*, 189–199.

Daoud, H., & Rouleau, G. A. (2011). Motor neuron disease: A role for ubiquilin 2 mutations in neurodegeneration. *Nature Reviews Neurology, 7*, 599–600.

De Vos, K., Severin, F., Van Herreweghe, F., Vancompernolle, K., Goossens, V., Hyman, A., & Grooten, J. (2000). Tumor necrosis factor induces hyperphosphorylation of kinesin light chain and inhibits kinesin-mediated transport of mitochondria. *The Journal of Cell Biology, 149*, 1207–1214.

De Vos, K. J., Chapman, A. L., Tennant, M. E., et al. (2007). Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. *Human Molecular Genetics, 16*, 2720–2728.

Deng, H.-X., Chen, W., Hong, S.-T., et al. (2011). Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature, 477*, 211.

Deshpande, D. M., Kim, Y.-S., Martinez, T., et al. (2006). Recovery from paralysis in adult rats using embryonic stem cells. *Annals of Neurology, 60*, 32–44.

Devos, D., Moreau, C., Lassalle, P., Perez, T., De Seze, J., Brunaud-Danel, V., Destée, A., Tonnel, A. B., & Just, N. (2004). Low levels of the vascular endothelial growth factor in CSF from early ALS patients. *Neurology, 62*, 2127–2129.

Dewil, M., dela Cruz, V. F., Van Den Bosch, L., & Robberecht, W. (2007). Inhibition of p38 mitogen activated protein kinase activation and mutant SOD1(G93A)-induced motor neuron death. *Neurobiology of Disease, 26*, 332–341.

Dobrogowska, D. H., Lossinsky, A. S., Tarnawski, M., & Vorbrodt, A. W. (1998). Increased blood–brain barrier permeability and endothelial abnormalities induced by vascular endothelial growth factor. *Journal of Neurocytology, 27*, 163–173.

Dodge, J. C., Haidet, A. M., Yang, W., et al. (2008). Delivery of AAV-IGF-1 to the CNS extends survival in ALS mice through modification of aberrant glial cell activity. *Molecular Therapy, 16*, 1056–1064.

Dupuis, L., & Loeffler, J.-P. (2009). Neuromuscular junction destruction during amyotrophic lateral sclerosis: Insights from transgenic models. *Current Opinion in Pharmacology, 9*, 341–346.

Dupuis, L., Gonzalez de Aguilar, J. L., Oudart, H., de Tapia, M., Barbeito, L., & Loeffler, J. P. (2004). Mitochondria in amyotrophic lateral sclerosis: A trigger and a target. *Neurodegenerative Diseases, 1*, 245–254.

Ebens, A., Brose, K., Leonardo, E. D., Hanson, M. G., Bladt, F., Birchmeier, C., Barres, B. A., & Tessier-Lavigne, M. (1996). Hepatocyte growth factor/scatter factor is an axonal chemoattractant and a neurotrophic factor for spinal motor neurons. *Neuron, 17*, 1157–1172.

Emoto, N., Gonzalez, A. M., Walicke, P. A., Wada, E., Simmons, D. M., Shimasaki, S., & Baird, A. (1989). Basic fibroblast growth factor (FGF) in the central nervous system: Identification of specific loci of basic FGF expression in the rat brain. *Growth Factors, 2*, 21–29.

Fernandez-Santiago, R., Sharma, M., Mueller, J. C., et al. (2006). Possible gender-dependent association of vascular endothelial growth factor (VEGF) gene and ALS. *Neurology, 66*, 1929–1931.

Ferraiuolo, L., Kirby, J., Grierson, A. J., Sendtner, M., & Shaw, P. J. (2011). Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. *Nature Reviews Neurology, 7*, 616–630.

Fischer, L. R., Culver, D. G., Tennant, P., Davis, A. A., Wang, M., Castellano-Sanchez, A., Khan, J., Polak, M. A., & Glass, J. D. (2004). Amyotrophic lateral sclerosis is a distal axonopathy: Evidence in mice and man. *Experimental Neurology, 185*, 232–240.

Fornai, F., Longone, P., Cafaro, L., et al. (2008). Lithium delays progression of amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences, 105*, 2052–2057.

Franz, C. K., Federici, T., Yang, J., et al. (2009). Intraspinal cord delivery of IGF-I mediated by adeno-associated virus 2 is neuroprotective in a rat model of familial ALS. *Neurobiology of Disease, 33*, 473–481.

Ganesalingam, J., An, J., Shaw, C. E., Shaw, G., Lacomis, D., & Bowser, R. (2011). Combination of neurofilament heavy chain and complement C3 as CSF biomarkers for ALS. *Journal of Neurochemistry, 117*, 528–537.

Genestine, M., Caricati, E., Fico, A., et al. (2011). Enhanced neuronal Met signalling levels in ALS mice delay disease onset. *Cell Death Disease, 2*, e130.

Geser, F., Prvulovic, D., O'Dwyer, L., Hardiman, O., Bede, P., Bokde, A. L., Trojanowski, J. Q., & Hampel, H. (2011). On the development of markers for pathological TDP-43 in amyotrophic lateral sclerosis with and without dementia. *Progress in Neurobiology, 95*, 649–662.

Gordon, P. H., Moore, D. H., Miller, R. G., et al. (2007). Efficacy of minocycline in patients with amyotrophic lateral sclerosis: A phase III randomised trial. *The Lancet Neurology, 6*, 1045–1053.

Gould, T. W., Buss, R. R., Vinsant, S., Prevette, D., Sun, W., Knudson, C. M., Milligan, C. E., & Oppenheim, R. W. (2006). Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *Journal of Neuroscience, 26*, 8774–8786.

Gros-Louis, F., Laurent, S., Lopes, A. A. S., Khoris, J., Meininger, V., Camu, W., & Rouleau, G. A. (2003). Absence of mutations in the hypoxia response element of VEGF in ALS. *Muscle and Nerve, 28*, 774–775.

Gros-Louis, F., Larivière, R., Gowing, G., Laurent, S., Camu, W., Bouchard, J.-P., Meininger, V., Rouleau, G. A., & Julien, J.-P. (2004). A frameshift deletion in peripherin gene associated with amyotrophic lateral sclerosis. *The Journal of Biological Chemistry, 279*, 45951–45956.

Grothe, C., Wewetzer, K., Lagrange, A., & Unsicker, K. (1991). Effects of basic fibroblast growth factor on survival and choline acetyltransferase development of spinal cord neurons. *Brain Research. Developmental Brain Research, 62*, 257–261.

Grothe, C., Haastert, K., & Jungnickel, J. (2006). Physiological function and putative therapeutic impact of the FGF-2 system in peripheral nerve regeneration–lessons from in vivo studies in mice and rats. *Brain Research Reviews, 51*, 293–299.

Grumbles, R. M., Sesodia, S., Wood, P. M., & Thomas, C. K. (2009). Neurotrophic factors improve motoneuron survival and function of muscle reinnervated by embryonic neurons. *Journal of Neuropathology and Experimental Neurology, 68*, 736–746.

Grundstrom, E., Lindholm, D., Johansson, A., Blennow, K., & Askmark, H. (2000). GDNF but not BDNF is increased in cerebrospinal fluid in amyotrophic lateral sclerosis. *Neuroreport, 11*, 1781–1783.

Guo, Y., Wang, Q., Zhang, K., An, T., Shi, P., Li, Z., Duan, W., & Li, C. (2012). HO-1 induction in motor cortex and intestinal dysfunction in TDP-43 A315T transgenic mice. *Brain Research, 1460*, 88–95.

Gurney, M. E., Pu, H., Chiu, A. Y., et al. (1994). Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science, 264*, 1772–1775.

Haase, G., Kennel, P., Pettmann, B., Vigne, E., Akli, S., Revah, F., Schmalbruch, H., & Kahn, A. (1997). Gene therapy of murine motor neuron disease using adenoviral vectors for neurotrophic factors. *Nature Medicine, 3*, 429–436.

Hallböök, F., Ibáñez, C. F., & Persson, H. (1991). Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. *Neuron, 6*, 845–858.

Henderson, C. E., Camu, W., Mettling, C., et al. (1993). Neurotrophins promote motor neuron survival and are present in embryonic limb bud. *Nature, 363*, 266–270.

Hobson, M. I., Green, C. J., & Terenghi, G. (2000). VEGF enhances intraneural angiogenesis and improves nerve regeneration after axotomy. *Journal of Anatomy, 197*(Pt 4), 591–605.

Hohn, A., Leibrock, J., Bailey, K., & Barde, Y. A. (1990). Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature, 344*, 339–341.

Holasek, S. S., Wengenack, T. M., Kandimalla, K. K., Montano, C., Gregor, D. M., Curran, G. L., & Poduslo, J. F. (2005). Activation of the stress-activated MAP kinase, p38, but not JNK in

cortical motor neurons during early presymptomatic stages of amyotrophic lateral sclerosis in transgenic mice. *Brain Research, 1045*, 185–198.

Howe, C. L., Bergstrom, R. A., & Horazdovsky, B. F. (2009). Subcutaneous IGF-1 is not beneficial in 2-year ALS trial. *Neurology, 73*, 1247; author reply 1247–1248.

Hwang, D. H., Lee, H. J., Park, I. H., Seok, J. I., Kim, B. G., Joo, I. S., & Kim, S. U. (2009). Intrathecal transplantation of human neural stem cells overexpressing VEGF provide behavioral improvement, disease onset delay and survival extension in transgenic ALS mice. *Gene Therapy, 16*, 1234–1244.

Igaz, L. M., Kwong, L. K., Lee, E. B., et al. (2011). Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. *The Journal of Clinical Investigation, 121*, 726–738.

Ikeda, K., Klinkosz, B., Greene, T., Cedarbaum, J. M., Wong, V., Lindsay, R. M., & Mitsumoto, H. (1995). Effects of brain-derived neurotrophic factor on motor dysfunction in wobbler mouse motor neuron disease. *Annals of Neurology, 37*, 505–511.

Ilieva, H., Polymenidou, M., & Cleveland, D. W. (2009). Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *The Journal of Cell Biology, 187*, 761–772.

Ince, P. G., Highley, J. R., Kirby, J., Wharton, S. B., Takahashi, H., Strong, M. J., & Shaw, P. J. (2011). Molecular pathology and genetic advances in amyotrophic lateral sclerosis: an emerging molecular pathway and the significance of glial pathology. *Acta Neuropathologica, 122*, 657–671.

Ip, N. Y., Stitt, T. N., Tapley, P., Klein, R., Glass, D. J., Fandl, J., Greene, L. A., Barbacid, M., & Yancopoulos, G. D. (1993). Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells. *Neuron, 10*, 137–149.

Ishigaki, A., Aoki, M., Nagai, M., Warita, H., Kato, S., Kato, M., Nakamura, T., Funakoshi, H., & Itoyama, Y. (2007). Intrathecal delivery of hepatocyte growth factor from amyotrophic lateral sclerosis onset suppresses disease progression in rat amyotrophic lateral sclerosis model. *Journal of Neuropathology and Experimental Neurology, 66*, 1037–1044.

Johansson, A., Larsson, A., Nygren, I., Blennow, K., & Askmark, H. (2003). Increased serum and cerebrospinal fluid FGF-2 levels in amyotrophic lateral sclerosis. *Neuroreport, 14*, 1867–1869.

Jung, W., Castren, E., Odenthal, M., Vande Woude, G. F., Ishii, T., Dienes, H. P., Lindholm, D., & Schirmacher, P. (1994). Expression and functional interaction of hepatocyte growth factor-scatter factor and its receptor c-met in mammalian brain. *The Journal of Cell Biology, 126*, 485–494.

Kadoyama, K., Funakoshi, H., Ohya, W., & Nakamura, T. (2007). Hepatocyte growth factor (HGF) attenuates gliosis and motoneuronal degeneration in the brainstem motor nuclei of a transgenic mouse model of ALS. *Neuroscience Research, 59*, 446–456.

Kalmar, B., Novoselov, S., Gray, A., Cheetham, M. E., Margulis, B., & Greensmith, L. (2008). Late stage treatment with arimoclomol delays disease progression and prevents protein aggregation in the SOD1 mouse model of ALS. *Journal of Neurochemistry, 107*, 339–350.

Kalra, S., Genge, A., & Arnold, D. L. (2003). A prospective, randomized, placebo-controlled evaluation of corticoneuronal response to intrathecal BDNF therapy in ALS using magnetic resonance spectroscopy: Feasibility and results. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 4*, 22–26.

Kanning, K. C., Kaplan, A., & Henderson, C. E. (2010). Motor neuron diversity in development and disease. *Annual Review of Neuroscience, 33*, 409–440.

Kano, O., Beers, D. R., Henkel, J. S., & Appel, S. H. (2012). Peripheral nerve inflammation in ALS mice: Cause or consequence. *Neurology, 78*, 833–835.

Kaspar, B. K. (2003). Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science, 301*, 839–842.

Kastin, A. J., Akerstrom, V., & Pan, W. (2003). Glial cell line-derived neurotrophic factor does not enter normal mouse brain. *Neuroscience Letters, 340*, 239–241.

Kato, S., Funakoshi, H., Nakamura, T., Kato, M., Nakano, I., Hirano, A., & Ohama, E. (2003). Expression of hepatocyte growth factor and c-Met in the anterior horn cells of the spinal cord in the patients with amyotrophic lateral sclerosis (ALS): Immunohistochemical studies on sporadic ALS and familial ALS with superoxide dismutase 1 gene mutation. *Acta Neuropathologica, 106*, 112–120.

Keller, B. A., Volkening, K., Droppelmann, C. A., Ang, L. C., Rademakers, R., & Strong, M. J. (2012). Co-aggregation of RNA binding proteins in ALS spinal motor neurons: Evidence of a common pathogenic mechanism. *Acta Neuropathologica, 124*, 733–747.

Kieran, D., Kalmar, B., Dick, J. R. T., Riddoch-Contreras, J., Burnstock, G., & Greensmith, L. (2004). Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. *Nature Medicine, 10*, 402–405.

Kiernan, M. C., Vucic, S., Cheah, B. C., Turner, M. R., Eisen, A., Hardiman, O., Burrell, J. R., & Zoing, M. C. (2011). Amyotrophic lateral sclerosis. *Lancet, 377*, 942–955.

Klimaschewski, L., Meisinger, C., & Grothe, C. (1999). Localization and regulation of basic fibroblast growth factor (FGF-2) and FGF receptor-1 in rat superior cervical ganglion after axotomy. *Journal of Neurobiology, 38*, 499–506.

Klopstock, T., Elstner, M., & Bender, A. (2011). Creatine in mouse models of neurodegeneration and aging. *Amino Acids, 40*, 1297–1303.

Koh, S.-H., Baik, W., Noh, M.-Y., Cho, G.-W., Kim, H. Y., Kim, K.-S., & Kim, S. H. (2012). The functional deficiency of bone marrow mesenchymal stromal cells in ALS patients is proportional to disease progression rate. *Experimental Neurology, 233*, 472–480.

Koliatsos, V. E., Clatterbuck, R. E., Winslow, J. W., Cayouette, M. H., & Price, D. L. (1993). Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons in vivo. *Neuron, 10*, 359–367.

Kosai, K., Matsumoto, K., Funakoshi, H., & Nakamura, T. (1999). Hepatocyte growth factor prevents endotoxin-induced lethal hepatic failure in mice. *Hepatology, 30*, 151–159.

Kraemer, B. C., Schuck, T., Wheeler, J. M., Robinson, L. C., Trojanowski, J. Q., Lee, V. M. Y., & Schellenberg, G. D. (2010). Loss of murine TDP-43 disrupts motor function and plays an essential role in embryogenesis. *Acta Neuropathologica, 119*, 409–419.

Krakora, D., Mulcrone, P., Meyer, M., et al. (2013). Synergistic effects of GDNF and VEGF on lifespan and disease progression in a familial ALS rat model. *Molecular Therapy, 21*, 1602–1610. [Epub 29 May, 2013].

Lacomblez, L., Bensimon, G., Leigh, P. N., Guillet, P., & Meininger, V. (1996). Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet, 347*, 1425–1431.

Lai, E. C., Felice, K. J., Festoff, B. W., et al. (1997). Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. The North America ALS/IGF-I Study Group. *Neurology, 49*, 1621–1630.

Lamballe, F., Genestine, M., Caruso, N., Arce, V., Richelme, S., Helmbacher, F., & Maina, F. (2011). Pool-specific regulation of motor neuron survival by neurotrophic support. *The Journal of Neuroscience, 31*, 11144–11158.

Lambrechts, D., Storkebaum, E., Morimoto, M., et al. (2003). VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. *Nature Genetics, 34*, 383–394.

Lambrechts, D., Poesen, K., Fernandez-Santiago, R., et al. (2009). Meta-analysis of vascular endothelial growth factor variations in amyotrophic lateral sclerosis: Increased susceptibility in male carriers of the -2578AA genotype. *Journal of Medical Genetics, 46*, 840–846.

Leitner, M. L., Molliver, D. C., Osborne, P. A., Vejsada, R., Golden, J. P., Lampe, P. A., Kato, A. C., Milbrandt, J., & Johnson, E. M. (1999). Analysis of the retrograde transport of glial cell line-derived neurotrophic factor (GDNF), neurturin, and persephin suggests that in vivo signaling for the GDNF family is GFRalpha coreceptor-specific. *The Journal of Neuroscience, 19*, 9322–9331.

Lepore, A. C., Haenggeli, C., Gasmi, M., Bishop, K. M., Bartus, R. T., Maragakis, N. J., & Rothstein, J. D. (2007). Intraparenchymal spinal cord delivery of adeno-associated virus IGF-1 is protective in the SOD1G93A model of ALS. *Brain Research, 1185*, 256–265.

Levi-Montalcini, R., & Cohen, S. (1960). Effects of the extract of the mouse submaxillary salivary glands on the sympathetic system of mammals. *Annals of the New York Academy of Sciences, 85*, 324–341.

Levi-Montalcini, R., & Hamburger, V. (1951). Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. *The Journal of Experimental Zoology, 116*, 321–361.

Li, L., Oppenheim, R. W., Lei, M., & Houenou, L. J. (1994). Neurotrophic agents prevent motoneuron death following sciatic nerve section in the neonatal mouse. *Journal of Neurobiology, 25*, 759–766.

Li, B., Xu, W., Luo, C., Gozal, D., & Liu, R. (2003). VEGF-induced activation of the PI3-K/Akt pathway reduces mutant SOD1-mediated motor neuron cell death. *Brain Research Molecular Brain Research, 111*, 155–164.

Li, W., Brakefield, D., Pan, Y., Hunter, D., Myckatyn, T. M., & Parsadanian, A. (2007). Muscle-derived but not centrally derived transgene GDNF is neuroprotective in G93A-SOD1 mouse model of ALS. *Experimental Neurology, 203*, 457–471.

Lin, L. F., Mismer, D., Lile, J. D., Armes, L. G., Butler, E. T., 3, Vannice, J. L., & Collins, F. (1989). Purification, cloning, and expression of ciliary neurotrophic factor (CNTF). *Science, 246*, 1023–1025.

Lin, L. F., Doherty, D. H., Lile, J. D., Bektesh, S., & Collins, F. (1993). GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science, 260*, 1130–1132.

Liu, H.-N., Tjostheim, S., Dasilva, K., et al. (2012). Targeting of monomer/misfolded SOD1 as a therapeutic strategy for amyotrophic lateral sclerosis. *Journal of Neuroscience, 32*, 8791–8799.

Lu, C.-H., Petzold, A., Kalmar, B., Dick, J., Malaspina, A., & Greensmith, L. (2012). Plasma neurofilament heavy chain levels correlate to markers of late stage disease progression and treatment response in SOD1(G93A) mice that model ALS. *PLoS ONE, 7*, e40998.

Ludolph, A. C., Bendotti, C., Blaugrund, E., et al. (2010). Guidelines for preclinical animal research in ALS/MND: A consensus meeting. *Amyotrophic Lateral Sclerosis, 11*, 38–45.

Ludolph, A. C., Brettschneider, J., & Weishaupt, J. H. (2012). Amyotrophic lateral sclerosis. *Current Opinion in Neurology, 25*, 530–535.

Lunetta, C., Serafini, M., Prelle, A., et al. (2012). Impaired expression of insulin-like growth factor-1 system in skeletal muscle of amyotrophic lateral sclerosis patients. *Muscle and Nerve, 45*, 200–208.

Madhavan, R., & Peng, H. B. (2006). HGF induction of postsynaptic specializations at the neuromuscular junction. *Journal of Neurobiology, 66*, 134–147.

Masu, Y., Wolf, E., Holtmann, B., Sendtner, M., Brem, G., & Thoenen, H. (1993). Disruption of the CNTF gene results in motor neuron degeneration. *Nature, 365*, 27–32.

Meier, C., Parmantier, E., Brennan, A., Mirsky, R., & Jessen, K. R. (1999). Developing Schwann cells acquire the ability to survive without axons by establishing an autocrine circuit involving insulin-like growth factor, neurotrophin-3, and platelet-derived growth factor-BB. *The Journal of Neuroscience, 19*, 3847–3859.

Messi, M. L., Clark, H. M., Prevette, D. M., Oppenheim, R. W., & Delbono, O. (2007). The lack of effect of specific overexpression of IGF-1 in the central nervous system or skeletal muscle on pathophysiology in the G93A SOD1 mouse model of ALS. *Experimental Neurology, 207*, 52–63.

Miller, R. G., Petajan, J. H., Bryan, W. W., et al. (1996). A placebo-controlled trial of recombinant human ciliary neurotrophic (rhCNTF) factor in amyotrophic lateral sclerosis. rhCNTF ALS Study Group. *Annals of Neurology, 39*, 256–260.

Mitsuma, N., Yamamoto, M., Li, M., Ito, Y., Mitsuma, T., Mutoh, T., Takahashi, M., & Sobue, G. (1999). Expression of GDNF receptor (RET and GDNFR-alpha) mRNAs in the spinal cord of patients with amyotrophic lateral sclerosis. *Brain Res, 820*, 77–85.

Mohajeri, M. H., Figlewicz, D. A., & Bohn, M. C. (1999). Intramuscular grafts of myoblasts genetically modified to secrete glial cell line-derived neurotrophic factor prevent motoneuron loss and disease progression in a mouse model of familial amyotrophic lateral sclerosis. *Human Gene Therapy, 10*, 1853–1866.

Moreno-Igoa, M., Calvo, A. C., Penas, C., et al. (2010). Fragment C of tetanus toxin, more than a carrier. Novel perspectives in non-viral ALS gene therapy. *Journal of Molecular Medicine (Berlin), 88*, 297–308.

Munch, C., Sedlmeier, R., Meyer, T., et al. (2004). Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. *Neurology, 63*, 724–726.

Mutoh, T., Sobue, G., Hamano, T., Kuriyama, M., Hirayama, M., Yamamoto, M., & Mitsuma, T. (2000). Decreased phosphorylation levels of TrkB neurotrophin receptor in the spinal cords from patients with amyotrophic lateral sclerosis. *Neurochemical Research, 25*, 239–245.

Nagano, I., Ilieva, H., Shiote, M., Murakami, T., Yokoyama, M., Shoji, M., & Abe, K. (2005a). Therapeutic benefit of intrathecal injection of insulin-like growth factor-1 in a mouse model of Amyotrophic Lateral Sclerosis. *Journal of Neurological Sciences, 235*, 61–68.

Nagano, I., Shiote, M., Murakami, T., et al. (2005b). Beneficial effects of intrathecal IGF-1 administration in patients with amyotrophic lateral sclerosis. *Neurological Research, 27*, 768–772.

Nakamura, T., Tomita, Y., Hirai, R., Yamaoka, K., Kaji, K., & Ichihara, A. (1985). Inhibitory effect of transforming growth factor-beta on DNA synthesis of adult rat hepatocytes in primary culture. *Biochemical and Biophysical Research Communications, 133*, 1042–1050.

Nakao, N., Odin, P., Lindvall, O., & Brundin, P. (1996). Differential trophic effects of basic fibroblast growth factor, insulin-like growth factor-1, and neurotrophin-3 on striatal neurons in culture. *Experimental Neurology, 138*, 144–157.

Ochs, G., Penn, R. D., York, M., et al. (2000). A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 1*, 201–206.

Okura, Y., Arimoto, H., Tanuma, N., Matsumoto, K., Nakamura, T., Yamashima, T., Miyazawa, T., & Matsumoto, Y. (1999). Analysis of neurotrophic effects of hepatocyte growth factor in the adult hypoglossal nerve axotomy model. *The European Journal of Neuroscience, 11*, 4139–4144.

Oosthuyse, B., Moons, L., Storkebaum, E., et al. (2001). Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. *Nature Genetics, 28*, 131–138.

Oppenheim, R. W., Yin, Q. W., Prevette, D., & Yan, Q. (1992). Brain-derived neurotrophic factor rescues developing avian motoneurons from cell death. *Nature, 360*, 755–757.

Pan, W., & Kastin, A. J. (2000). Interactions of IGF-1 with the blood–brain barrier in vivo and in situ. *Neuroendocrinology, 72*, 171–178.

Pan, W., Kastin, A. J., Maness, L. M., & Brennan, J. M. (1999). Saturable entry of ciliary neurotrophic factor into brain. *Neuroscience Letters, 263*, 69–71.

Pan, W., Yu, Y., Yemane, R., Cain, C., Yu, C., & Kastin, A. J. (2006). Permeation of hepatocyte growth factor across the blood–brain barrier. *Experimental Neurology, 201*, 99–104.

Park, S., Kim, H.-T., Yun, S., Kim, I.-S., Lee, J., Lee, I.-S., & Park, K. I. (2009). Growth factor-expressing human neural progenitor cell grafts protect motor neurons but do not ameliorate motor performance and survival in ALS mice. *Experimental & Molecular Medicine, 41*, 487–500.

Perlson, E., Jeong, G.-B., Ross, J. L., Dixit, R., Wallace, K. E., Kalb, R. G., & Holzbaur, E. L. F. (2009). A switch in retrograde signaling from survival to stress in rapid-onset neurodegeneration. *The Journal of Neuroscience, 29*, 9903–9917.

Petri, S., Krampfl, K., Kuhlemann, K., Dengler, R., & Grothe, C. (2009). Preserved expression of fibroblast growth factor (FGF)-2 and FGF receptor 1 in brain and spinal cord of amyotrophic lateral sclerosis patients. *Histochemistry and Cell Biology, 131*, 509–519.

Peviani, M., Caron, I., Pizzasegola, C., Gensano, F., Tortarolo, M., & Bendotti, C. (2010). Unraveling the complexity of amyotrophic lateral sclerosis: recent advances from the transgenic mutant SOD1 mice. *CNS & Neurological Disorders Drug Targets, 9*, 491–503.

Piehl, F., Ji, R. R., Cullheim, S., Hokfelt, T., Lindholm, D., & Hughes, R. A. (1995). Fibroblast growth factors regulate calcitonin gene-related peptide mRNA expression in rat motoneurons after lesion and in culture. *The European Journal of Neuroscience, 7*, 1739–1750.

Poduslo, J. F., & Curran, G. L. (1996). Permeability at the blood–brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. *Brain Research Molecular Brain Research, 36*, 280–286.

Poo, M. M. (2001). Neurotrophins as synaptic modulators. *Nature Review Neuroscience, 2*, 24–32.

Puls, I., Jonnakuty, C., LaMonte, B. H., et al. (2003). Mutant dynactin in motor neuron disease. *Nature Genetics, 33*, 455–456.

Reaume, A. G., Elliott, J. L., Hoffman, E. K., et al. (1996). Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nature Genetics, 13*, 43–47.

Reddy, L. V., Koirala, S., Sugiura, Y., Herrera, A. A., & Ko, C. P. (2003). Glial cells maintain synaptic structure and function and promote development of the neuromuscular junction in vivo. *Neuron, 40*, 563–580.

Renton, A. E., Majounie, E., Waite, A., et al. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron, 72*, 257–268.

Riethmacher, D., Sonnenberg-Riethmacher, E., Brinkmann, V., Yamaai, T., Lewin, G. R., & Birchmeier, C. (1997). Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. *Nature, 389*, 725–730.

Rind, H. B., & von Bartheld, C. S. (2002). Anterograde axonal transport of internalized GDNF in sensory and motor neurons. *Neuroreport, 13*, 659–664.

Robinson, R. (2012). Antisense therapy for ALS found safe in small trial, but more safety data are sought. *Neurology Today, 12*, 4–5.

Rogers, M.-L., & Rush, R. A. (2012). Non-viral gene therapy for neurological diseases, with an emphasis on targeted gene delivery. *Journal of Controlled Release, 157*, 183–189.

Rotwein, P., Burgess, S. K., Milbrandt, J. D., & Krause, J. E. (1988). Differential expression of insulin-like growth factor genes in rat central nervous system. *Proceedings of the National Academy of Sciences of the United States of America, 85*, 265–269.

Roux, S., Saint Cloment, C., Curie, T., Girard, E., Mena, F.-J. M., Barbier, J., Osta, R., Molgó, J., & Brûlet, P. (2006). Brain-derived neurotrophic factor facilitates in vivo internalization of tetanus neurotoxin C-terminal fragment fusion proteins in mature mouse motor nerve terminals. *European Journal of Neuroscience, 24*, 1546–1554.

Rowland, L. P. (2001). How amyotrophic lateral sclerosis got its name: The clinical-pathologic genius of Jean-Martin Charcot. *Archives of Neurology, 58*, 512–515.

Rudnicki, S. A., Berry, J. D., Ingersoll, E., Archibald, D., Cudkowicz, M. E., Kerr, D. A., & Dong, Y. (2013). Dexpramipexole effects on functional decline and survival in subjects with amyotrophic lateral sclerosis in a Phase II study: Subgroup analysis of demographic and clinical characteristics. *Amyotrophic Lateral Sclerosis & Frontotemporal Degeneration, 14*, 44–51.

Sagot, Y., Rossé, T., Vejsada, R., Perrelet, D., & Kato, A. C. (1998). Differential effects of neurotrophic factors on motoneuron retrograde labeling in a murine model of motoneuron disease. *The Journal of Neuroscience, 18*, 1132–1141.

Scott, S., Kranz, J. E., Cole, J., et al. (2008). Design, power, and interpretation of studies in the standard murine model of ALS. *Amyotrophic Lateral Sclerosis: Official Publication of the World Federation of Neurology Research Group on Motor Neuron Diseases, 9*, 4–15.

Selvaraj, B. T., Frank, N., Bender, F. L. P., Asan, E., & Sendtner, M. (2012). Local axonal function of STAT3 rescues axon degeneration in the pmn model of motoneuron disease. *The Journal of Cell Biology, 199*, 437–451.

Sendtner, M., Kreutzberg, G. W., & Thoenen, H. (1990). Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy. *Nature, 345*, 440–441.

Sendtner, M., Schmalbruch, H., Stöckli, K. A., Carroll, P., Kreutzberg, G. W., & Thoenen, H. (1992). Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuronopathy. *Nature, 358*, 502–504.

Shemer, J., Raizada, M. K., Masters, B. A., Ota, A., & LeRoith, D. (1987). Insulin-like growth factor I receptors in neuronal and glial cells. Characterization and biological effects in primary culture. *The Journal of Biological Chemistry, 262*, 7693–7699.

Shi, P., Wei, Y., Zhang, J., Gal, J., & Zhu, H. (2010). Mitochondrial dysfunction is a converging point of multiple pathological pathways in amyotrophic lateral sclerosis. *Journal of Alzheimer's Disease, 20*(Suppl 2), S311–S324.

Simon, C. M., Jablonka, S., Ruiz, R., Tabares, L., & Sendtner, M. (2010). Ciliary neurotrophic factor-induced sprouting preserves motor function in a mouse model of mild spinal muscular atrophy. *Human Molecular Genetics, 19*, 973–986.

Sondell, M., Sundler, F., & Kanje, M. (2000). Vascular endothelial growth factor is a neurotrophic factor which stimulates axonal outgrowth through the flk-1 receptor. *The European Journal of Neuroscience, 12*, 4243–4254.

Sorenson, E. J., Windbank, A. J., Mandrekar, J. N., et al. (2008). Subcutaneous IGF-1 is not beneficial in 2-year ALS trial. *Neurology, 71*, 1770–1775.

Stevenson, A., Yates, D. M., Manser, C., De Vos, K. J., Vagnoni, A., Leigh, P. N., McLoughlin, D. M., & Miller, C. C. J. (2009). Riluzole protects against glutamate-induced slowing of neurofilament axonal transport. *Neuroscience Letters, 454*, 161–164.

Storkebaum, E., Lambrechts, D., Dewerchin, M., et al. (2005). Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. *Nature Neuroscience, 8*, 85–92.

Sun, W., Funakoshi, H., & Nakamura, T. (2002). Overexpression of HGF retards disease progression and prolongs life span in a transgenic mouse model of ALS. *The Journal of Neuroscience, 22*, 6537–6548.

Sutedja, N. A., Fischer, K., Veldink, J. H., van der Heijden, G. J., Kromhout, H., Heederik, D., Huisman, M. H., Wokke, J. J., & van den Berg, L. H. (2009). What we truly know about occupation as a risk factor for ALS: A critical and systematic review. *Amyotrophic Lateral Sclerosis, 10*, 295–301.

Suzuki, M., Mchugh, J., Tork, C., Shelley, B., Klein, S. M., Aebischer, P., & Svendsen, C. N. (2007). GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. *PLoS ONE, 2*, e689.

Suzuki, M., Mchugh, J., Tork, C., Shelley, B., Hayes, A., Bellantuono, I., Aebischer, P., & Svendsen, C. N. (2008). Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS. *Molecular Therapy, 16*, 2002–2010.

Takeuchi, S., Fujiwara, N., Ido, A., et al. (2010). Induction of protective immunity by vaccination with wild-type apo superoxide dismutase 1 in mutant SOD1 transgenic mice. *Journal of Neuropathology and Experimental Neurology, 69*, 1044–1056.

Tatsumi, R., Sankoda, Y., Anderson, J. E., et al. (2009). Possible implication of satellite cells in regenerative motoneuritogenesis: HGF upregulates neural chemorepellent Sema3A during myogenic differentiation. *American Journal of Physiology. Cell Physiology, 297*, C238–C252.

Thau, N., Jungnickel, J., Knippenberg, S., Ratzka, A., Dengler, R., Petri, S., & Grothe, C. (2012). Prolonged survival and milder impairment of motor function in the SOD1 ALS mouse model devoid of fibroblast growth factor 2. *Neurobiology of Disease, 47*, 248–257.

The BDNF Study Group. (1999). A controlled trial of recombinant methionyl human BDNF in ALS: The BDNF Study Group (Phase III). *Neurology, 52*, 1427–1433.

Tolosa, L., Mir, M., Asensio, V. J., Olmos, G., & Lladó, J. (2008). Vascular endothelial growth factor protects spinal cord motoneurons against glutamate-induced excitotoxicity via phosphatidylinositol 3-kinase. *Journal of Neurochemistry, 105*, 1080–1090.

Tsuboi, Y., Kakimoto, K., Akatsu, H., Daikuhara, Y., & Yamada, T. (2002). Hepatocyte growth factor in cerebrospinal fluid in neurologic disease. *Acta Neurologica Scandinavica, 106*, 99–103.

Turner, B., & Talbot, K. (2008). Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. *Progress in Neurobiology, 85*, 94–134.

Upton-Rice, M. N., Cudkowicz, M. E., Warren, L., Mathew, R. K., Ren, J. M., Finklestein, S. P., & Brown, R. H., Jr. (1999). Basic fibroblast growth factor does not prolong survival in a transgenic model of familial amyotrophic lateral sclerosis. *Annals of Neurology, 46*, 934.

Vande Velde, C., Dion, P. A., & Rouleau, G. A. (2011). Amyotrophic lateral sclerosis: New genes, new models, and new mechanisms. *F1000 Biology Reports, 3*, 18.

Verstraete, E., Veldink, J. H., Huisman, M. H. B., et al. (2012). Lithium lacks effect on survival in amyotrophic lateral sclerosis: A phase IIb randomised sequential trial. *Journal of Neurology, Neurosurgery, and Psychiatry, 83*, 557–564.

Wang, W., Merrill, M. J., & Borchardt, R. T. (1996). Vascular endothelial growth factor affects permeability of brain microvessel endothelial cells in vitro. *The American Journal of Physiology, 271*, C1973–C1980.

Wang, Y., Ou Mao, X., Xie, L., Banwait, S., Marti, H. H., Greenberg, D. A., & Jin, K. (2007). Vascular endothelial growth factor overexpression delays neurodegeneration and prolongs survival in amyotrophic lateral sclerosis mice. *Journal of Neuroscience, 27*, 304–307.

Wilczak, N., de Vos, R. A. I., & De Keyser, J. (2003). Free insulin-like growth factor (IGF)-I and IGF binding proteins 2, 5, and 6 in spinal motor neurons in amyotrophic lateral sclerosis. *Lancet, 361*, 1007–1011.

Wong, V., Glass, D. J., Arriaga, R., Yancopoulos, G. D., Lindsay, R. M., & Conn, G. (1997). Hepatocyte growth factor promotes motor neuron survival and synergizes with ciliary neurotrophic factor. *The Journal of Biological Chemistry, 272*, 5187–5191.

Woolley, A. G., Sheard, P. W., & Duxson, M. J. (2005). Neurotrophin-3 null mutant mice display a postnatal motor neuropathy. *The European Journal of Neuroscience, 21*, 2100–2110.

Wu, F. T. H., Stefanini, M. O., Mac Gabhann, F., & Popel, A. S. (2009). A compartment model of VEGF distribution in humans in the presence of soluble VEGF receptor-1 acting as a ligand trap. *PLoS ONE, 4*, e5108.

Wu, L. S., Cheng, W. C., Hou, S. C., Yan, Y. T., Jiang, S. T., & Shen, C. K. (2010). TDP-43, a neuro-pathosignature factor, is essential for early mouse embryogenesis. *Genesis, 48*, 56–62.

Xu, L., Yan, J., Chen, D., Welsh, A. M., Hazel, T., Johe, K., Hatfield, G., & Koliatsos, V. E. (2006). Human neural stem cell grafts ameliorate motor neuron disease in SOD1 transgenic rats. *Transplantation, 82*, 865–875.

Xu, L., Ryugo, D. K., Pongstaporn, T., Johe, K., & Koliatsos, V. E. (2009). Human neural stem cell grafts in the spinal cord of SOD1 transgenic rats: Differentiation and structural integration into the segmental motor circuitry. *The Journal of Comparative Neurology, 514*, 297–309.

Xu, L., Shen, P., Hazel, T., Johe, K., & Koliatsos, V. E. (2011). Dual transplantation of human neural stem cells into cervical and lumbar cord ameliorates motor neuron disease in SOD1 transgenic rats. *Neuroscience Letters, 494*, 222–226.

Yamanaka, K., Chun, S.-J., Boillée, S., Fujimori-Tonou, N., Yamashita, H., Gutmann, D. H., Takahashi, R., Misawa, H., & Cleveland, D. W. (2008). Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nature Neuroscience, 11*, 251–253.

Yan, Q., Elliott, J., & Snider, W. D. (1992). Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature, 360*, 753–755.

Yan, Q., Elliott, J. L., Matheson, C., Sun, J., Zhang, L., Mu, X., Rex, K. L., & Snider, W. D. (1993). Influences of neurotrophins on mammalian motoneurons in vivo. *Journal of Neurobiology, 24*, 1555–1577.

Yan, J., Xu, L., Welsh, A. M., Chen, D., Hazel, T., Johe, K., & Koliatsos, V. E. (2006). Combined immunosuppressive agents or CD4 antibodies prolong survival of human neural stem cell grafts and improve disease outcomes in amyotrophic lateral sclerosis transgenic mice. *Stem Cells (Dayton, Ohio), 24*, 1976–1985.

Yanpallewar, S. U., Barrick, C. A., Buckley, H., Becker, J., & Tessarollo, L. (2012). Deletion of the BDNF truncated receptor TrkB.T1 delays disease onset in a mouse model of amyotrophic lateral sclerosis. *PLoS ONE, 7*, e39946.

Yasuda, T., & Mochizuki, H. (2010). Use of growth factors for the treatment of Parkinson's disease. *Expert Review of Neurotherapeutics, 10*, 915–924.

Zhang, Y., & Pardridge, W. M. (2009). Near complete rescue of experimental Parkinson's disease with intravenous, non-viral GDNF gene therapy. *Pharmaceutical Research, 26*, 1059–1063.

Zhang, B., Tu, P., Abtahian, F., Trojanowski, J. Q., & Lee, V. M. (1997). Neurofilaments and orthograde transport are reduced in ventral root axons of transgenic mice that express human SOD1 with a G93A mutation. *The Journal of Cell Biology, 139*, 1307–1315.

Zhang, Y., Zhang, H., Fu, Y., Song, H., Wang, L., Zhang, J., & Fan, D. (2006). VEGF C2578A polymorphism does not contribute to amyotrophic lateral sclerosis susceptibility in sporadic Chinese patients. *Amyotrophic Lateral Sclerosis, 7*, 119–122.

Zheng, C., Nennesmo, I., Fadeel, B., & Henter, J. (2004). Vascular endothelial growth factor prolongs survival in a transgenic mouse model of ALS. *Annals of Neurology, 56*, 564–567.

Zheng, C., Sköld, M. K., Li, J., Nennesmo, I., Fadeel, B., & Henter, J.-I. (2007). VEGF reduces astrogliosis and preserves neuromuscular junctions in ALS transgenic mice. *Biochemical and Biophysical Research Communications, 363*, 989–993.

Zhu, S., Stavrovskaya, I. G., Drozda, M., et al. (2002). Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature, 417*, 74–78.

Zinman, L., & Cudkowicz, M. (2011) Emerging targets and treatments in amyotrophic lateral sclerosis. *Lancet Neurol, 10*, 481–490.

Neurotrophins and p75NTR in Axonal Regeneration and Myelination

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Contents

Abstract

The neurotrophins are powerful, secreted signaling molecules that regulate the survival, proliferation, and function of neural cells. Neurotrophin-dependent signaling is not only critical during neurodevelopment but also plays an important role in the adult, where it mediates processes of plasticity and repair in both the central and peripheral nervous systems. The roles that the four known neurotrophins and their receptors play in regulating the cellular processes required for repair has been the subject of a significant body of research and has produced several promising avenues for treatment. This chapter reviews the current understanding of Neurotrophin-dependent signaling and how this evolving understanding can facilitate the development of future strategies to address

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neurodegeneration and neurotrauma. Of particular interest in this context is the p75 pan-neurotrophin receptor, a mercurial molecule that binds not only the neurotrophins but also the other neurotrophin receptors in order to modulate these signaling cascades. P75-dependant signaling has been shown to be essential in the regulation of processes such as neurite outgrowth, axonal regeneration, cell survival, cellular proliferation, and myelination; these processes are critical to successful neural repair. As we learn more about the neurotrophins and p75 through future research, novel therapeutic targets for the treatment of damage to the nervous system will continue to be revealed.

Keywords

Brain-derived neurotrophic factor • Dorsal root entry zone • Dorsal root ganglion • Eph/ephrin • LINGO-1 • Myelination • Nerve growth factor • Nerve growth factor receptor • Neurotrophin-3 • NogoA receptor • Oligodendrocyte • Semaphorin • Sortilin • Trk receptor

1 Introduction

Since the discovery of nerve growth factor (Cohen et al. 1954), neurotrophins (NTs) have been touted as potential remedies for neurotrauma. For injuries involving peripheral nerves or the spinal cord, the problem is inefficient or ineffective growth of damaged axons. The promise of NTs stems from the notion that disability may be reversed by providing neurons and their associated glia with adequate trophic support. While much work has been aimed at exploiting the function of spared axons as a possible solution to incomplete neurotrauma (Ramer 2010), true functional recovery entails regeneration of damaged axons and their reconnection with appropriate targets. This is not all, however, since regenerated axons must be myelinated to reliably conduct action potentials. It is therefore not insignificant that NTs provoke multiple responses from myelinating glia.

In this chapter the biology of NTs and their receptors will be reviewed, with a particular emphasis on the pan-NT receptor p75NTR, given its pivotal role in a growing number of signalling mechanisms. The effects of NTs and their receptors on regeneration and myelination, mainly in the context of sensory neurons of the dorsal root ganglion (DRG), will be discussed. By virtue of their anatomical arrangement (with axons in both the peripheral and central nervous systems) and well-characterized responses to NTs, DRG neurons have proven particularly informative with respect to the mechanisms by which NTs mediate both regeneration and myelination.

2 The Neurotrophins

Neurotrophic factors are characterized by their ability to support the survival and growth of specific neuronal populations. The neurotrophic hypothesis holds that

target-derived neurotrophins, retrogradely transported to cell bodies, are responsible for maintenance of neuronal phenotype in adulthood. The most complete existing data relates to the NTs (Bradbury et al. 2000; Boucher et al. 2000). There are four known NTs: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), all of which form homodimers (Chao 2003). Each NT is derived from a precursor molecule (pro-NT) that can be cleaved by a furan or convertase to produce a mature NT which is subsequently released into the extracellular space. Although this posttranslational modification occurs intracellularly, unprocessed pro-NTs are also released to the extracellular space via the constitutive secretory pathway and – in the case of pro-BDNF – via a regulated secretory pathway (Mowla et al. 2001). The mature forms are non-covalently bound dimers that share 90 % homology, including a cysteine-rich motif within the structural core of the dimer and three beta hairpin loops forming the distinctive regions involved in determining receptor specificity (Wiesmann and de Vos 2001).

3 Neurotrophin Receptors

3.1 Trk Receptors

NT signals are received and transduced by the tropomyosin-related kinase (Trk) A, B, and C receptors as well as the p75 neurotrophin receptor (p75NTR), the latter being a member of the tumor necrosis factor receptor (TNFR) family. Each Trk receptor is a cell surface transmembrane protein that possesses ligand-dependant tyrosine kinase activity. However, alternative splicing of TrkB (TrkBT1, TrkBT2, and TrkBT4) and TrkC ($TrkCTK^-$) produces additional truncated isoforms of these receptors that lack the tyrosine kinase domain (Squinto et al. 1991; Valenzuela et al. 1993; Forooghian et al. 2001). Each full-length receptor and truncated isoform share a conserved extracellular domain that includes two immunoglobulin-like C2 domains that not only prevent the autoactivation of the full-length receptors (Arevalo et al. 2000) but also determine neurotrophin binding specificity (Urfer et al. 1995, 1997). This specificity has translated into high-affinity interactions of NGF with TrkA, BDNF and NT-4/5 with TrkB isoforms, and NT-3 with TrkC isoforms (Chao 2003). Thus in the majority of cases, the cellular sensitivity and responsiveness to neurotrophins is dictated by the expression of their respective Trk receptors (although NT-bound p75NTR can participate in NT signalling through alternate pathways (Naska et al. 2010)).

3.2 p75NTR

Perhaps the most interesting member of the neurotrophin-signalling suite is p75NTR, whose many roles in cellular signalling continue to emerge. p75NTR was first identified in 1973 (Herrup and Shooter 1973) and was originally named the nerve growth factor receptor (NGFR); the name was later changed when it was

realized that p75NTR had affinity not only for NGF but also for the other NTs. p75NTR was subsequently characterized as a 75 kDa, type I transmembrane protein (Grob et al. 1985). p75NTR has been simplistically described as a regulator of cell survival or death, but in reality its biological function is much more subtle and nuanced. In fact, p75NTR signalling can have effects as wide reaching as cell death, proliferation, differentiation, survival, neurite outgrowth/collapse, and even the modulation of synaptic connectivity, plasticity, and pruning (Skeldal et al. 2011; Garcia et al. 2011).

3.3 p75NTR Signalling Partners

There are several signalling modules in which p75NTR participates. First, p75NTR has been shown to greatly increase the binding affinity of the Trk receptors for their cognate NTs (Esposito et al. 2001). The Trk receptors form receptor dimers upon ligand binding, as do most receptor tyrosine kinases, and trans-autophosphorylate one another to activate downstream signalling pathways (Chao 2003). The association of p75NTR with these Trk dimers creates high-affinity binding sites, increasing both the sensitivity and degree of Trk signalling. In this way p75NTR is capable of positively regulating Trk signalling. The converse, however, is also possible. With proteolytic cleavage at a juxtamembrane α-secretase site, the extracellular domain of p75NTR is released into the extracellular space where it can bind competitively with available NTs. The consequent dearth of NTs available for the Trk receptors results in decreased Trk signalling.

Depending on the NTs present, the Trk receptors being expressed, and the type of cleavage (if any) that p75NTR undergoes, a whole multitude of functional outcomes is possible. Each of the different Trk receptors activates different downstream signalling pathways, which are often dependent on p75NTR. For example, TrkA is not able to activate a PI3-kinase/Akt signalling cascade without associating with p75NTR, but can activate a MAP-kinase cascade with or without p75NTR (Nykjaer et al. 2005). The Trk receptors are broadly involved in cell proliferation, differentiation, and survival, and p75NTR serves to enhance or mute these effects when it does or does not associate with them.

The Trk receptors are not p75NTR's only bedfellows, however. p75NTR also forms a complex with sortilin, a protein usually involved in intracellular vesicle trafficking, at the plasma membrane (Nykjaer et al. 2004). The association of sortilin with p75NTR greatly increases the affinity of p75NTR for the pro-NTs over mature NTs. It was shown that the interaction between p75NTR and sortilin is necessary for pro-NGF- and pro-BDNF-induced apoptosis (Nykjaer et al. 2004). In this context p75NTR is no longer a peripheral player, serving to modulate the signalling of another receptor, but is rather the receptor that transduces the signal to the cytosol. Upon binding of a pro-NT by the p75NTR/sortilin complex, the intracellular domain of p75NTR is cleaved and induces apoptosis via a Jun N-terminal kinase (JNK)-dependent mechanism (Barker 2004; Kenchappa et al. 2010).

A third signalling module that p75NTR is involved in is a membrane-bound complex with *LINGO-1* and, in neurons, the NogoA receptor (NgR) (Mi et al. 2004). This complex does not signal in response to NT or pro-NT signals, but rather responds to myelin-associated inhibitors (MAIs). These include Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp) (Wang et al. 2002). In this circumstance p75NTR (or the related molecule Troy) (Park et al. 2005; Shao et al. 2005) is again required for signalling. Although LINGO-1 possesses a tyrosine kinase phosphorylation site in its cytoplasmic aspect – indicating that it is likely able to interact with downstream intracellular signalling cascades (Mi et al. 2008) – it is unable to induce signalling in the absence of p75NTR, despite being able to bind its ligands. In neurons, the functional effect of signalling through the p75NTR/NgR/LINGO-1 complex is inhibition of neurite outgrowth via collapse of extending axons' growth cones (Yamashita and Tohyama 2003). In recent work, Park et al. (Park et al. 2010) showed that developmental MAI-dependent axonal pruning is also p75NTR dependent.

p75NTR is also connected to a fourth receptor complex, involved in Eph/ephrin signalling. In 2001, Kong et al. (Kong et al. 2001) cloned a novel transmembrane protein they called ARMS (ankyrin repeat-rich membrane spanning) downstream of Eph and neurotrophin signalling. ARMS interacted with both TrkA and p75NTR and could be phosphorylated following NT or ephrin B treatment of cultured cells. This was an exciting result, particularly since ephrin B3 was subsequently shown to be another MAI (Benson et al. 2005): p75NTR/ephrin B signalling may participate in ephrin B3-mediated neurite growth inhibition. Bolstering this notion, in sympathetic neurons p75NTR is required for ephrin B-mediated growth cone collapse (Naska et al. 2010). Other recent and compelling evidence of inhibitory p75NTR/ephrin A "reverse" signalling has emerged (Lim et al. 2008). Ephrin A lacks a transmembrane domain, but in the retinotectal system, it couples with p75NTR to mediate retinal axon repulsion during topographic map formation.

Inhibitory semaphorin signalling in growing axons also seems to involve p75NTR, although the data are not as clear-cut. p75NTR has been reported to bind plexins and neuropilins, both receptor components for semaphorin family members (Ben Zvi et al. 2007, but see Naska et al. 2010). In the absence of p75NTR, sensory neurons are hypersensitive to Sema 3A, an effect thought to be the result of enhanced association of neuropilins with plexins. Hypersensitivity of p75NTR sympathetic axons to Sema 3A was also shown in p75NTR−/− hearts, where the high-to-low gradient of sympathetic innervation from epi- to endocardium – shaped by endocardial Sema 3A expression during development (Ieda et al. 2007) – was greatly exaggerated (Lorentz et al. 2010). This same study also showed increased Sema 3A-mediated inhibition of outgrowth from p75NTR−/− sympathetic ganglion explants. These findings stand in contrast to another showing that functional p75NTR, bound to a NT ligand, is actually required for the growth cone collapsing effects of Sema 3 family members on sympathetic neurons (Naska et al. 2010). Although it seems clear that p75NTR participates in semaphorin signalling, there is not yet a complete picture of how it does so.

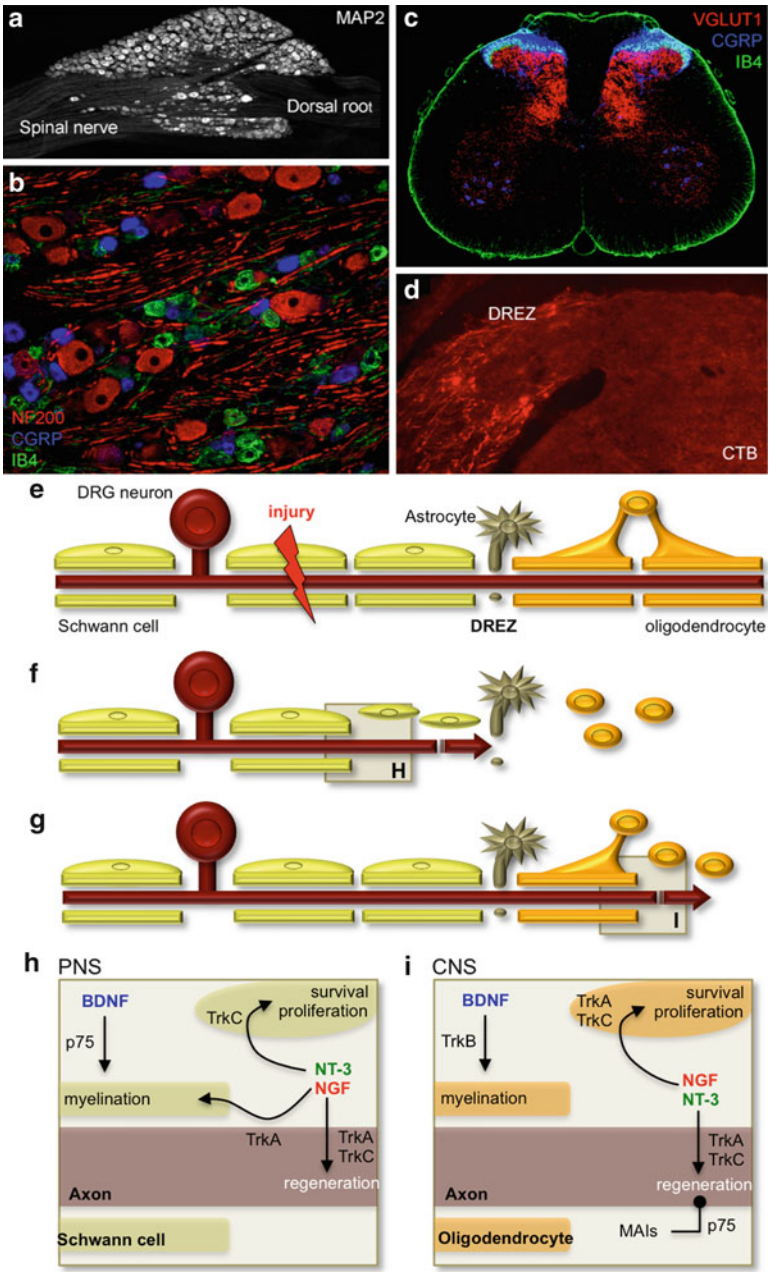

Fig. 1 Anatomy and selected NT responses of sensory neurons and associated glia. (**a**) A lumbar DRG section from a rat. (**b**) Subtypes of DRG neurons. Blue and green neurons, nociceptors; red neurons, mechano-/proprioceptors. (**c**) Central projections of sensory neurons. Terminal projections of subpopulations correspond to colors used in **B** (pia mater also binds IB4, and some motoneurons also express CGRP). (**d**) Large-diameter DRG neurons injured in the dorsal roots 1

4 Neurotrophins and Sensory Axon Regeneration

The tenets of the neurotrophic hypothesis have been most extensively demonstrated for sensory neurons of the dorsal root ganglion (DRG) (McMahon et al. 1995; Verge et al. 1995; Munson et al. 1997). As such, DRG neurons are ideal for assessing the role of neurotrophic factors and their receptors in regeneration. The anatomical arrangement of DRG neurons makes them ideal for experimental manipulation: they are pseudounipolar in that each has a single axon which emanates from the soma and bifurcates within the DRG, sending one branch peripherally in the peripheral nerves and the other centrally in the dorsal roots (Fig. 1). The dorsal root axons pass from the PNS into the CNS at a well-defined border, the dorsal root entry zone (DREZ). Once beyond the DREZ, most axons bifurcate again, forming longitudinal bundles. These are the ascending dorsal columns, large myelinated axons which terminate in the brainstem, and Lissauer's tract, a primarily unmyelinated propriospinal tract. From the longitudinally projecting axons extend branches which terminate in the grey matter of the dorsal and ventral horns. These are clustered around the axon's point of entry into the cord, but can occupy several segments rostrally and caudally. Thus, sensory axons can be injured in the peripheral nerve, in the dorsal roots (by rhizotomy), or within the spinal cord (Bradbury et al. 2000).

Sensory neurons come in several phenotypes, and this heterogeneity has been investigated in exquisite detail. About two thirds of all DRG neurons have unmyelinated or thinly myelinated axons, and more than 90 % of these innervate nociceptors peripherally. Roughly half of these are "peptidergic," as they express peptide neurotransmitters substance P and calcitonin gene-related peptide (CGRP). The remaining third of DRG neurons have rapidly conducting myelinated axons and subserve mechanoreception and proprioception.

All of the receptor components for neurotrophins are expressed in the adult DRG. Interestingly, small, substance P and CGRP-expressing nociceptive neurons virtually all express NGF receptor components – TrkA and p75NTR. The non-peptidergic group expresses none of the full-length Trk receptors, nor p75NTR (Molliver et al. 1997; Bennett et al. 1998). More than half of the large light neurons express TrkC and p75NTR, while few adult DRG neurons express TrkB (McMahon et al. 1994). Thus, the majority of sensory neurons respond to either NGF or NT-3.

NTs promote outgrowth of the expected phenotypes of adult sensory neurons (based on receptor expression in vivo) in dissociated culture (Lindsay 1988;

Fig. 1 (continued) week earlier and halted at the DREZ. (**e–g**) Stages in successful regeneration across the DREZ following dorsal root injury (indicated in **G**). (**h, i**) NT responses of axons, Schwann cells, and oligodendrocytes (and their precursors) and the NT receptors involved. Abbreviations: MAP2, microtubule-associated protein 2; NF200, 200 kDa neurofilament; CGRP, calcitonin gene-related peptide; IB4, lectin from *Bandeiraea simplicifolia*; VGLUT1, vesicular glutamate transporter 1; CTB, cholera toxin B fragment; MAIs, myelin-associated inhibitors

Hu-Tsai et al. 1994; Tuttle and Matthew 1995; Kimpinski et al. 1997; Gavazzi et al. 1999): NGF and NT-3 enhance outgrowth from peptidergic nociceptors and NF200-positive mechano-/proprioceptors, respectively. BDNF seems to have varied effects on sensory neurons in vitro, in some cases promoting outgrowth and in others inhibiting it (Gavazzi et al. 1999). In vivo, BDNF is ineffective at promoting regeneration of large-diameter axons (Bradbury et al. 1999; Ramer et al. 2000); furthermore, it inhibits intraspinal collateral sprouting of most primary afferent phenotypes (Ramer et al. 2007; Soril et al. 2008).

While the in vitro data show that NTs are clearly sufficient to elicit neurite outgrowth, there appears to be little necessity for endogenous NTFs in naturally occurring regeneration of peripheral nerves. For example, axotomized TrkA- and TrkC-expressing nociceptive and mechanosensitive axons reinnervate their peripheral targets normally in the presence of antibodies which block NGF and NT-3 signalling (Diamond et al. 1987; Yasargil et al. 1988; Diamond et al. 1992). Surprisingly little is known about the efficacy of exogenous NTs in enhancing regeneration of sensory axons following peripheral nerve injury. This is possibly because peripheral nerve regeneration actually happens, making subtle increases in regeneration distance or numbers of regenerating axons difficult to detect.

The dorsal rhizotomy model is particularly attractive for studying regeneration by virtue of the fact that regeneration proceeds in the permissive Schwann cell environment of the injured dorsal root, but halts upon contact with the astrocytic glia limitans at the PNS-CNS interface. Thus the role of neurotrophins and their receptors can be examined on a background of unequivocal regeneration failure. Using this model, Ramer et al. (Ramer et al. 2000) found that intrathecal delivery of NGF and NT-3, but not BDNF, resulted in regeneration of injured sensory axons beyond the DREZ and into the spinal cord. As might be expected from prior in vitro work, the phenotype of regenerating axons corresponded to Trk expression: NGF resulted in ingrowth of CGRP-expressing (TrkA-positive) primary afferents, and NT-3 resulted in regeneration of (TrkC-expressing) NF200-positive axons. The axons which regenerated were able to synaptically drive second-order neurons (Ramer et al. 2000, 2002), and at least in the case of NT-3, there was ultrastructural evidence of new synapses with neurons in the dorsal grey matter (Ramer et al. 2002). NGF administration was accompanied by the reemergence of responses to heat and noxious pressure (Ramer et al. 2000), and in NT-3-treated animals, behaviors requiring proprioception such as paw placement and walking across a beam and across a horizontal ladder returned (Ramer et al. 2002).

5 p75NTR and NT-Mediated Sensory Axon Regeneration

An attractive model for p75NTR/Trk interactions in axonal regeneration was posited in the late 1980s (Johnson, Jr. et al. 1988). This involved a "presentation" mechanism, by which Schwann cell-expressed p75NTR generated an NT gradient which could be followed by axons. This hypothetical framework led to the widely accepted notion that the powerful chemotactic effects of NTs on axons are mediated

by p75NTR (Huang and Reichardt 2003). However, several studies have pointed to a negative role of p75NTR in NT signalling and neurite outgrowth. Most notably, regeneration of motor axons following peripheral nerve injury is more robust in p75NTR knockouts than in wild-type mice (Ferri et al. 1998; Boyd and Gordon 2001, but see Song et al. 2009).

These data, combined with the possibility that α-secretase disrupts any substrate-bound gradient by liberating the ECD of p75NTR (Zampieri et al. 2005), led to questions regarding the validity of the presentation model. In fact, in p75NTR−/− mice (exon III deletion), injured sensory axons were able to not only penetrate the dorsal root entry zone and enter the CNS following dorsal rhizotomy but also drive CNS neurons and restore sensory function (Scott and Ramer 2010). Successful regeneration was dependent on endogenous NGF and NT-3, as the regeneration of both small-caliber and large-diameter axons was prevented in p75NTR−/− mice by NGF- and NT-3 sequestration by TrkA-Fc and TrkC-Fc, respectively. Importantly, p75NTR−/− axons could not penetrate the astrocytic scar unless their associated Schwann cells were also p75NTR−/−, and in tissue culture experiments of Schwann cells and DRG neurons, it was shown that Schwann cell-expressed p75NTR reduced NT-dependent neurite outgrowth. Since NGF and NT-3 levels were equivalent in wild-type and p75NTR−/− mice, these data showed that rather than presenting neurotrophins to regenerating axons, Schwann cell p75NTR restricts NT availability and limits regeneration into the cord.

It is important to contrast this work with studies on the effects of p75NTR following injury to sensory axons within the CNS. Since p75NTR transduces inhibitory signalling by MAIs, it is reasonable to hypothesize that its absence might relieve inhibition. However, Song et al. (Song et al. 2004) showed that sensory axons injured in the dorsal columns of p75NTR−/− mice (the same exon III null mutants used in the rhizotomy studies) failed to regenerate beyond the glial scar. The crucial difference between this and the rhizotomy model is the lack of reactive and NT-producing Schwann cells following direct CNS lesions. That is, regeneration did not fail following dorsal column injury because p75NTR was unable to mediate MAI signalling, but because trophic support was inadequate. Indeed, grafts of p75NTR−/− peripheral nerve are better able to support growth of injured dorsal column axons than are wild-type grafts (Scott and Ramer 2010). Together, these studies cast doubt on the role of all known p75NTR-interacting inhibitory molecules produced in the injured spinal cord (including NogoA, MAG, OMgp, ephrin B3, and Semaphorin 3A) in blocking regeneration of injured axons. In fact, a recent study using mice lacking all three known NgR ligands showed a frank absence of regeneration, reinforcing these uncertainties (Lee et al. 2010).

6 Neurotrophins, p75NTR, and Myelination

Successful establishment of axonal conduction during development, and successful recovery of function following neurotrauma, requires myelination. Some of the most

promising experimental strategies for promoting axonal outgrowth following CNS injury include manipulating neurotrophic factor levels and antagonizing signalling by MAIs (although in the latter case "outgrowth" is restricted to plasticity of spared fibers rather than regeneration of injured axons (Lee et al. 2010)). Despite its importance to functional recovery, it has yet to be determined in any meaningful way whether myelination accompanies the axonal regeneration induced by these manipulations.

In the PNS, axonal internodes are myelinated by Schwann cells (One Schwann cell per internode), and in the central nervous system (CNS), this task is performed by oligodendrocytes (multiple internodes per oligodendrocyte). Although similar in many respects, there are some very marked differences between these two cell types beyond their location and structure. One such difference is the expression of p75NTR following injury (Zhou and Li 2007). In Schwann cells p75NTR is dramatically upregulated following injury and remains so until axon/Schwann cell contact is reestablished, while in oligodendrocytes this is not the case. It has been suggested throughout the literature that this difference may be one key aspect that prevents CNS damage from being repaired in the same way as damage to the PNS, although the proposed mechanisms by which this might be accomplished vary.

6.1 Schwann Cells

In Schwann cells, Trk signalling is responsible for regulating proliferation and differentiation, as well as elongation and axon ensheathment. Cosgaya et al. (2002) demonstrated that NT-3, via TrkC, has a powerful inhibitory effect on myelination, maintaining Schwann cells in a proliferative state. NT-3 signalling via p75NTR also negatively affects Schwann cell migration (Bentley and Lee 2000; Yamauchi et al. 2004; Skeldal et al. 2011). Once NT-3 levels begin to drop, however, BDNF is able to upregulate myelin-specific genes in Schwann cells. BDNF is central to myelination by Schwann cells both in development and after injury: BDNF derived from sensory axons promotes Schwann cell myelination during development (Tolwani et al. 2004; Ng et al. 2007), and, following peripheral nerve injury, BDNF is required for myelination of regenerating axons (Zhang et al. 2000). Crucially, in all cases BDNF exerts its effects on Schwann cells via p75NTR (Cosgaya et al. 2002; Song et al. 2006).

NGF also promotes myelination in DRG-Schwann cell cocultures (Chan et al. 2004), although this is mediated not by a direct action of NGF on Schwann cells, but via TrkA expressed on sensory axons. It was speculated that some of the effects might have been due to an increase in axonal diameter, but it is well known that NGF potently upregulates BDNF selectively in TrkA-expressing DRG neurons and their processes (Michael et al. 1997). The potent effects of BDNF on myelination by Schwann cells probably underpin these indirect effects of NGF.

Schwann cell p75NTR not only is involved in the initiation of the glial myelination program and the maintenance of cell survival but appears to be involved in the actual act of ensheathment as well. Chan and colleagues (Chan et al. 2006) demonstrated that p75NTR is actively recruited to the axon-glial junction by the

polarity protein Par-3 at the commencement of ensheathment. Moreover, they showed that axon-Schwann cell contact was necessary to induce this recruitment and localization of p75NTR and proposed that this positions p75NTR precisely on the plasma membrane of the Schwann cell to receive pro-myelinating signals.

6.2 Oligodendrocytes

Neurotrophin signalling also operates in the oligodendrocyte lineage. Functional Trk receptors are expressed on these cells (Barres et al. 1994; Condorelli et al. 1995; Cohen et al. 1996; Kumar and de Vellis 1996); NGF and NT-3 promote oligodendrocyte precursor cell (OPC) survival in vitro and, when combined with platelet-derived growth factor, NT-3 induces their proliferation (Barres et al. 1994). NGF inhibits oligodendrocyte myelination of sensory axons in culture, but this occurs through modulating the "receptivity" of TrkA-expressing axons rather than via direct effects on OPCs (Chan et al. 2004). NT-3 withdrawal from culture results in OPC differentiation into pre-myelinating oligodendrocytes, and this is generally accepted to be the result of a cell-autonomous cycle-counting mechanism (Barres and Raff 1994; Durand and Raff 2000). In contrast to the survival- and proliferation-enhancing effects of NT-3, BDNF initiates the myelination program (via TrkB), and, accordingly, BDNF heterozygous mice exhibit delayed developmental myelination (Xiao et al. 2010). Unlike in Schwann cells, BDNF promotes oligodendrocyte myelination equally well in the presence or absence of p75NTR (Du et al. 2006; Xiao et al. 2010).

This is not to say that NGF and NT-3 never push oligodendrocytes toward differentiation. NT-3 and NGF have been shown to increase the expression of myelin basic protein and proteolipid protein, both components of mature myelin, in basal forebrain oligodendrocytes (Du et al. 2006). Unlike BDNF, they do so in part via p75NTR. For NT-3 at least, this occurs through activation of myelin protein translation, rather than by increasing myelin gene transcription (Coelho et al. 2009). Additionally, high concentrations of NT-3 are capable of transiently inducing OPC differentiation into oligodendrocytes (Heinrich et al. 1999).

Much of what is known about NT effects on oligodendrocytes comes from in vitro studies and developmental assessment of myelination in transgenic mouse studies; very little is known about NT effects on oligodendrocytes, their progenitors, or myelination of axons in the adult animal following neurotrauma. The dorsal rhizotomy model may again be particularly informative here, since Schwann cells do not invade the CNS when the injury occurs in the dorsal roots. In quantitative stereological TEM studies on rhizotomized rats, it was shown that 1 week's intrathecal NT-3 treatment, which promotes regeneration of sensory axons into the spinal cord, also results in a near doubling of oligodendroglial tissue in the CNS part of the DREZ (Hanna-Mitchell et al. 2008). How NT treatment affects myelination of regenerated axons in this model has not yet been worked out, but this task should be made easier by the fact that rhizotomy – unlike direct CNS trauma– does not result in oligodendrocyte progenitor death or demyelination of spared axons (Sun et al. 2010).

p75NTR/LINGO-1 signalling also has a role to play in oligodendrocytes, but it is distinct from that in neurons. In oligodendrocytes this signal inhibits differentiation of OPCs into pre-myelinating oligodendrocytes (Mi et al. 2005). LINGO-1 signalling via p75NTR seems to be essential to oligodendrocyte process outgrowth (and by extension, myelination), since the inhibitory effects of LINGO-1 are reversed by p75NTR antagonists (Bourikas et al. 2010). Intriguingly, axonal LINGO-1 also inhibits oligodendrocyte differentiation, an effect that is potentiated by NGF acting through axonal TrkA (Lee et al. 2007). LINGO-1 is expressed in white matter of the injured spinal cord (Barrette et al. 2007), so it is reasonable to speculate that myelin-derived inhibitory proteins released into the extracellular space following injury inhibit nearby OPCs from differentiating into myelinating oligodendrocytes. This intriguing hypothesis has yet to be tested, however.

7 Conclusion

The literature reviewed in this chapter illustrates the wide-ranging effects of NTs on axonal regeneration and myelination, both prerequisites for functional recovery in the wake of neurotrauma. These have largely been examined in isolation, however, and the time has come to develop strategies aimed at optimizing both processes. Although details vary, the following generalizations can be safely made (Fig. 1): NGF and NT-3 promote regeneration of sensory axons, and while NGF promotes peripheral myelination, NT-3 inhibits it. NGF and NT-3 also tend to enhance OPC survival and proliferation. BDNF, on the other hand, not only promotes myelination by both Schwann cells and oligodendrocytes but also inhibits plasticity of sensory axons. p75NTR is required for appropriate peripheral myelination, but may act to prevent oligodendrocyte myelination through its association with LINGO-1. For regeneration in the CNS – one of the toughest challenges in neuroscience – one might envision a sequential approach using NTs, first targeting sensory axonal regeneration and then initiating the myelination program.

The hope that neurotrophins hold for repairing the damaged nervous system must be tempered by the understanding that developmental mechanisms underlying neurotrophin-mediated axon growth and myelination are unlikely to be faithfully recapitulated in the adult. However, manipulations involving powerful experimental models – such as primary afferent neurons and their processes – will continue to shed light on how neurotrophin treatment might be refined to achieve all requirements of functional restoration.

References

Arevalo, J. C., Conde, B., Hempstead, B. L., Chao, M. V., Martin-Zanca, D., & Perez, P. (2000). TrkA immunoglobulin-like ligand binding domains inhibit spontaneous activation of the receptor. *Molecular and Cellular Biology, 20*, 5908–5916.

Barker, P. A. (2004). p75NTR is positively promiscuous: novel partners and new insights. *Neuron, 42*, 529–533.

Barres, B. A., & Raff, M. C. (1994). Control of oligodendrocyte number in the developing rat optic nerve. *Neuron, 12*, 935–942.

Barres, B. A., Raff, M. C., Gaese, F., Bartke, I., Dechant, G., & Barde, Y. A. (1994). A crucial role for neurotrophin-3 in oligodendrocyte development. *Nature, 367*, 371–375.

Barrette, B., Vallieres, N., Dube, M., & Lacroix, S. (2007). Expression profile of receptors for myelin-associated inhibitors of axonal regeneration in the intact and injured mouse central nervous system. *Molecular and Cellular Neuroscience, 34*, 519–538.

Ben Zvi, A., Ben Gigi, L., Klein, H., & Behar, O. (2007). Modulation of Semaphorin3A activity by p75 neurotrophin receptor influences peripheral axon patterning. *The Journal of Neuroscience, 27*, 13000–13011.

Bennett, D. L., Michael, G. J., Ramachandran, N., Munson, J. B., Averill, S., Yan, Q., et al. (1998). A distinct subgroup of small DRG cells express GDNF receptor components and GDNF is protective for these neurons after nerve injury. *The Journal of Neuroscience, 18*, 3059–3072.

Benson, M. D., Romero, M. I., Lush, M. E., Lu, Q. R., Henkemeyer, M., & Parada, L. F. (2005). Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 10694–10699.

Bentley, C. A., & Lee, K. F. (2000). p75 is important for axon growth and Schwann cell migration during development. *The Journal of Neuroscience, 20*, 7706–7715.

Boucher, T. J., Kerr, B. J., Ramer, M. S., Thompson, S. W., & McMahon, S. B. (2000). Neurotrophic factor effects on pain signaling systems. In M. Devor, M. C. Rowbotham, & Z. Wiesenfeld-Hallin (Eds.), *Proceedings of the 9th world congress on pain* (pp. 175–190). Seattle: IASP Press.

Bourikas, D., Mir, A., & Walmsley, A. R. (2010). LINGO-1-mediated inhibition of oligodendrocyte differentiation does not require the leucine-rich repeats and is reversed by p75(NTR) antagonists. *Molecular and Cellular Neuroscience, 45*, 363–369.

Boyd, J. G., & Gordon, T. (2001). The neurotrophin receptors, TrkB and p75, differentially regulate motor axonal regeneration. *Journal of Neurobiology, 49*, 314–325.

Bradbury, E. J., Khemani, S., Von King, R., Priestley, J. V., & McMahon, S. B. (1999). NT-3 promotes growth of lesioned adult rat sensory axons ascending in the dorsal columns of the spinal cord. *The European Journal of Neuroscience, 11*, 3873–3883.

Bradbury, E. J., McMahon, S. B., & Ramer, M. S. (2000). Keeping in touch: sensory neurone regeneration in the CNS. *Trends in Pharmacological Sciences, 21*, 389–394.

Chan, J. R., Watkins, T. A., Cosgaya, J. M., Zhang, C., Chen, L., Reichardt, L. F., et al. (2004). NGF controls axonal receptivity to myelination by Schwann cells or oligodendrocytes. *Neuron, 43*, 183–191.

Chan, J. R., Jolicoeur, C., Yamauchi, J., Elliott, J., Fawcett, J. P., Ng, B. K., et al. (2006). The polarity protein Par-3 directly interacts with p75NTR to regulate myelination. *Science, 314*, 832–836.

Chao, M. V. (2003). Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nature Reviews Neuroscience, 4*, 299–309.

Coelho, R. P., Yuelling, L. M., Fuss, B., & Sato-Bigbee, C. (2009). Neurotrophin-3 targets the translational initiation machinery in oligodendrocytes. *Glia, 57*, 1754–1764.

Cohen, S., Levi-Montalcini, R., & Hamburger, V. (1954). A nerve growth-stimulating factor isolated from sarcomas 37 and 180. *Proceedings of the National Academy of Sciences of the United States of America, 40*, 1014–1018.

Cohen, R. I., Marmur, R., Norton, W. T., Mehler, M. F., & Kessler, J. A. (1996). Nerve growth factor and neurotrophin-3 differentially regulate the proliferation and survival of developing rat brain oligodendrocytes. *The Journal of Neuroscience, 16*, 6433–6442.

Condorelli, D. F., Salin, T., Dell' Albani, P., Mudo, G., Corsaro, M., Timmusk, T., et al. (1995). Neurotrophins and their Trk receptors in cultured cells of the glial lineage and in white matter of the central nervous system. *Journal of Molecular Neuroscience, 6*, 237–248.

Cosgaya, J. M., Chan, J. R., & Shooter, E. M. (2002). The neurotrophin receptor p75NTR as a positive modulator of myelination. *Science, 298*, 1245–1248.

Diamond, J., Coughlin, M., Macintyre, L., Holmes, M., & Visheau, B. (1987). Evidence that endogenous beta nerve growth factor is responsible for the collateral sprouting, but not the regeneration, of nociceptive axons in adult rats. *Proceedings of the National Academy of Sciences of the United States of America, 84*, 6596–6600.

Diamond, J., Foerster, A., Holmes, M., & Coughlin, M. (1992). Sensory nerves in adult rats regenerate and restore sensory function to the skin independently of endogenous NGF. *The Journal of Neuroscience, 12*, 1467–1476.

Du, Y., Fischer, T. Z., Clinton-Luke, P., Lercher, L. D., & Dreyfus, C. F. (2006). Distinct effects of p75 in mediating actions of neurotrophins on basal forebrain oligodendrocytes. *Molecular and Cellular Neuroscience, 31*, 366–375.

Durand, B., & Raff, M. (2000). A cell-intrinsic timer that operates during oligodendrocyte development. *Bioessays, 22*, 64–71.

Esposito, D., Patel, P., Stephens, R. M., Perez, P., Chao, M. V., Kaplan, D. R., et al. (2001). The cytoplasmic and transmembrane domains of the p75 and Trk A receptors regulate high affinity binding to nerve growth factor. *The Journal of Biological Chemistry, 276*, 32687–32695.

Ferri, C. C., Moore, F. A., & Bisby, M. A. (1998). Effects of facial nerve injury on mouse motoneurons lacking the p75 low-affinity neurotrophin receptor. *Journal of Neurobiology, 34*, 1–9.

Forooghian, F., Kojic, L., Gu, Q., & Prasad, S. S. (2001). Identification of a novel truncated isoform of TrkB in the kitten primary visual cortex. *Journal of Molecular Neuroscience, 17*, 81–88.

Garcia, N., Tomas, M., Santafe, M. M., Lanuza, M. A., Besalduch, N., & Tomas, J. (2011). Blocking p75 NTR receptors alters polyinnervationz of neuromuscular synapses during development. *Journal of Neuroscience Research, 89*, 1331–1341.

Gavazzi, I., Kumar, R. D., McMahon, S. B., & Cohen, J. (1999). Growth responses of different subpopulations of adult sensory neurons to neurotrophic factors in vitro. *The European Journal of Neuroscience, 11*, 3405–3414.

Grob, P. M., Ross, A. H., Koprowski, H., & Bothwell, M. (1985). Characterization of the human melanoma nerve growth factor receptor. *The Journal of Biological Chemistry, 260*, 8044–8049.

Hanna-Mitchell, A. T., O'Leary, D., Mobarak, M. S., Ramer, M. S., McMahon, S. B., Priestley, J. V., et al. (2008). The impact of neurotrophin-3 on the dorsal root transitional zone following injury. *Spinal Cord, 46*, 804–810.

Heinrich, M., Gorath, M., & Richter-Landsberg, C. (1999). Neurotrophin-3 (NT-3) modulates early differentiation of oligodendrocytes in rat brain cortical cultures. *Glia, 28*, 244–255.

Herrup, K., & Shooter, E. M. (1973). Properties of the beta nerve growth factor receptor of avian dorsal root ganglia. *Proceedings of the National Academy of Sciences of the United States of America, 70*, 3884–3888.

Huang, E. J., & Reichardt, L. F. (2003). Trk receptors: roles in neuronal signal transduction. *Annual Review of Biochemistry, 72*, 609–642.

Hu-Tsai, M., Winter, J., Emson, P. C., & Woolf, C. J. (1994). Neurite outgrowth and GAP-43 mRNA expression in cultured adult rat dorsal root ganglion neurons: effects of NGF or prior peripheral axotomy. *Journal of Neuroscience Research, 39*, 634–645.

Ieda, M., Kanazawa, H., Kimura, K., Hattori, F., Ieda, Y., Taniguchi, M., et al. (2007). Sema3a maintains normal heart rhythm through sympathetic innervation patterning. *Nature Medicine, 13*, 604–612.

Johnson, E. M., Jr., Taniuchi, M., & DiStefano, P. S. (1988). Expression and possible function of nerve growth factor receptors on Schwann cells. *Trends in Neurosciences, 11*, 299–304.

Kenchappa, R. S., Tep, C., Korade, Z., Urra, S., Bronfman, F. C., Yoon, S. O., et al. (2010). p75 Neurotrophin receptor-mediated apoptosis in sympathetic neurons involves a biphasic activation of JNK and up-regulation of tumor necrosis factor-alpha-converting enzyme/ADAM17. *The Journal of Biological Chemistry, 285*, 20358–20368.

Kimpinski, K., Campenot, R. B., & Mearow, K. (1997). Effects of the neurotrophins nerve growth factor, neurotrophin-3, and brain-derived neurotrophic factor (BDNF) on neurite growth from adult sensory neurons in compartmented cultures. *Journal of Neurobiology, 33*, 395–410.

Kong, H., Boulter, J., Weber, J. L., Lai, C., & Chao, M. V. (2001). An evolutionarily conserved transmembrane protein that is a novel downstream target of neurotrophin and ephrin receptors. *The Journal of Neuroscience, 21*, 176–185.

Kumar, S., & de Vellis, J. (1996). Neurotrophin activates signal transduction in oligodendroglial cells: expression of functional TrkC receptor isoforms. *Journal of Neuroscience Research, 44*, 490–498.

Lee, X., Yang, Z., Shao, Z., Rosenberg, S. S., Levesque, M., Pepinsky, R. B., et al. (2007). NGF regulates the expression of axonal LINGO-1 to inhibit oligodendrocyte differentiation and myelination. *The Journal of Neuroscience, 27*, 220–225.

Lee, J. K., Geoffroy, C. G., Chan, A. F., Tolentino, K. E., Crawford, M. J., Leal, M. A., et al. (2010). Assessing spinal axon regeneration and sprouting in Nogo-, MAG-, and OMgp-deficient mice. *Neuron, 66*, 663–670.

Lim, Y. S., McLaughlin, T., Sung, T. C., Santiago, A., Lee, K. F., & O'Leary, D. D. (2008). p75(NTR) mediates ephrin-A reverse signaling required for axon repulsion and mapping. *Neuron, 59*, 746–758.

Lindsay, R. M. (1988). Nerve growth factors (NGF, BDNF) enhance axonal regeneration but are not required for survival of adult sensory neurons. *The Journal of Neuroscience, 8*, 2394–2405.

Lorentz, C. U., Alston, E. N., Belcik, T., Lindner, J. R., Giraud, G. D., & Habecker, B. A. (2010). Heterogeneous ventricular sympathetic innervation, altered beta-adrenergic receptor expression, and rhythm instability in mice lacking the p75 neurotrophin receptor. *American Journal of Physiology. Heart and Circulation Physiology, 298*, H1652–H1660.

McMahon, S. B., Armanini, M. P., Ling, L. H., & Phillips, H. S. (1994). Expression and coexpression of Trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets. *Neuron, 12*, 1161–1171.

McMahon, S. B., Bennett, D. L., Priestley, J. V., & Shelton, D. L. (1995). The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule. *Nature Medicine, 1*, 774–780.

Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., et al. (2004). LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nature Neuroscience, 7*, 221–228.

Mi, S., Miller, R. H., Lee, X., Scott, M. L., Shulag-Morskaya, S., Shao, Z., et al. (2005). LINGO-1 negatively regulates myelination by oligodendrocytes. *Nature Neuroscience, 8*, 745–751.

Mi, S., Sandrock, A., & Miller, R. H. (2008). LINGO-1 and its role in CNS repair. *The International Journal of Biochemistry & Cell Biology, 40*, 1971–1978.

Michael, G. J., Averill, S., Nitkunan, A., Rattray, M., Bennett, D. L., Yan, Q., et al. (1997). Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. *The Journal of Neuroscience, 17*, 8476–8490.

Molliver, D. C., Wright, D. E., Leitner, M. L., Parsadanian, A. S., Doster, K., Wen, D., et al. (1997). IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. *Neuron, 19*, 849–861.

Mowla, S. J., Farhadi, H. F., Pareek, S., Atwal, J. K., Morris, S. J., Seidah, N. G., et al. (2001). Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *The Journal of Biological Chemistry, 276*, 12660–12666.

Munson, J. B., Shelton, D. L., & McMahon, S. B. (1997). Adult mammalian sensory and motor neurons: roles of endogenous neurotrophins and rescue by exogenous neurotrophins after axotomy. *The Journal of Neuroscience, 17*, 470–476.

Naska, S., Lin, D. C., Miller, F. D., & Kaplan, D. R. (2010). p75NTR is an obligate signaling receptor required for cues that cause sympathetic neuron growth cone collapse. *Molecular and Cellular Neuroscience, 45*, 108–120.

Ng, B. K., Chen, L., Mandemakers, W., Cosgaya, J. M., & Chan, J. R. (2007). Anterograde transport and secretion of brain-derived neurotrophic factor along sensory axons promote Schwann cell myelination. *The Journal of Neuroscience, 27*, 7597–7603.

Nykjaer, A., Lee, R., Teng, K. K., Jansen, P., Madsen, P., Nielsen, M. S., et al. (2004). Sortilin is essential for proNGF-induced neuronal cell death. *Nature, 427*, 843–848.

Nykjaer, A., Willnow, T. E., & Petersen, C. M. (2005). p75NTR–live or let die. *Current Opinion in Neurobiology, 15*, 49–57.

Park, J. B., Yiu, G., Kaneko, S., Wang, J., Chang, J., & He, Z. (2005). A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. *Neuron, 45*, 345–351.

Park, K. J., Grosso, C. A., Aubert, I., Kaplan, D. R., & Miller, F. D. (2010). p75NTR-dependent, myelin-mediated axonal degeneration regulates neural connectivity in the adult brain. *Nature Neuroscience, 13*, 559–566.

Ramer, M. S. (2010). Endogenous neurotrophins and plasticity following spinal deafferentation. *Experimental Neurology, 235*, 70–77.

Ramer, M. S., Priestley, J. V., & McMahon, S. B. (2000). Functional regeneration of sensory axons into the adult spinal cord. *Nature, 403*, 312–316.

Ramer, M. S., Bishop, T., Dockery, P., Mobarak, M. S., O'Leary, D., Fraher, J. P., et al. (2002). Neurotrophin-3-mediated regeneration and recovery of proprioception following dorsal rhizotomy. *Molecular and Cellular Neuroscience, 19*, 239–249.

Ramer, L. M., McPhail, L. T., Borisoff, J. F., Soril, L. J., Kaan, T. K., Lee, J. H., et al. (2007). Endogenous TrkB ligands suppress functional mechanosensory plasticity in the deafferented spinal cord. *The Journal of Neuroscience, 27*, 5812–5822.

Scott, A. L., & Ramer, M. S. (2010). Schwann cell p75NTR prevents spontaneous sensory reinnervation of the adult spinal cord. *Brain, 133*(2), 421–432.

Shao, Z., Browning, J. L., Lee, X., Scott, M. L., Shulga-Morskaya, S., Allaire, N., et al. (2005). TAJ/TROY, an orphan TNF receptor family member, binds Nogo-66 receptor 1 and regulates axonal regeneration. *Neuron, 45*, 353–359.

Skeldal, S., Matusica, D., Nykjaer, A., & Coulson, E. J. (2011). Proteolytic processing of the p75 neurotrophin receptor: a prerequisite for signalling?: Neuronal life, growth and death signalling are crucially regulated by intra-membrane proteolysis and trafficking of p75(NTR). *Bioessays, 33*, 614–625.

Song, X. Y., Zhong, J. H., Wang, X., & Zhou, X. F. (2004). Suppression of p75NTR does not promote regeneration of injured spinal cord in mice. *The Journal of Neuroscience, 24*, 542–546.

Song, X. Y., Zhou, F. H., Zhong, J. H., Wu, L. L., & Zhou, X. F. (2006). Knockout of p75(NTR) impairs re-myelination of injured sciatic nerve in mice. *Journal of Neurochemistry, 96*, 833–842.

Song, X. Y., Zhang, F. H., Zhou, F. H., Zhong, J., & Zhou, X. F. (2009). Deletion of p75NTR impairs regeneration of peripheral nerves in mice. *Life Sciences, 84*, 61–68.

Soril, L. J., Ramer, L. M., McPhail, L. T., Kaan, T. K., & Ramer, M. S. (2008). Spinal brain-derived neurotrophic factor governs neuroplasticity and recovery from cold-hypersensitivity following dorsal rhizotomy. *Pain, 138*, 98–110.

Squinto, S. P., Stitt, T. N., Aldrich, T. H., Davis, S., Bianco, S. M., Radziejewski, C., et al. (1991). TrkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. *Cell, 65*, 885–893.

Sun, F., Lin, C. L., McTigue, D., Shan, X., Tovar, C. A., Bresnahan, J. C., et al. (2010). Effects of axon degeneration on oligodendrocyte lineage cells: dorsal rhizotomy evokes a repair response while axon degeneration rostral to spinal contusion induces both repair and apoptosis. *Glia, 58*, 1304–1319.

Tolwani, R. J., Cosgaya, J. M., Varma, S., Jacob, R., Kuo, L. E., & Shooter, E. M. (2004). BDNF overexpression produces a long-term increase in myelin formation in the peripheral nervous system. *Journal of Neuroscience Research, 77*, 662–669.

Tuttle, R., & Matthew, W. D. (1995). Neurotrophins affect the pattern of DRG neurite growth in a bioassay that presents a choice of CNS and PNS substrates. *Development, 121*, 1301–1309.

Urfer, R., Tsoulfas, P., O'Connell, L., Shelton, D. L., Parada, L. F., & Presta, L. G. (1995). An immunoglobulin-like domain determines the specificity of neurotrophin receptors. *The EMBO Journal, 14*, 2795–2805.

Urfer, R., Tsoulfas, P., O'Connell, L., & Presta, L. G. (1997). Specificity determinants in neurotrophin-3 and design of nerve growth factor-based trkC agonists by changing central beta-strand bundle residues to their neurotrophin-3 analogs. *Biochemistry, 36*, 4775–4781.

Valenzuela, D. M., Maisonpierre, P. C., Glass, D. J., Rojas, E., Nunez, L., Kong, Y., et al. (1993). Alternative forms of rat TrkC with different functional capabilities. *Neuron, 10*, 963–974.

Verge, V. M., Richardson, P. M., Wiesenfeld-Hallin, Z., & Hokfelt, T. (1995). Differential influence of nerve growth factor on neuropeptide expression in vivo: a novel role in peptide suppression in adult sensory neurons. *The Journal of Neuroscience, 15*, 2081–2096.

Wang, K. C., Kim, J. A., Sivasankaran, R., Segal, R., & He, Z. (2002). P75 Interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature, 420*, 74–78.

Wiesmann, C., & de Vos, A. M. (2001). Nerve growth factor: structure and function. *Cellular and Molecular Life Sciences, 58*, 748–759.

Xiao, J., Wong, A. W., Willingham, M. M., van den, B. M., Kilpatrick, T. J., & Murray, S. S. (2010). Brain-derived neurotrophic factor promotes central nervous system myelination via a direct effect upon oligodendrocytes. *Neurosignals, 18*, 186–202.

Yamashita, T., & Tohyama, M. (2003). The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. *Nature Neuroscience, 6*, 461–467.

Yamauchi, J., Chan, J. R., & Shooter, E. M. (2004). Neurotrophins regulate Schwann cell migration by activating divergent signaling pathways dependent on Rho GTPases. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 8774–8779.

Yasargil, G. M., Macintyre, L., Doucette, R., Visheau, B., Holmes, M., & Diamond, J. (1988). Axonal domains within shared touch domes in the rat: a comparison of their fate during conditions favoring collateral sprouting and following axonal regeneration. *The Journal of Comparative Neurology, 270*, 301–312.

Zampieri, N., Xu, C. F., Neubert, T. A., & Chao, M. V. (2005). Cleavage of p75 neurotrophin receptor by alpha-secretase and gamma-secretase requires specific receptor domains. *The Journal of Biological Chemistry, 280*, 14563–14571.

Zhang, J. Y., Luo, X. G., Xian, C. J., Liu, Z. H., & Zhou, X. F. (2000). Endogenous BDNF is required for myelination and regeneration of injured sciatic nerve in rodents. *The European Journal of Neuroscience, 12*, 4171–4180.

Zhou, X. F., & Li, H. Y. (2007). Roles of glial p75NTR in axonal regeneration. *Journal of Neuroscience Research, 85*, 1601–1605.

Neurotrophins and Pain

Ru-Ping Dai and Xin-Fu Zhou

Contents

Abstract

There is ample evidence suggesting that neurotrophins in particular nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) regulate pain processing as the modulators. NGF released from local tissue and dorsal root ganglia (DRG) binds to tropomyosin-related kinase (Trk) A receptors and

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regulates many membrane receptors, ion channels, and signaling molecules contributing to pain processing. Molecules antagonizing NGF-TrkA signaling have been developed and attenuate chronic pain in the patients. However, anti-NGF therapy leads to bone death in some patients suggesting that the therapy antagonizing NGF-TrkA signaling should be cautious to control pain in patients with chronic pain. On the other hand, brain-derived neurotrophic factor (BDNF) may regulate pain as a central modulator. BDNF is released when nociceptors are activated and regulated by NGF. This chapter reviews the roles and the underlying mechanisms by which NGF and BDNF regulate pain processing. The clinical trial of anti-NGF therapy is also discussed.

Keywords

Brain derived neutrophic factor • Central sensitization • Nerve growth factor • Neurotrophin • Pain • Spinal cord

List of Abbreviations

5-HT receptor	5-Hydroxytryptamine receptor
ASIC	Acid-sensing ion channels
B_2R	Bradykinin (BK) receptors
BDNF	Brain-derived neurotrophic factor
CGRP	Calcitonin gene-related peptide
CREB	cAMP response element-binding protein
DRG	Dorsal root ganglia
EPSCs	Excitatory postsynaptic currents
ERK	Extracellular regulated kinase
GABA	γ-aminobutyric acid
NGF	Nerve growth factor
NMDA receptor	*N*-methyl-D-aspartate receptor
NSAID	Nonsteroidal anti-inflammatory drugs
NT	Neurotrophin factor
$p75^{NTR}$	p75 neurotrophin receptor
SP	Substance P
TNF-α	Tumor necrosis factor-α
TRK	Tropomyosin kinase
TRPV	Transient receptor potential vanilloid

1 Introduction

Pain is an unpleasant feeling often caused by noxious stimuli such as trauma, stubbing a toe, or burning the finger. As defined by the International Association for the Study of Pain, pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such

damage" (Bonica 1979). It can motivate the individual to withdraw from the damaged situation and is essential for the avoidance of tissue injury and promotes healing after injury (Lynn 1983). Pain is usually transitory and disappears once the noxious stimuli are removed or the injured tissue is healed. However, in many situations, pain can be persistent even after removal of the noxious stimuli or the healing of the body. The development of pathological or chronic pain will severely affect the patients' life quality and general functions (Raj 2007). As a matter of fact, pain is the most common reason for physician consultation in the United States and Europe, and management of pathological pain is still a major health challenge throughout the world (Breivik et al. 2006; Hardt et al. 2008). This is because there is still a lack of effective treatments of chronic pain without unwanted side effects and/or abusive liability.

Currently, nonsteroidal anti-inflammatory drugs (NSAID) such as ibuprofen and cyclooxygenase-2 inhibitors and opioids such as tramadol or morphine are most widely used for pain control in clinical practice. However, the concerns of gastrointestinal and renal side effects of NSAIDs and of cardiovascular risk of cyclooxygenase-2 inhibitors limit their use in many medical conditions (Whelton 2000). On the other hand, despite the fact that opioids are an effective treatment in the many types of chronic pain, their side effects and liabilities, including loss of drug effectiveness, constipation (the most common long-term side effect causing noncompliance), drug diversion, respiratory depression and accidental death caused by overdose, are major concerns in clinical practice (Chou et al. 2009). Therefore, development of new agents for chronic pain control without severe side effects remains a significant, unmet clinical need.

Neurotrophins are a family of growth factors containing the following members: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin factor (NT), and NT-4/5. All neurotrophins are synthesized as a proform of approximately 30 kDa and cleaved to a mature form of approximately 13 kDa (Barbacid 1995; Reichardt 2006). Each neurotrophin binds with high affinity to their specific receptors, one of the tropomyosin kinase (Trk) family of transmembrane receptors. For example, NGF binds to TrkA, BDNF and NT-3/4 bind to TrkB, and NT-3 binds to TrkC (and to TrkA with lower affinity). Conversely all neurotrophins have a common low-affinity binding transmembrane receptor, p75 neurotrophin receptor ($p75^{NTR}$). Through binding to their high-affinity receptors and activating the downstream signaling, neurotrophins can regulate the survival, development, and function of neuronal subpopulations that express appropriate receptors (Barbacid 1995). In the absence of Trk or if the ratio of p75/TrkA is high, neurotrophins can activate $p75^{NTR}$ and subsequently the downstream signaling (JNK, Nf-kB) and promote apoptosis. In addition to regulating the survival and function of the nervous system, there is ample evidence indicating that NGF and BDNF play important roles in development of various types of pain (Pezet and McMahon 2006; Mantyh et al. 2011). In this chapter, the evidence that NGF and BDNF act as the pain mediators and modulators will be discussed (Fig. 1a). In addition, the clinical trials of management of chronic pain by neutralizing NGF are also introduced.

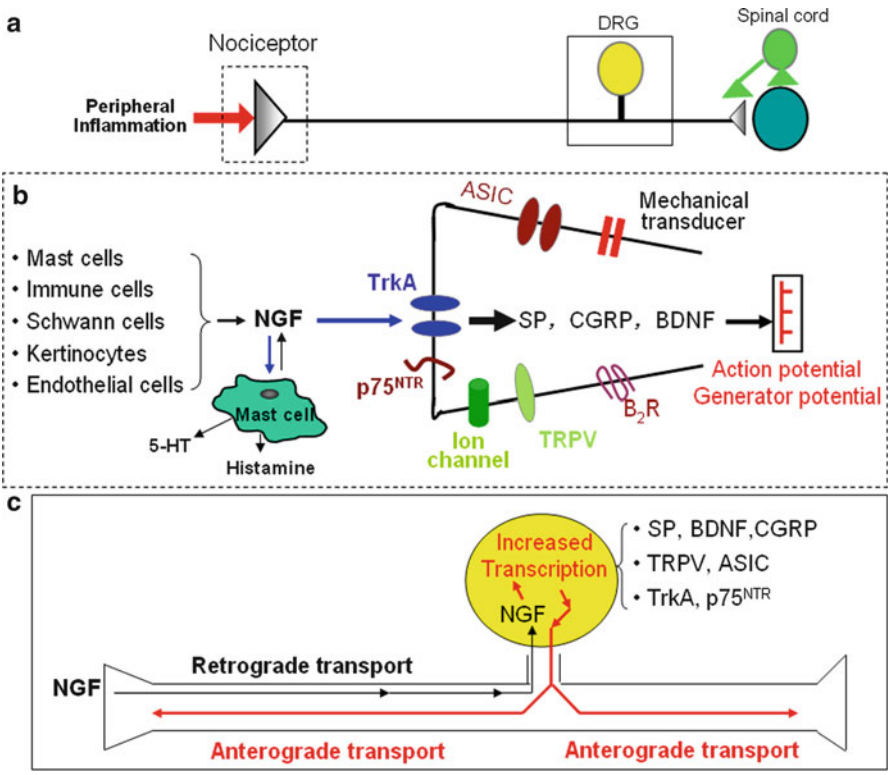

Fig. 1 Summary of the mechanisms by which NGF exerts its neuromodulator in pain processing. (**a**) Schematic summary of pain processing from nerve terminals, the primary sensory neuron (DRG), and spinal cord (second-order neuron). (**b**) Acute phase of NGF exerting pain hypersensitivity through peripheral sensitization. In response to peripheral inflammation, NGF released from many types of cells sensitizes many membrane surface receptors (TRPV, ASIC, etc.) and releases pain mediators (SP, CGRP, and BDNF), resulting in mechanical allodynia and thermal hyperalgesia. (**c**) Later phase of NGF exerting pain hypersensitivity through peripheral and central sensitizations. Released NGF can transport to DRG through a retrograde transport way. In the DRG, many genes are activated and the increased pain mediators such as SP, BDNF, and TRPV can anterogradely transport to peripheral tissue or spinal cord which contributes to central sensitization. The process may take hours to days

2 Nerve Growth Factor

Nerve growth factor is the first identified neurotrophin and can be isolated into two forms, 7S or 2.5S (S is defined as the sedimentation coefficient) from male mouse salivary gland. Mature NGF is an approximately 12.5 kDa protein and usually forms homodimers after being cleaved from pro NGF which may have independent biological activity through activating $p75^{NTR}$. NGF binds to TrkA, which directly

activates the downstream signaling (Levi-Montalcini 1998). The complexity of NGF-TrkA can be internalized and retrogradely transported to the cell bodies from the nerve terminals (Mantyh et al. 2011).

2.1 Deprivation of NGF Results in Pain Insensitivity

NGF as a pain modulator has been identified by the finding that selective mutations in NGF or TrkA genes cause congenital insensitivity to pain in humans and loss of pain behaviors in genetically altered mice (Crowley et al. 1994; Einarsdottir et al. 2004). In NGF or TrkA knockout mice, most sensory neurons expressing TrkA die and are profoundly unresponsive to pain noxious stimuli (Crowley et al. 1994; Smeyne et al. 1994; Einarsdottir et al. 2004). In addition, patients with a mutation in TrkA gene have normal proprioception and normal sensation to innocuous pressure but present pain insensitivity to thermal stimuli (Indo 2002). Other studies observed that immunization against NGF in the critical period of the development, i.e., in the utero or prenatal period, results in depletion of substance P in dorsal root ganglia (DRG) neurons in animals (Ross et al. 1981; Johnson et al. 1989). Delivery of anti-NGF antibody in the early postnatal development causes a developmental switch of high-threshold mechanoreceptors to sensitive mechanoreceptors (Ritter et al. 1991).

Together, deprivation of NGF using genetic or immunological methods shows that NGF is essential for the survival of the sensory neurons in the prenatal development phase. However, NGF is not required for the survival of sensory neurons in the postnatal phase; instead, NGF mainly maintains the peptidergic phenotype of primary neuronal afferents.

2.2 NGF in Inflammatory Pain

Ample evidence shows that NGF is involved in the induction and persistence of various types of pain including inflammatory pain, cancer pain, neuropathic pain, and surgical pain. In these types of pathological pain, NGF and its high-affinity receptor TrkA are upregulated in the local region and DRG. Manipulating NGF or TrkA and downstream signaling could attenuate pain hypersensitivity in pathological pain. Given that the role of NGF signaling has been extensively studied in the inflammatory pain, thus the role of NGF as an inflammatory mediator that regulates pain process firstly and then its role in other types of pain will be discussed.

In inflammatory pain including carrageenan, formalin, and complete Freund's adjuvants as well as in models of autoimmune arthritis (Shelton et al. 2005) and ultraviolet B radiation-induced acute inflammation (Bishop et al. 2007), NGF and TrkA are increased in the inflamed tissue and DRG. The local increase of NGF is mainly released from the inflammatory cells, immune cells, Schwann cells, as well as noninflammatory cells such as keratinocytes and endothelial cells (Tron et al. 1990). The released NGF binds to TrkA on mast cells resulting in the release of inflammatory mediators such as histamine, serotonin (5-HT), and NGF

itself (Fig. 1b). When NGF binds to TrkA on the peptidergic fiber terminals, it activates the downstream intracellular signaling pathways, which subsequently activate or modulate many membrane surface receptors including bradykinin receptors; ion channels including transient receptor potential vanilloid 1 (TRPV1), acid-sensing ion channels (ASIC) 2/3, voltage-gated sodium (Na_v) or calcium (Ca_v) ion channels, and delayed rectified potassium (K^+) currents; and putative mechanotransducers (Mantyh et al. 2011). The rapid modification of membrane surface receptors in the primary afferent terminals leads to sensitization of primary afferent terminals to mechanical or thermal stimuli (Bennett et al. 1998; Shu and Mendell 1999). The peripheral sensitization is rapid taking from minutes to hours (Fig. 1b). When the inflammation is persistent (e.g., inflammation induced by complete Freund's adjuvant), NGF and TrkA are transported to the DRG in a retrograde way (Ji et al. 2002). There NGF-TrkA signaling results in the production and release of neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) and the other neurotrophin, BDNF, some of which are transported to peripheral tissue or the spinal cord (Lindsay and Harmar 1989; Woolf and Costigan 1999; Ji et al. 2002). The procedure of central sensitization may take several hours to days since it is involved in the retrograde transport, gene expression, anterograde transport, phenotype switch, and so on (Woolf and Costigan 1999) (Fig. 1c). A typical example is that NGF-induced pain hypersensitivity is selectively blocked by MK 801, a noncompetitive antagonist of *N*-methyl-D-aspartate (NMDA) receptor, which plays a critical role in the development of windup and central sensitization (Woolf 1996; Yaksh et al. 1999).

2.3 NGF in Other Types of Pain

NGF not only acts as an inflammatory mediator and results in peripheral and central sensitization in inflammatory pain but is also involved in other types of pain. In a mouse model of bone cancer, sustained delivery of anti-NGF antibody could inhibit the development of cancer pain, along with blocking the pathological sprouting of sensory and sympathetic nerve fibers, the formation of neuroma-like structures (Halvorson et al. 2005; Mantyh et al. 2010). This study suggests that NGF is released during the development of cancer pain. The released local NGF induces a remarkable reorganization of sensory and sympathetic nerve fibers, resulting in the formation of neuroma-like structure and sympathetic sprouting (Mantyh et al. 2010). Further study demonstrates that the source of NGF may be mainly from tumor-associated stromal, inflammatory, and immune cells (Ehrhard et al. 1993; Artico et al. 2008; Jimenez-Andrade et al. 2010). In this regard, injection of prostate cells which do not express detectable levels of NGF gene results in sclerotic bone lesions. In this model of bone cancer, TrkA-positive sensory and sympathetic nerve fibers are also observed to undergo the reorganization of pathological sprouting. Administration of anti-NGF to sequester the increased NGF in the early phase of prostate-induced bone cancer pain could block the nerve fibers sprouting and dramatically attenuate the development of cancer pain (Jimenez-Andrade et al. 2010). The findings that NGF

contributes to the sprouting of sensory and sympathetic nerve fibers in cancer pain suggest that preemptive anti-NGF therapy not only attenuates the attendant pain but also inhibits the pathological changes of sensory and sympathetic nerve fibers, which may be critical for the development of chronic pain hypersensitivity. Therefore, anti-NGF therapy may be effective in treating various types of cancer pain which predicts the occurrence of tissue/nerve injury.

Recent studies have shown that NGF also regulates the postsurgical pain (Zahn et al. 2004; Wu et al. 2007). Hind paw incision (a common animal model of surgical pain) results in the increased NGF in the incised tissue, but not DRG. Anti-NGF treatment could attenuate the guarding pain- and heat hyperalgesia induced by hind paw incision although it would not affect the C-fiber sensitization (Zahn et al. 2004; Wu et al. 2007). Further study showed that the increased NGF after hind paw incision is mainly localized in nerve bundles including Schwann cells and axons (Wu et al. 2007). Thus, NGF may be taken up by the axon and transported into the DRG and exerts its biological function, similar to that in inflammatory pain. These findings suggest that NGF may be a potential target to treat postsurgical pain (Wu et al. 2007).

The role of NGF in the neuropathic pain has not been well established. Neuropathic pain is one of the greatest challenges in clinical practice and refractory to current therapies. Neuropathic pain is normally caused by nerve injury induced by various etiologies including diabetes, infection such as postherpetic neuralgia, drug treatment-induced neuropathy, and traumatic injury in the peripheral nerves or spinal cord (Pezet and McMahon 2006). In the traumatic injury-induced neuropathic pain models such as spinal nerve ligation or sciatic constriction injury, some axons have lesions while the other axons are intact. The role of NGF may be distinct in the development of this type of neuropathic pain. This is because in the injured axons, it undergoes Wallerian degeneration which may lead to the retrograde neurodegeneration to the spinal cord and loss of the contact with their peripheral target. NGF has been reported to exert neuroprotective effect on small-diameter nociceptive sensory neurons and therefore reverse sensory hyperalgesia (McMahon and Cafferty 2004). However, the clinical trial in diabetic neuropathy does not show any neuroprotective effect (Apfel 2002). In contrast, the injured Schwann cell or recruited immune cells may release NGF which would sensitize the intact axons or transport to the DRG in a retrograde way (Pezet and McMahon 2006). Thus, blocking NGF may be able to attenuate neuropathic pain. Several studies indeed have shown that anti-NGF could attenuate the pain hypersensitivity in rat models of sciatic constriction injury and spinal cord injury (Ramer and Bisby 1999; Deng et al. 2000; Gwak et al. 2003). Further studies are needed to elucidate how NGF is involved in the development of neuropathic pain.

2.4 Anti-NGF Therapy in Clinical Trials

Ample experimental evidence has shown that blocking NGF or its high-affinity receptor TrkA could greatly inhibit the progress of chronic pain, in particular pain

induced by inflammatory injury. For example, systemic anti-NGF treatment prevents thermal and mechanical hyperalgesia in a complete Freund's adjuvant-induced inflammatory pain (Woolf et al. 1994). Administration of Trk-IgG fusion protein attenuates pain hypersensitivity induced by carrageenan (McMahon et al. 1995; Koltzenburg et al. 1999). In visceral inflammatory pain model, administration of anti-NGF antibody or Trk-IgG fusion protein markedly reduced the pain hypersensitivity. In addition, anti-NGF therapy also reversed the prolonged hyperalgesia in autoimmune arthritis (Shelton et al. 2005). Further studies have shown that anti-NGF therapy reduces pain caused by bone fracture or incisional pain suggesting that anti-NGF therapy is also effective for other types of pain (Jimenez-Andrade et al. 2007; Wu et al. 2007; Ghilardi et al. 2011).

The encouraging data of anti-NGF therapy in animal studies drive the scientists to investigate the role of NGF in humans. In the patient with chronic pain including arthritis, fibromyalgia, or peripheral nerve injury, NGF expression is increased locally (Aloe et al. 1992; Anand et al. 1997; Sarchielli et al. 2007). In addition, subcutaneous injection of NGF leads to persistent mechanical hyperalgesia in humans (Svensson et al. 2003; Rukwied et al. 2010). These studies suggest that increased local NGF may be involved in the development of chronic pain. There are several strategies to develop the anti-NGF-TrkA signaling for the treatment of chronic pain. These include NGF sequestration by anti-NGF antibody, inhibiting NGF binding to TrkA, blocking TrkA kinase activity by TrkA-Fc fusion protein (Shelton et al. 1995; Winston et al. 2003).

One typical molecule is a humanized anti-NGF monoclonal antibody tanezumab (RN-624, PF-04383119) (Cattaneo 2010). An early study has shown that, as compared to the placebo, tanezumab dose-dependently reduces the knee pain whilst walking in the patients with osteoarthritis, a form of arthritis in which the cartilage breaks down in the joint (Lane et al. 2010). The clinical trial of 450 patients shows that tanezumab along with NSAIDs reduces pain by 45–62 %, while a placebo only reduces pain by 22 % (Lane et al. 2010). In addition, tanezumab was also associated with dramatically greater improvement as measured by the patients' global assessment. However, phase 3 osteoarthritis trial was halted because 16 patients taking tanezumab were observed to have bone tissue death in their hip and knee joints and required the joint replacement (Garber 2011). Several possibilities are raised including the combined use of tanezumab and NSAIDs and reduced pain leading to much more movement of the joints (Lane et al. 2010; Garber 2011). Thus, it is still an important issue to address the safety of anti-NGF therapy in patients with chronic pain (Ackermann 2012).

3 Brain-Derived Neurotrophic Factor

Another neurotrophin involved in pain is brain-derived neurotrophic factor (BDNF). BDNF is a 12.4 kDa basic protein and the second identified neurotrophin isolated from pig brain in 1982 (Barde et al. 1982). It is well known that BDNF plays a critical role for cell differentiation, neuronal survival, migration, dendritic

arborization, synaptogenesis, and activity-dependent synaptic plasticity in the central nervous system (Greenberg et al. 2009). Dysfunction of BDNF in the central nervous system would lead to cognitive dysfunction and anxiety/depression behaviors (Greenberg et al. 2009). There is ample evidence showing that BDNF also regulates pain processing as a central modulator (Pezet and McMahon 2006).

3.1 Expression Pattern of BDNF and TrkB

Besides widely expressed in the brain, BDNF is also expressed in the tissues involving in the pain processing including the DRG and spinal cord. In the DRG, BDNF is constitutively expressed in the small- and medium-sized peptidergic neurons, which accounts for 10–40 % in the DRG neurons (Wetmore and Olson 1995; Luo et al. 2001). The synthesis of BDNF in the DRG neurons anterogradely transports into central terminals of primary afferent fibers in spinal cord dorsal horns. In the spinal cord, BDNF is mainly located in the large vesicles which also contain substance P, the common pain mediator. The BDNF contained in the sensory neurons and terminals in the spinal cord are believed to be released with activity. TrkB is also expressed in a subpopulation of DRG and the terminals in the dorsal horns of spinal cord (Zhou et al. 1993; Kashiba et al. 2003). In addition, TrkB is also expressed in nodose and trigeminal neurons and their terminals in trigeminal nucleus (Behnia et al. 2003). TrkB immunoreactive cells are also observed in the neurons of spinothalamic tract, which is associated with the transmission of painful information to supraspinal regions (Slack et al. 2005).

In addition to its expression in sensory neurons and central nervous system, BDNF gene is also expressed in the peripheral tissue. The expression of BDNF is higher in visceral tissues (such as the bladder, lung, and colon) than in the somatic tissues (Braun et al. 1999; Lommatzsch et al. 1999). In inflammatory or disease situations, BDNF is upregulated locally. For example, in the lung by allergen challenges, BDNF level is increased and expressed in the inflammatory infiltrates in subsets of T cells and the activated alveolar macrophages (Braun et al. 2004). Sequestration of the upregulated BDNF blocked the enhanced airway reactivity and persistent airway obstruction (Braun et al. 2004). In patients with chronic pancreatitis, BDNF is also greatly increased in ductural complexes and perineurium of the enlarged nerve. The increased level of BDNF is well correlated with the intensity of pain in the patients (Zhu et al. 2001).

3.2 Effect of Exogenous BDNF in Pain

There is accumulating evidence showing that BDNF regulates various types of pain states in different anatomical sites including peripheral tissues, DRG, spinal cord, and supraspinal levels. First, intrathecal delivery of exogenous BDNF could induce mechanical allodynia and hyperalgesia (Coull et al. 2005). Second, BDNF superfusion dramatically increases C-fiber-mediated reflexes which is greatly

inhibited in the BDNF-deficient mice (Kerr et al. 1999). In the inflammatory state, BDNF significantly increases the frequency of miniature excitatory postsynaptic currents (EPSCs) of the neurons in the spinal dorsal horns suggesting that BDNF activates nociceptive second-order neurons (Matayoshi et al. 2005). Third, BDNF-TrkB signaling activates the signaling closely associated with pain sensitization. For example, BDNF, after binding to TrkB, could potentiate glutaminergic neurotransmission, in particular *N*-methyl-D-aspartate (NMDA) receptor response (Slack et al. 2004). NMDA receptor is an ionotropic glutamate receptor and is believed to play a central role in central sensitization (South et al. 2003). Furthermore, intraspinal injection of BDNF can release the neuropeptide SP and induce the activation of extracellular regulated kinase (ERK) in the spinal cord (Kawasaki et al. 2004). Activation of ERK in the DRG and spinal cord has been reported to contribute to the development of pain such as inflammatory pain and neuropathic pain (Obata et al. 2004). BDNF may be able to activate cAMP response element-binding protein (CREB) and early gene c-fos, both of which are sensitive and specific markers in the spinal cord for the noxious stimuli (Obata et al. 2004; Jongen et al. 2005). More recent study suggests that the increased BDNF in the spinal microglia induces the depolarizing shift in the anion reversal potential and subsequently converts the GABA (γ-aminobutyric acid) inhibitory neurons to excitatory neurons (Coull et al. 2005).

3.3 Role of BDNF in Inflammatory Pain

As described above, pharmacological, biochemical, and neurophysiological evidence demonstrates that exogenous BDNF promotes pain processing. Extensive studies have shown that endogenous BDNF is increased in the DRG and spinal cord, and contributes to pain hypersensitivity in inflammatory pain. First, BDNF is increased in the DRG and spinal cord following peripheral inflammation including intraplantar formalin, CFA, and carrageenan (Pezet et al. 2002). The upregulation of BDNF is partly mediated by NGF since anti-NGF antibodies prevent the activation of BDNF in the spinal cord (Michael et al. 1997). In addition, a recent study also reports that TNF-α also contributes to the upregulation of BDNF and TrkB in the DRG (Onda et al. 2004). In DRG primary culture, chronic TNF-α treatment also upregulates the expression of BDNF and TrkB accompanied by the increased expression of CGRP and SP (Lin et al. 2011). In addition, tooth pulp inflammation increases BDNF expression in trigeminal ganglion (Kashiba et al. 2003). The increased BDNF is co-localized with CGRP and TRPV1 (Duric and McCarson 2007; Lin et al. 2011). Second, intrathecal injection of TrkB-IgG fusion protein dramatically ameliorates the pain behavior in response to peripheral inflammation suggesting that BDNF-TrkB signaling in the DRG and spinal cord contributes to inflammatory pain hypersensitivity (Lin et al. 2011). BDNF-TrkB signaling exerts their pro-nociceptive effect through multiple signaling such as activation of ERK1/2 signaling and CGRP, the well-known inflammatory pain mediator (Pezet and McMahon 2006) (Fig. 2).

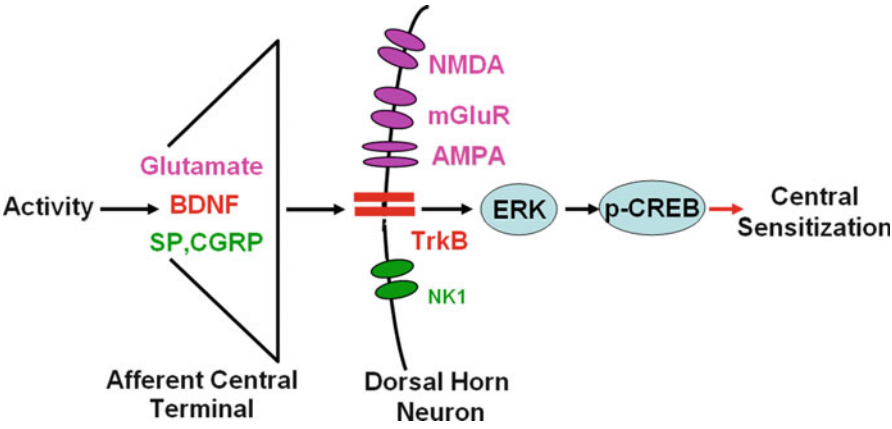

Fig. 2 Schematic summary of the mechanisms by which BDNF regulates pain processing as a central modulator in the spinal cord. Noxious stimuli induce the release of BDNF and neurotransmitters including glutamate, SP, and CGRP. BDNF can bind to TrkB and subsequently activate ERK which contributes to the central sensitization. Glutamate can bind to its receptors including NMDA, mGluR, and AMPA receptors. BDNF also phosphorylates the subunit of NMDA receptor, NR1. SP binds its receptor, NK1. These pain neurotransmitters also play critical role for the central sensitization

BDNF-TrkB signaling is also observed in the spinal cord or peripheral tissue in the inflammatory visceral pain. In a bladder inflammation model, TrkB is increased in the bladder, pelvic ganglion, and DRG (Qiao and Vizzard 2002). In addition, BDNF level is increased in the rectosigmoid biopsies which correlates with the pain score in the patients with irritable bowel syndrome (Yu et al. 2012). In experimental inflammation of mouse gut, BDNF is upregulated in the DRG in the experimental colitis (Yang et al. 2010). The pain intensity in this inflammatory visceral pain is reduced in BDNF (+/−) mice as compared with BDNF (+/+) mice (Yu et al. 2012). Our recent study showed that the modulation of BDNF in visceral pain is sex specific. Anti-BDNF antibody pretreatment attenuates the nocifensive response in the males, whilst exacerbates the nocifensive response in the females in visceral pain induced by acetic acid intraperitoneal injection (Li et al. 2010). The upregulation of BDNF in the pancreas has also been reported in the patients with chronic pancreatitis, and the level of BDNF correlated with pain intensity (Zhu et al. 2001). All of these studies strongly indicate that BDNF regulates inflammatory pain through peripheral and central mechanisms.

3.4 Role of BDNF in Neuropathic Pain and Surgical Pain

In neuropathic pain, BDNF expression is also dysregulated in the sensory neurons. However, the expression of BDNF in the sensory neurons is different in the different neuropathic pain models. For example, in the axotomy model induced by sciatic nerve section, BDNF level is upregulated in the medium- and large-diameter DRG

neurons and the central terminals in the deep layers of the dorsal horns in the spinal cord (Michael et al. 1999). However, the expression of BDNF in the small-diameter neurons is decreased probably due to the neurodegeneration induced by the axotomy. In another neuropathic pain model induced by sciatic nerve constriction injury, BDNF is upregulated in the small-diameter neurons. The increased BDNF in the small-diameter neurons may be mediated by TNF-α through inducing NGF expression (Onda et al. 2004). A series of studies have shown that the activated microglia can release BDNF in the spinal nerve ligation-induced neuropathic pain model (Onda et al. 2004). The increased BDNF in microglia activates TrkB, which, subsequently, converts inhibitory neurons to excitatory function through inverts the polarity of currents induced by γ-aminobutyric acid (GABA) (Coull et al. 2005). The disinhibition of GABA receptor-mediated neurons would contribute to the peripheral nerve injury-induced tactile allodynia (Coull et al. 2005). The upstream signaling of microglial BDNF includes ATP receptors, in particular P2X4 receptors through P2X4R-evoked increase in $Ca^{(2+)}$ and the activation of p38 mitogen activation protein kinase (Trang et al. 2009a). Thus, in peripheral nerve injury-induced neuropathic pain, the upregulation BDNF can be from the neurons or microglia and maintains the persistent pain hypersensitivity after nerve injury.

Our previous study has shown that BDNF is also increased in the DRG and spinal cord after surgical incision. The increased BDNF is mainly localized in the neurons as well as glial cells. Intrathecal delivery of anti-BDNF could dramatically reduce the incision-evoked pain hypersensitivity suggesting that BDNF in the DRG and spinal cord also contribute to pain hypersensitivity in surgical pain (Li et al. 2008). BDNF is also reported to be involved in cancer-induced pain through modulating NMDA receptors (Wang et al. 2012). Minocycline, a potent microglia inhibitor, could prevent the activation of BDNF in the model of bone cancer pain (Wang et al. 2012). This suggests that BDNF released from microglia contributes to cancer-induced bone pain.

3.5 Antinociceptive Effect of BDNF

Interestingly, in inflammatory pain, the expression of BDNF in the hippocampus is downregulated (Duric and McCarson 2007). Midbrain injection of BDNF, but not NGF, significantly exerts analgesia by elevating the tail-flick response latency and the latency in the hot-plate experiment (Siuciak et al. 1994, 1995). In addition, midbrain injection of BDNF also reduces the paw flinch response in the formalin intraplantar injection-induced pain (Siuciak et al. 1994, 1995). The analgesic effect of BDNF in the midbrain is associated with serotonergic neurons and can be prevented by naloxone (Siuciak et al. 1994, 1995). In addition, heterozygous BDNF(+/−) mice develops the hyperalgesia accompanied with the premature age-associated decreased forebrain 5-HT level and fiber density (Lyons et al. 1999). These findings suggest that supraspinal BDNF may exert antinociceptive effect through regulating the function of serotonergic neurons and levels of endogenous opioid receptors.

4 The Involvement of P75^{NTR} in Pain

Unlike TrkA and TrkB, p75^{NTR} is a low-affinity binding receptor for the whole family of neurotrophins. However, p75^{NTR} is the high-affinity binding receptor of precursor of neurotrophins such as pro NGF or proBDNF (precursor of BDNF). p75^{NTR} has been reported to exert multiple neuronal functions in the presence of Trk receptors (see review (Lu et al. 2005)). In the absence of Trks, neurotrophin acts on p75^{NTR} and promotes apoptosis. In a neuropathic pain model, p75^{NTR} expression is increased in the axon transport and uninjured primary afferents (Obata et al. 2006). Functional inhibition of p75^{NTR} in the DRG could reverse neuropathic pain after nerve injury. Further study shows that p75^{NTR} promotes neuropathic pain through enhancing TrkA signaling and activating p38 mitogen-activated protein kinase and TPRV1. These findings indicate that p75^{NTR} contributes to nerve injury-induced neuropathic pain (Obata et al. 2006). More recent study shows that morphine tolerance is attenuated in the wild-type but not p75^{NTR} (−/−) mice in which the induction of opioid physical dependence is not developed.

5 Conclusion

Neurotrophins, in particular NGF and BDNF, regulate and drive acute and chronic pain as pain modulators in adults. NGF contributes to pain processing mainly acting as peripheral inflammatory mediators. It can directly sensitize the peripheral nociceptive afferents and lead to peripheral sensitization rapidly. It can also retrogradely transport to sensory neurons and activate downstream signaling and gene expression. Anti-NGF therapy for treatment of chronic pain represents a novel class of analgesic therapy, but its safety is still a major challenge for the clinical application.

BDNF regulates pain processing as central modulators. BDNF is increased in the primary sensory neurons and spinal cord in the various types of pain. BDNF binds to TrkB and activates many pain mediators such as NMDA receptors, neuropeptides, and ERK, leading to the central sensitization. Sequestration of the increased BDNF through intrathecal delivery attenuates most types of pain. BDNF in the supraspinal level is downregulated in inflammatory pain, and midbrain infusion of BDNF exerts analgesic effect.

References

Ackermann, P. W., (2012). Katz et al. Efficacy and safety of tanezumab in the treatment of chronic low back pain [*Pain* 2011;152, 2248–2258] and Hill, Blocking the effects of NGF as a route to safe and effective pain relief – Fact or fancy? [*Pain* 2011;152, 2200–2201]. *Pain*, *153*, 1128–1129.

Aloe, L., Tuveri, M. A., Carcassi, U., & Levi-Montalcini, R. (1992). Nerve growth factor in the synovial fluid of patients with chronic arthritis. *Arthritis and Rheumatism, 35*, 351–355.

Anand, P., Terenghi, G., Birch, R., Wellmer, A., Cedarbaum, J. M., Lindsay, R. M., Williams-Chestnut, R. E., & Sinicropi, D. V. (1997). Endogenous NGF and CNTF levels in human peripheral nerve injury. *Neuroreport, 8*, 1935–1938.

Apfel, S. C. (2002). Nerve growth factor for the treatment of diabetic neuropathy: What went wrong, what went right, and what does the future hold? *International Review of Neurobiology, 50*, 393–413.

Artico, M., Bronzetti, E., Felici, L. M., Alicino, V., Ionta, B., Bronzetti, B., Magliulo, G., Grande, C., Zamai, L., Pasquantonio, G., & De Vincentiis, M. (2008). Neurotrophins and their receptors in human lingual tonsil: An immunohistochemical analysis. *Oncology Reports, 20*, 1201–1206.

Barbacid, M. (1995). Neurotrophic factors and their receptors. *Current Opinion in Cell Biology, 7*, 148–155.

Barde, Y. A., Edgar, D., & Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *The EMBO Journal, 1*, 549–553.

Behnia, A., Zhang, L., Charles, M., & Gold, M. S. (2003). Changes in TrkB-like immunoreactivity in rat trigeminal ganglion after tooth injury. *Journal of Endodontia, 29*, 135–140.

Bennett, D. L., Koltzenburg, M., Priestley, J. V., Shelton, D. L., & McMahon, S. B. (1998). Endogenous nerve growth factor regulates the sensitivity of nociceptors in the adult rat. *The European Journal of Neuroscience, 10*, 1282–1291.

Bishop, T., Hewson, D. W., Yip, P. K., Fahey, M. S., Dawbarn, D., Young, A. R., & McMahon, S. B. (2007). Characterisation of ultraviolet-B-induced inflammation as a model of hyperalgesia in the rat. *Pain, 131*, 70–82.

Bonica, J. J. (1979). The need of a taxonomy. *Pain, 6*, 247–248.

Braun, A., Lommatzsch, M., Mannsfeldt, A., Neuhaus-Steinmetz, U., Fischer, A., Schnoy, N., Lewin, G. R., & Renz, H. (1999). Cellular sources of enhanced brain-derived neurotrophic factor production in a mouse model of allergic inflammation. *American Journal of Respiratory Cell and Molecular Biology, 21*, 537–546.

Braun, A., Lommatzsch, M., Neuhaus-Steinmetz, U., Quarcoo, D., Glaab, T., McGregor, G. P., Fischer, A., & Renz, H. (2004). Brain-derived neurotrophic factor (BDNF) contributes to neuronal dysfunction in a model of allergic airway inflammation. *British Journal of Pharmacology, 141*, 431–440.

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *European Journal of Pain, 10*, 287–333.

Cattaneo, A. (2010). Tanezumab, a recombinant humanized mAb against nerve growth factor for the treatment of acute and chronic pain. *Current Opinion in Molecular Therapeutics, 12*, 94–106.

Chou, R., Fanciullo, G. J., Fine, P. G., Adler, J. A., Ballantyne, J. C., Davies, P., Donovan, M. I., Fishbain, D. A., Foley, K. M., Fudin, J., Gilson, A. M., Kelter, A., Mauskop, A., O'Connor, P. G., Passik, S. D., Pasternak, G. W., Portenoy, R. K., Rich, B. A., Roberts, R. G., Todd, K. H., & Miaskowski, C. (2009). Clinical guidelines for the use of chronic opioid therapy in chronic noncancer pain. *The Journal of Pain, 10*, 113–130.

Coull, J. A., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M. W., & De Koninck, Y. (2005). BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature, 438*, 1017–1021.

Crowley, C., Spencer, S. D., Nishimura, M. C., Chen, K. S., Pitts-Meek, S., Armanini, M. P., Ling, L. H., McMahon, S. B., Shelton, D. L., Levinson, A. D., et al. (1994). Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. *Cell, 76*, 1001–1011.

Deng, Y. S., Zhong, J. H., & Zhou, X. F. (2000). Effects of endogenous neurotrophins on sympathetic sprouting in the dorsal root ganglia and allodynia following spinal nerve injury. *Experimental Neurology, 164*, 344–350.

Duric, V., & McCarson, K. E. (2007). Neurokinin-1 (NK-1) receptor and brain-derived neurotrophic factor (BDNF) gene expression is differentially modulated in the rat spinal dorsal horn and hippocampus during inflammatory pain. *Molecular Pain, 3*, 32.

Ehrhard, P. B., Erb, P., Graumann, U., & Otten, U. (1993). Expression of nerve growth factor and nerve growth factor receptor tyrosine kinase Trk in activated CD4-positive T-cell clones. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 10984–10988.

Einarsdottir, E., Carlsson, A., Minde, J., Toolanen, G., Svensson, O., Solders, G., Holmgren, G., Holmberg, D., & Holmberg, M. (2004). A mutation in the nerve growth factor beta gene (NGFB) causes loss of pain perception. *Human Molecular Genetics, 13*, 799–805.

Foster, P. A., Costa, S. K., Poston, R., Hoult, J. R., & Brain, S. D. (2003). Endothelial cells play an essential role in the thermal hyperalgesia induced by nerve growth factor. *The FASEB Journal, 17*, 1703–1705.

Garber, K. (2011). Fate of novel painkiller mAbs hangs in balance. *Nature Biotechnology, 29*, 173–174.

Ghilardi, J. R., Freeman, K. T., Jimenez-Andrade, J. M., Mantyh, W. G., Bloom, A. P., Bouhana, K. S., Trollinger, D., Winkler, J., Lee, P., Andrews, S. W., Kuskowski, M. A., & Mantyh, P. W. (2011). Sustained blockade of neurotrophin receptors TrkA, TrkB and TrkC reduces non-malignant skeletal pain but not the maintenance of sensory and sympathetic nerve fibers. *Bone, 48*, 389–398.

Greenberg, M. E., Xu, B., Lu, B., & Hempstead, B. L. (2009). New insights in the biology of BDNF synthesis and release: Implications in CNS function. *The Journal of Neuroscience, 29*, 12764–12767.

Gwak, Y. S., Nam, T. S., Paik, K. S., Hulsebosch, C. E., & Leem, J. W. (2003). Attenuation of mechanical hyperalgesia following spinal cord injury by administration of antibodies to nerve growth factor in the rat. *Neuroscience Letters, 336*, 117–120.

Halvorson, K. G., Kubota, K., Sevcik, M. A., Lindsay, T. H., Sotillo, J. E., Ghilardi, J. R., Rosol, T. J., Boustany, L., Shelton, D. L., & Mantyh, P. W. (2005). A blocking antibody to nerve growth factor attenuates skeletal pain induced by prostate tumor cells growing in bone. *Cancer Research, 65*, 9426–9435.

Hardt, J., Jacobsen, C., Goldberg, J., Nickel, R., & Buchwald, D. (2008). Prevalence of chronic pain in a representative sample in the United States. *Pain Medicine, 9*, 803–812.

Indo, Y. (2002). Genetics of congenital insensitivity to pain with anhidrosis (CIPA) or hereditary sensory and autonomic neuropathy type IV. Clinical, biological and molecular aspects of mutations in TRKA(NTRK1) gene encoding the receptor tyrosine kinase for nerve growth factor. *Clinical Autonomic Research, 12*(Suppl 1), I20–I32.

Ji, R. R., Samad, T. A., Jin, S. X., Schmoll, R., & Woolf, C. J. (2002). p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron, 36*, 57–68.

Jimenez-Andrade, J. M., Martin, C. D., Koewler, N. J., Freeman, K. T., Sullivan, L. J., Halvorson, K. G., Barthold, C. M., Peters, C. M., Buus, R. J., Ghilardi, J. R., Lewis, J. L., Kuskowski, M. A., & Mantyh, P. W. (2007). Nerve growth factor sequestering therapy attenuates non-malignant skeletal pain following fracture. *Pain, 133*, 183–196.

Jimenez-Andrade, J. M., Bloom, A. P., Stake, J. I., Mantyh, W. G., Taylor, R. N., Freeman, K. T., Ghilardi, J. R., Kuskowski, M. A., & Mantyh, P. W. (2010). Pathological sprouting of adult nociceptors in chronic prostate cancer-induced bone pain. *The Journal of Neuroscience, 30*, 14649–14656.

Johnson, E. M., Jr., Osborne, P. A., & Taniuchi, M. (1989). Destruction of sympathetic and sensory neurons in the developing rat by a monoclonal antibody against the nerve growth factor (NGF) receptor. *Brain Research, 478*, 166–170.

Jongen, J. L., Haasdijk, E. D., Sabel-Goedknegt, H., van der Burg, J., Vecht Ch, J., & Holstege, J. C. (2005). Intrathecal injection of GDNF and BDNF induces immediate early gene expression in rat spinal dorsal horn. *Experimental Neurology, 194*, 255–266.

Kashiba, H., Uchida, Y., & Senba, E. (2003). Distribution and colocalization of NGF and GDNF family ligand receptor mRNAs in dorsal root and nodose ganglion neurons of adult rats. *Brain Research. Molecular Brain Research, 110*, 52–62.

Kawasaki, Y., Kohno, T., Zhuang, Z. Y., Brenner, G. J., Wang, H., Van Der Meer, C., Befort, K., Woolf, C. J., & Ji, R. R. (2004). Ionotropic and metabotropic receptors, protein kinase A,

protein kinase C, and Src contribute to C-fiber-induced ERK activation and cAMP response element-binding protein phosphorylation in dorsal horn neurons, leading to central sensitization. *The Journal of Neuroscience, 24*, 8310–8321.

Kerr, B. J., Bradbury, E. J., Bennett, D. L., Trivedi, P. M., Dassan, P., French, J., Shelton, D. B., McMahon, S. B., & Thompson, S. W. (1999). Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord. *The Journal of Neuroscience, 19*, 5138–5148.

Koltzenburg, M., Bennett, D. L., Shelton, D. L., & McMahon, S. B. (1999). Neutralization of endogenous NGF prevents the sensitization of nociceptors supplying inflamed skin. *The European Journal of Neuroscience, 11*, 1698–1704.

Lane, N. E., Schnitzer, T. J., Birbara, C. A., Mokhtarani, M., Shelton, D. L., Smith, M. D., & Brown, M. T. (2010). Tanezumab for the treatment of pain from osteoarthritis of the knee. *The New England Journal of Medicine, 363*, 1521–1531.

Levi-Montalcini, R. (1998). The saga of the nerve growth factor. *Neuroreport, 9*, R71–R83.

Li, C. Q., Xu, J. M., Liu, D., Zhang, J. Y., & Dai, R. P. (2008). Brain derived neurotrophic factor (BDNF) contributes to the pain hypersensitivity following surgical incision in the rats. *Molecular Pain, 4*, 27.

Li, F., Zhang, J. W., Wei, R., Luo, X. G., Zhang, J. Y., Zhou, X. F., Li, C. Q., & Dai, R. P. (2010). Sex-differential modulation of visceral pain by brain derived neurotrophic factor (BDNF) in rats. *Neuroscience Letters, 478*, 184–187.

Lin, Y. T., Ro, L. S., Wang, H. L., & Chen, J. C. (2011). Up-regulation of dorsal root ganglia BDNF and TrkB receptor in inflammatory pain: An in vivo and in vitro study. *Journal of Neuroinflammation, 8*, 126.

Lindsay, R. M., & Harmar, A. J. (1989). Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. *Nature, 337*, 362–364.

Lommatzsch, M., Braun, A., Mannsfeldt, A., Botchkarev, V. A., Botchkareva, N. V., Paus, R., Fischer, A., Lewin, G. R., & Renz, H. (1999). Abundant production of brain-derived neurotrophic factor by adult visceral epithelia. Implications for paracrine and target-derived neurotrophic functions. *The American Journal of Pathology, 155*, 1183–1193.

Lu, B., Pang, P. T., & Woo, N. H. (2005). The yin and yang of neurotrophin action. *Nature Reviews Neuroscience, 6*, 603–614.

Luo, X. G., Rush, R. A., & Zhou, X. F. (2001). Ultrastructural localization of brain-derived neurotrophic factor in rat primary sensory neurons. *Neuroscience Research, 39*, 377–384.

Lynn, B. (1983). *Cutaneous nociceptors*. Manchester: Manchester University Press.

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., Wihler, C., Koliatsos, V. E., & Tessarollo, L. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 15239–15244.

Mantyh, W. G., Jimenez-Andrade, J. M., Stake, J. I., Bloom, A. P., Kaczmarska, M. J., Taylor, R. N., Freeman, K. T., Ghilardi, J. R., Kuskowski, M. A., & Mantyh, P. W. (2010). Blockade of nerve sprouting and neuroma formation markedly attenuates the development of late stage cancer pain. *Neuroscience, 171*, 588–598.

Mantyh, P. W., Koltzenburg, M., Mendell, L. M., Tive, L., & Shelton, D. L. (2011). Antagonism of nerve growth factor-TrkA signaling and the relief of pain. *Anesthesiology, 115*, 189–204.

Matayoshi, S., Jiang, N., Katafuchi, T., Koga, K., Furue, H., Yasaka, T., Nakatsuka, T., Zhou, X. F., Kawasaki, Y., Tanaka, N., & Yoshimura, M. (2005). Actions of brain-derived neurotrophic factor on spinal nociceptive transmission during inflammation in the rat. *The Journal of Physiology, 569*, 685–695.

McMahon, S. B., & Cafferty, W. B. (2004). Neurotrophic influences on neuropathic pain. *Novartis Foundation Symposium, 261*, 68–92. discussion 92–102, 149–154.

McMahon, S. B., Bennett, D. L., Priestley, J. V., & Shelton, D. L. (1995). The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule. *Nature Medicine, 1*, 774–780.

Michael, G. J., Averill, S., Nitkunan, A., Rattray, M., Bennett, D. L., Yan, Q., & Priestley, J. V. (1997). Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. *The Journal of Neuroscience, 17*, 8476–8490.

Michael, G. J., Averill, S., Shortland, P. J., Yan, Q., & Priestley, J. V. (1999). Axotomy results in major changes in BDNF expression by dorsal root ganglion cells: BDNF expression in large TrkB and trkC cells, in pericellular baskets, and in projections to deep dorsal horn and dorsal column nuclei. *The European Journal of Neuroscience, 11*, 3539–3551.

Obata, K., Yamanaka, H., Kobayashi, K., Dai, Y., Mizushima, T., Katsura, H., Fukuoka, T., Tokunaga, A., & Noguchi, K. (2004). Role of mitogen-activated protein kinase activation in injured and intact primary afferent neurons for mechanical and heat hypersensitivity after spinal nerve ligation. *The Journal of Neuroscience, 24*, 10211–10222.

Obata, K., Katsura, H., Sakurai, J., Kobayashi, K., Yamanaka, H., Dai, Y., Fukuoka, T., & Noguchi, K. (2006). Suppression of the p75 neurotrophin receptor in uninjured sensory neurons reduces neuropathic pain after nerve injury. *The Journal of Neuroscience, 26*, 11974–11986.

Onda, A., Murata, Y., Rydevik, B., Larsson, K., Kikuchi, S., & Olmarker, K. (2004). Infliximab attenuates immunoreactivity of brain-derived neurotrophic factor in a rat model of herniated nucleus pulposus. *Spine, 29*, 1857–1861 (Phila Pa 1976).

Pezet, S., & McMahon, S. B. (2006). Neurotrophins: Mediators and modulators of pain. *Annual Review of Neuroscience, 29*, 507–538.

Pezet, S., Malcangio, M., & McMahon, S. B. (2002). BDNF: A neuromodulator in nociceptive pathways? *Brain Research. Brain Research Reviews, 40*, 240–249.

Qiao, L., & Vizzard, M. A. (2002). Up-regulation of tyrosine kinase (Trka, Trkb) receptor expression and phosphorylation in lumbosacral dorsal root ganglia after chronic spinal cord (T8-T10) injury. *The Journal of Comparative Neurology, 449*, 217–230.

Raj, P. (2007). Taxonomy and classification of pain. In D. K. S. Niv, B. Diego, & A. Lamberto (Eds.), *The handbook of chronic pain*. New York: Nova Biomedical Books.

Ramer, M. S., & Bisby, M. A. (1999). Adrenergic innervation of rat sensory ganglia following proximal or distal painful sciatic neuropathy: Distinct mechanisms revealed by anti-NGF treatment. *The European Journal of Neuroscience, 11*, 837–846.

Reichardt, L. F. (2006). Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 361*, 1545–1564.

Ritter, A. M., Lewin, G. R., Kremer, N. E., & Mendell, L. M. (1991). Requirement for nerve growth factor in the development of myelinated nociceptors in vivo. *Nature, 350*, 500–502.

Ross, M., Lofstrandh, S., Gorin, P. D., Johnson, E. M., & Schwartz, J. P. (1981). Use of an experimental autoimmune model to define nerve growth factor dependency of peripheral and central substance P-containing neurons in the rat. *The Journal of Neuroscience, 1*, 1304–1311.

Rukwied, R., Mayer, A., Kluschina, O., Obreja, O., Schley, M., & Schmelz, M. (2010). NGF induces non-inflammatory localized and lasting mechanical and thermal hypersensitivity in human skin. *Pain, 148*, 407–413.

Sarchielli, P., Mancini, M. L., Floridi, A., Coppola, F., Rossi, C., Nardi, K., Acciarresi, M., Pini, L. A., & Calabresi, P. (2007). Increased levels of neurotrophins are not specific for chronic migraine: Evidence from primary fibromyalgia syndrome. *The Journal of Pain, 8*, 737–745.

Shelton, D. L., Sutherland, J., Gripp, J., Camerato, T., Armanini, M. P., Phillips, H. S., Carroll, K., Spencer, S. D., & Levinson, A. D. (1995). Human Trks: Molecular cloning, tissue distribution, and expression of extracellular domain immunoadhesins. *The Journal of Neuroscience, 15*, 477–491.

Shelton, D. L., Zeller, J., Ho, W. H., Pons, J., & Rosenthal, A. (2005). Nerve growth factor mediates hyperalgesia and cachexia in auto-immune arthritis. *Pain, 116*, 8–16.

Shu, X., & Mendell, L. M. (1999). Nerve growth factor acutely sensitizes the response of adult rat sensory neurons to capsaicin. *Neuroscience Letters, 274*, 159–162.

Siuciak, J. A., Altar, C. A., Wiegand, S. J., & Lindsay, R. M. (1994). Antinociceptive effect of brain-derived neurotrophic factor and neurotrophin-3. *Brain Research, 633*, 326–330.

Siuciak, J. A., Wong, V., Pearsall, D., Wiegand, S. J., & Lindsay, R. M. (1995). BDNF produces analgesia in the formalin test and modifies neuropeptide levels in rat brain and spinal cord areas associated with nociception. *The European Journal of Neuroscience, 7*, 663–670.

Slack, S. E., Pezet, S., McMahon, S. B., Thompson, S. W., & Malcangio, M. (2004). Brain-derived neurotrophic factor induces NMDA receptor subunit one phosphorylation via ERK and PKC in the rat spinal cord. *The European Journal of Neuroscience, 20*, 1769–1778.

Slack, S. E., Grist, J., Mac, Q., McMahon, S. B., & Pezet, S. (2005). TrkB expression and phospho-ERK activation by brain-derived neurotrophic factor in rat spinothalamic tract neurons. *The Journal of Comparative Neurology, 489*, 59–68.

Smeyne, R. J., Klein, R., Schnapp, A., Long, L. K., Bryant, S., Lewin, A., Lira, S. A., & Barbacid, M. (1994). Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. *Nature, 368*, 246–249.

South, S. M., Kohno, T., Kaspar, B. K., Hegarty, D., Vissel, B., Drake, C. T., Ohata, M., Jenab, S., Sailer, A. W., Malkmus, S., Masuyama, T., Horner, P., Bogulavsky, J., Gage, F. H., Yaksh, T. L., Woolf, C. J., Heinemann, S. F., & Inturrisi, C. E. (2003). A conditional deletion of the NR1 subunit of the NMDA receptor in adult spinal cord dorsal horn reduces NMDA currents and injury-induced pain. *The Journal of Neuroscience, 23*, 5031–5040.

Svensson, P., Cairns, B. E., Wang, K., & Arendt-Nielsen, L. (2003). Injection of nerve growth factor into human masseter muscle evokes long-lasting mechanical allodynia and hyperalgesia. *Pain, 104*, 241–247.

Trang, T., Beggs, S., Wan, X., & Salter, M. W. (2009a). P2X4-Receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. *The Journal of Neuroscience, 29*, 3518–3528.

Trang, T., Koblic, P., Kawaja, M., & Jhamandas, K. (2009b). Attenuation of opioid analgesic tolerance in p75 neurotrophin receptor null mutant mice. *Neuroscience Letters, 451*, 69–73.

Tron, V. A., Coughlin, M. D., Jang, D. E., Stanisz, J., & Sauder, D. N. (1990). Expression and modulation of nerve growth factor in murine keratinocytes (PAM 212). *The Journal of Clinical Investigation, 85*, 1085–1089.

Wang, L. N., Yang, J. P., Zhan, Y., Ji, F. H., Wang, X. Y., Zuo, J. L., & Xu, Q. N. (2012). Minocycline-induced reduction of brain-derived neurotrophic factor expression in relation to cancer-induced bone pain in rats. *Journal of Neuroscience Research, 90*, 672–681.

Wetmore, C., & Olson, L. (1995). Neuronal and nonneuronal expression of neurotrophins and their receptors in sensory and sympathetic ganglia suggest new intercellular trophic interactions. *The Journal of Comparative Neurology, 353*, 143–159.

Whelton, A. (2000). Renal and related cardiovascular effects of conventional and COX-2-specific NSAIDs and non-NSAID analgesics. *American Journal of Therapy, 7*, 63–74.

Winston, J. H., Toma, H., Shenoy, M., He, Z. J., Zou, L., Xiao, S. Y., Micci, M. A., & Pasricha, P. J. (2003). Acute pancreatitis results in referred mechanical hypersensitivity and neuropeptide up-regulation that can be suppressed by the protein kinase inhibitor k252a. *The Journal of Pain, 4*, 329–337.

Woolf, C. J. (1996). Windup and central sensitization are not equivalent. *Pain, 66*, 105–108.

Woolf, C. J., & Costigan, M. (1999). Transcriptional and posttranslational plasticity and the generation of inflammatory pain. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 7723–7730.

Woolf, C. J., Safieh-Garabedian, B., Ma, Q. P., Crilly, P., & Winter, J. (1994). Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. *Neuroscience, 62*, 327–331.

Wu, C., Boustany, L., Liang, H., & Brennan, T. J. (2007). Nerve growth factor expression after plantar incision in the rat. *Anesthesiology, 107*, 128–135.

Yaksh, T. L., Hua, X. Y., Kalcheva, I., Nozaki-Taguchi, N., & Marsala, M. (1999). The spinal biology in humans and animals of pain states generated by persistent small afferent input. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 7680–7686.

Yang, J., Yu, Y., Yu, H., Zuo, X., Liu, C., Gao, L., Chen, Z. Y., & Li, Y. (2010). The role of brain-derived neurotrophic factor in experimental inflammation of mouse gut. *European Journal of Pain, 14*, 574–579.

Yu, Y. B., Zuo, X. L., Zhao, Q. J., Chen, F. X., Yang, J., Dong, Y. Y., Wang, P., & Li, Y. Q. (2012). Brain-derived neurotrophic factor contributes to abdominal pain in irritable bowel syndrome. *Gut, 61*, 685–694.

Zahn, P. K., Subieta, A., Park, S. S., & Brennan, T. J. (2004). Effect of blockade of nerve growth factor and tumor necrosis factor on pain behaviors after plantar incision. *The Journal of Pain, 5*, 157–163.

Zhou, X. F., Parada, L. F., Soppet, D., & Rush, R. A. (1993). Distribution of TrkB tyrosine kinase immunoreactivity in the rat central nervous system. *Brain Research, 622*, 63–70.

Zhou, X. F., Chie, E. T., Deng, Y. S., Zhong, J. H., Xue, Q., Rush, R. A., & Xian, C. J. (1999). Injured primary sensory neurons switch phenotype for brain-derived neurotrophic factor in the rat. *Neuroscience, 92*, 841–853.

Zhu, Z. W., Friess, H., Wang, L., Zimmermann, A., & Buchler, M. W. (2001). Brain-derived neurotrophic factor (BDNF) is upregulated and associated with pain in chronic pancreatitis. *Digestive Diseases and Sciences, 46*, 1633–1639.

Neurotrophin Signaling in Cancer

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Contents

Abstract

Neurotrophins are growth factors that play a major role in neuron survival, proliferation, differentiation, and apoptosis. There are four neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) which function by interacting with Trk (tropomyosin-related kinase) tyrosine kinase receptors,

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TrkA, TrkB, TrkC, or p75NTR receptor. Binding of neurotrophins with their cognate Trk receptors activates Ras/mitogen-activated protein kinase (MAPK) pathway, Phosphatidylinositol-3 kinase (PI3K)/Akt pathway, and Phospholipase C-γ (PLC-γ) pathway which are involved in cell survival, proliferation, differentiation, and apoptosis. Activation of the pan-neurotrophin receptor, p75NTR, leads to JNK pathway activation that induces cell apoptosis or activates a NF-κB cell survival pathway. Although neurotrophins were originally found to act on neurons, studies indicate they also have activities in nonneuronal cells. Trk activation or mutation occurs in tumors of neuronal origin, like neuroblastoma and medulloblastoma, as well as in nonneuronal tumors like thyroid, breast, lung, and prostate cancer. In neuroblastoma, expression of TrkA occurs in tumors that are biologically favorable and prone to spontaneous regression or differentiation which may be due to the absence or presence of its ligand (NGF) in the microenvironment. Expression of TrkB and/or its ligand BDNF in neuroblastoma tumors is often associated with chemo-resistance, metastasis, and a poor prognosis. In breast cancer, overexpression of TrkA promotes cell growth, migration and invasion in vitro or tumor growth, metastasis, and angiogenesis. Based on these studies, small molecule inhibitors targeting Trk receptors or downstream targets in their signaling pathways have been developed. The Trk selective inhibitor CEP-701 (Cephalon) or the Akt inhibitor perifosine are both in phase 1 and 2 clinical trials. The effects of these small molecule inhibitors, alone or in combination with chemotherapeutic drugs, are discussed.

List of Abbreviations

BDNF	Brain-derived neurotrophic factor
ERK	Extracellular signal-regulated kinase
Grb2	Growth factor receptor-bound protein 2
HGF	Hepatocyte growth factor
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinases
MEK	MAP kinase kinase
mTOR	Mammalian target of rapamycin
NGF	Nerve growth factor
NSCLC	Non-small cell lung cancer
NT-3	Neurotrophin 3
NT-4/5	Neurotrophin 4/5
P75NTR	P75 neurotrophin receptor
PI3K	Phosphatidylinositol-3 kinase
PLC-γ	Phospholipase C-γ
PTB domain	Phosphotyrosine-binding domain
SCLC	Small cell lung cancer
Shc	Src-homology collagen protein
SOS	Son of sevenless
Trk	Tropomyosin-related kinase

1 Introduction

Neurotrophin are growth factors implicated in several different functions in the nervous system, including survival, proliferation, differentiation, myelination, apoptosis, axonal growth, and synaptic plasticity. In mammals, the neurotrophin family consists of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5) (Snider 1994). With the exception of NT-4/5, which is not detected in avian species, the neurotrophin sequences are evolutionarily conserved in vertebrates. The *nt-6* and *nt-7* genes encode neurotrophins NT-6 and NT-7 and have been identified only in fish, and do not appear to have mammalian orthologs (Gotz et al. 1994; Nilsson et al. 1998).

The signal transduction pathways that mediate the diverse biological functions of the neurotrophins are initiated through interactions with two categories of cell surface receptors: the selective tropomyosin-related kinase (Trk) tyrosine kinase receptors and the "shared" p75 neurotrophin receptor (p75NTR). These two types of receptors share no sequence similarity in either ligand-binding or cytoplasmic domains, and activate distinct neurotrophin-dependent signaling pathways. In many instances, Trk receptors and p75NTR not only activate autonomous pathways but also collaborate to mediate effects of the neurotrophins. There are three Trk receptors: TrkA, TrkB, TrkC. The Trk receptor was originally discovered as a rearrangement of non-muscle tropomyosin and a then unknown tyrosine kinase. This tyrosine kinase was referred to as Trk and subsequently identified as a receptor for NGF. Trk became TrkA with the indentification of TrkB and TrkC which were based on their DNA sequence similarity to Trk (Hempstead et al. 1991; Klein et al. 1989, 1991; Lamballe et al. 1991). Each neurotrophin binds with high affinity to Trk receptors (Fig. 1): NGF to TrkA, BDNF to TrkB, NT-3 to TrkC. NT-4/5 binds TrkB, too. NT-3 also binds to TrkA and TrkB receptors that contain an additional short insert in the extracellular domain (Clary and Reichardt 1994; Strohmaier et al. 1996). The p75NTR belongs to the tumor necrosis factor (TNF) superfamily and was the first identified receptor for NGF (Chao et al. 1986; Radeke et al. 1987). All neurotrophins bind to the common p75NTR receptor with low affinity. Recently, pro-neurotrophins (the precursors of the neurotrophins: pro-NGF, pro-BDNF, pro-NT-3, pro-NT-4/5) have been identified as high-affinity ligands for p75NTR in complex with a co-receptor, sortilin (Lee et al. 2001; Nykjaer et al. 2004; Teng et al. 2005) (Fig. 1).

Disturbances in neurotrophins, their receptors, or neurotrophin/receptor-initiated signaling pathways have been associated not only with various neurodegenerative disorders (e.g., Alzheimer's disease, Parkinson's disease), but also to several types cancers of neuronal (e.g., neuroblastoma, medulloblastoma, gliomas) and nonneuronal origin (e.g., breast, lung, prostate cancers).

This chapter will describe neurotrophins, their receptors, and the signaling pathways initiated by binding of neurotrophins to Trk or p75NTR receptors. The expression of neurotrophins and/or their receptors in neuronal and nonneuronal cancers, as well as the potential to target these neurotrophins/receptors signaling pathways in the treatment of cancers, will be described.

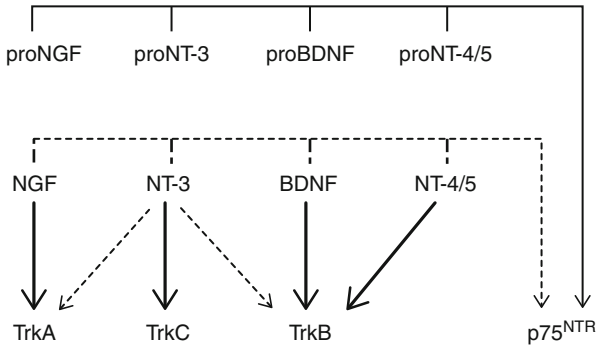

Fig. 1 Neurotrophins and their receptors: Neurotrophins preferentially bind to Trk receptors: NGF binds specifically to TrkA; BDNF and NT-4/5 recognize TrkB; NT-3 activates TrkC. NT-3 is also able to activate TrkA and TrkB with less efficiency. Neurotrophins bind p75NTR with equal affinity. Pro-neurotrophins bind p75NTR with high affinity

2 Neurotrophins

NGF was the first member of the neurotrophin family to be identified. NGF activity was initially observed in a mouse sarcoma that induced the dramatic growth of peripheral nerve fibers when transplanted into chick embryos. NGF was purified from mouse submandibular gland, which expressed extremely high levels of the factor. A dimer of 13.3 kDa polypeptides, termed the β subunit, was found to contain the biological activity of NGF. The β-NGF polypeptide chain is produced as a larger propeptide that is proteolytically cleaved to generate the mature form of a homodimeric polypeptide of about 26 kDa. A major biological function of NGF is the maintenance and survival of post-mitotic neurons; it is normally produced by the synaptic target field in limiting amounts to attract susceptible growth cones to functional site. NGF is a major player in the development of several peripheral nervous system subpopulations, as well as several central nervous system regions; as such, it has been considered a strong candidate for the treatment of several neurodegenerative conditions. In addition to its role in the maturation and maintenance or neuronal cells, NGF also has significant effects on nonneuronal cells. For example, it triggers chemotaxis in melanocytes, inhibits the migration of Schwann cells, has been reported to stimulate the proliferation of chromaffin cells, and is also an autocrine survival factor for B lymphocytes (Butte 2001; Reichardt 2006; Sofroniew et al. 2001; Wiesmann and de Vos 2001).

BDNF was the second neurotrophin to be discovered after its purification from pig brain in 1982. BDNF was produced initially as pro-BDNF (M.W. ~30 kDa), then cleaved to generate the mature form (M.W. ~14 kDa) which shared about 50 % amino acid identity with NGF. BDNF has survival- and growth-promoting actions on a variety of neurons, including dorsal root ganglion cells and hippocampal and cortical neurons. Certain peripheral sensory neurons, especially those in vestibular and nodose-petrosal ganglia, depend on

the presence of BDNF because BDNF homozygous (−/−) knockout mice lack these neurons. Unlike NGF, neither sympathetic or motor neurons are affected by BDNF. BDNF also plays a role in brain development and synaptic plasticity (Binder and Scharfman 2004; Reichardt 2006). As neural crest cells coalesce to form sympathetic ganglia, TrkB-positive cells are seen in both chicken and mouse embryos. The coexpression of TrkB and HNK-1 confirms that they are migrating neural crest cells. Subsequently, TrkB-positive cells begin to express the neural-specific markers Hu C/D and Islet-1, eventually commence neural differentiation (Straub et al. 2007).

NT-3 was identified using degenerate oligonucleotides to amplify by PCR novel cDNA sequences related to NGF and BDNF. The NT-3 cDNA encoded a mature polypeptide of predicted molecular weight 13.6 kDa. Recombinant NT-3 supports the survival of and neurite outgrowth from chick embryonic neurons, a result that established its neurotrophic activity. A PCR strategy similar to that used for NT-3 was used to identify a fourth mammalian neurotrophin, NT-4 (also known as NT-4/5), which was also shown to possess neurotrophic activity (Reichardt 2006).

3 Neurotrophin Receptors

3.1 Trk Receptors

Trk receptors are prototypical receptor tyrosine kinases that contain an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain is composed of a cysteine-rich domain followed by three leucine-rich domain, another cysteine-rich domain, and two immunoglobulin-like C2 type domains (Ig-C2). The cytoplasmic region contains a kinase domain (Fig. 2)(Brodeur et al. 2009; Gills and Dennis 2009; Reichardt 2006; Thiele et al. 2009). Binding of neurotrophins to Trk receptors occurs mainly through the Ig-C2 domains, with the domain closer to the transmembrane region playing a more prominent role in binding. The leucine-rich domains and the cysteine domains may also be involved in binding with neurotrophins. In addition to ligand binding, the Ig-C2 domains can also stabilize the monomeric form of the Trk receptor to prevent spontaneous dimerization and activation in the absence of neurotrophins. Deletions, chimeric receptors, or point mutations that disrupt the structure of the first or second Ig-C2 domain allow ligand-independent receptor activation. Differential splicing that leads to a loss of the first Ig-C2 domain of TrkA exhibits ligand-independent signaling and transformation activity when transfected into NIH-3T3 cells (Tacconelli et al. 2004).

The generation of alternatively spliced isoforms of Trk genes leads to functionally distinct receptors (Fig. 2). TrkA has three isoforms, TrkA-I, TrkA-II, and TrkA-III. TrkA-II is the isoform which is expressed in neuronal cells. TrkA-I is a nonneuronal TrkA isoform that lacks exon 9. Exon 9 encodes 6 amino acids in the extracellular domain near the transmembrane region (insertion) in TrkA and does not alter the reading frame. This does not affect NGF binding or receptor activation, but the TrkA-II isoform has enhanced responsiveness to NT3. TrkA

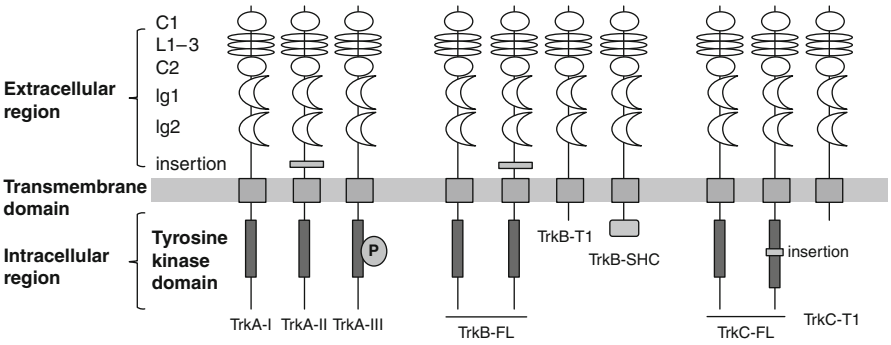

Fig. 2 Structures of the Trk receptors: The domain structures of the Trk receptors are schematized. Each Trk receptor has an extracellular domain, transmembrane domain, and an intracellular tyrosine kinase domain. The locations of two cysteine-rich domains (C1, C2), three leucine-rich domains (L1-3), and two immunoglobulin-like C2 type domains (Ig1, Ig2) in the extracellular domain of Trk receptors are depicted. There is an insert in the extracellular domain near the transmembrane region in TrkA or TrkB receptor. The insert of TrkC receptor is in the intracellular tyrosine kinase domain. Several truncated isoforms of TrkB and TrkC that lack the tyrosine kinase domain have been identified

isoforms lacking this sequence interact normally with NGF but show reduced NT-3 binding (Clary and Reichardt 1994). There is also an isoform, TrkA-III that is expressed early during development and splices out exons 6, 7, and 9. TrkA-III is constitutively active, independent of ligand (Tacconelli et al. 2004). Enhanced expression of TrkAIII occurs under hypoxia. TrkB is expressed as both full-length (kinase-intact) and truncated (kinase-deleted) isoforms. Full-length TrkB receptors have alternatively spliced product lacking 6–9 amino acids in the extracellular juxtamembrane region, like TrkA. TrkB variants lacking this region bind BDNF normally but show decreased affinity for NT-3 and NT-4/5. There are at least two kinase-deleted isoforms of TrkB that are truncated just beyond the transmembrane domain and differ by the absence (TrkB-T1) or presence (TrkB-SHC) of the SHC-binding site (Brodeur et al. 2009; Reichardt 2006; Thiele et al. 2009). Studies indicating that expression of truncated TrkB receptors inhibits BDNF induced neurite activity suggest that such isoforms may act as dominant-negative inhibitors of the TrkB kinase. This inhibition may occur by ligand sequestration or by formation of functionally inactive heterodimers. TrkC has full-length and truncated isoforms. Full-length TrkC receptors can be produced with amino acid inserts (14, 25, or 39 residues) in their kinase domain. Forms of TrkC containing insertions within the kinase domain retain their ability to autophosphorylate in response to neurotrophin-3, but cannot mediate proliferation in fibroblasts or neuronal differentiation in PC12 cells (Valenzuela et al. 1993). Truncated TrkC is the isoform without the intracellular kinase domain which may act as a dominant-negative inhibitor of kinase-intact TrkC. Thus, alternative splicing of the Trk receptors affects the specificity of neuronal responsiveness to neurotrophins.

3.2 p75NTR receptor

p75NTR is a 399-amino acid Type I transmembrane receptor which contains an extracellular, transmembrane, and intracellular domain. The extracellular domain contains four cysteine-rich domains (CR1-4). The intracellular domain contains a death domain which made it the first identified but last accepted member into the Death Receptor Family (TNF and TRAIL) (Fig. 3). Experimental and structural modeling studies have suggested that CR3 may be primarily responsible for interaction with neurotrophins (Yan and Chao 1991). p75NTR has both full-length and truncated isoforms. The p75ntr gene gives rise to a full-length receptor (p75NTR.FL) and an alternatively spliced isoform (p75NTR.T) lacking exon 3 (CR2-4). The full-length p75NTR receptor is also cleaved by a constitutively active metalloproteinase to generate a soluble extracellular domain that is capable of binding neurotrophins, and a receptor fragment containing transmembrane and intracellular domain (Fig. 3) (Zupan et al. 1989). The soluble form of p75NTR is produced at very high levels during development and following peripheral nerve injury, but the biological role(s) of the proteolytic products remain poorly defined (Zupan et al. 1989).

All neurotrophins bind to p75NTR receptor with similar affinity. The affinity of neurotrophins to TrkA and TrkB receptors is modulated by the presence of p75NTR, which enhances specificity for their primary ligands NGF and BDNF, respectively (Benedetti et al. 1993). Formation of high-affinity binding sites for NGF requires the presence of a correct ratio of full p75NTR and Trk receptors, as mutations of the cytoplasmic or transmembrane domains of either TrkA or p75NTR abolish the configuration of high-affinity binding sites. At the same time, p75NTR reduces the ability of NT-3 to activate TrkA, and NT-3 and NT-4 to activate TrkB.

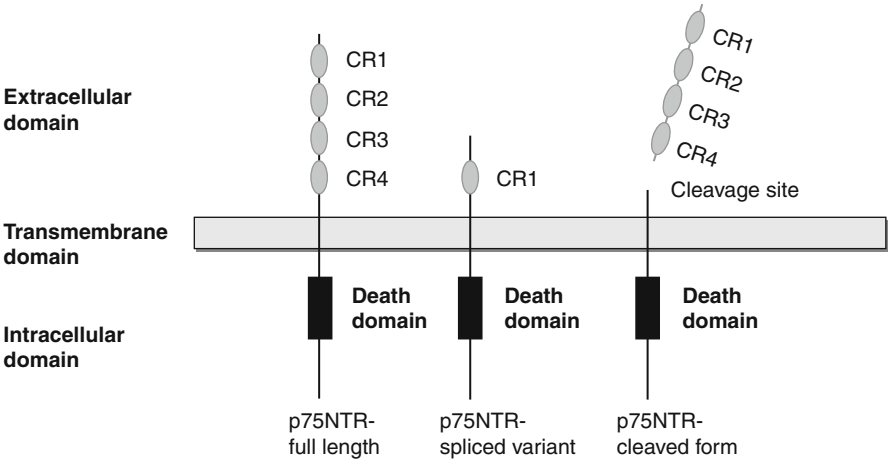

Fig. 3 Structure of p75NTR: p75NTR has an extracellular domain which consists of four cysteine-rich domain (CR1, CR2, CR3, CR4), a transmembrane domain, and an intracellular death domain. p75NTR has a spliced variant that lost the CR2 to CR4, and a cleaved form

This effect has been recently demonstrated in vivo in sympathetic neurons, where NT-3 can signal locally via TrkA receptors in the absence of p75NTR. Once axons reach their final target and receive NGF, TrkA retrograde signaling upregulates p75NTR, and the cells no longer respond to NT-3.

4 Neurotrophin Signaling Pathways

4.1 Neurotrophin Signaling Pathways Mediated by Trk Receptors

Binding of neurotrophin homodimers causes receptor dimerization, autophosphorylation on tyrosine (Y) residues within the activation loop (Y670, Y674, and Y675 relative to human TrkA), recruitment of different cytosolic adaptor proteins and enzymes which leads to the activation of several different signaling pathways. Among the signaling pathways activated by Trk receptors in response to neurotrophins, the Ras/mitogen-activated protein kinase (MAPK) pathway, phosphatidylinositol-3 kinase (PI3K) pathway, and PLCγ-protein kinase C (PLCγ) pathways are primarily studied (Fig. 4) (Kaplan and Miller 2000; Reichardt 2006). Most of the work describing these signaling pathways has been performed in PC12 cells, a rat adrenal pheochromocytoma cell line that responds to NGF and in many respects resembles sympathetic neurons. These signaling pathways have also been studied largely in the context of NGF activation of TrkA and extrapolated to the other Trk receptors.

4.1.1 Ras/MAPK Signaling Pathway

After binding to neurotrophins, Trk receptor tyrosine kinases are autophosphorylated and activated which recruits the adaptor protein Shc (Src-homology collagen protein) to phospho-Y490 on Trk receptors via interactions with the Shc PTB (phosphotyrosine binding) domain. Trk-mediated phosphorylation of Shc creates a phosphotyrosine site on Shc that recruits the adaptor Grb2 (growth factor receptor-bound protein 2), which is bound to the Ras exchange factor son of sevenless (SOS). These adaptors promote Ras activation which phosphorylates and activates Raf. Raf then phosphorylates and activates MEK (MAP kinase kinase), which in turn induces the phosphorylation and activation of ERK (extracellular signal-regulated kinase) (Fig. 4). Activated ERK translocates to the nucleus and transactivates transcription factors such as STAT1/3, Elk1, Myc, modulating gene expression to promote growth, differentiation, or development (Arevalo and Wu 2006; Reichardt 2006).

4.1.2 PI3K/Akt Pathway

PI3-kinases are activated through Ras-dependent and Ras-independent pathways, and the comparative importance of these two mechanisms differs in various subsets of neurons. In many neurons, Ras-mediated activation of PI3-kinase initiates the major pathways through which survival signals are conveyed. In addition, PI3-kinase can

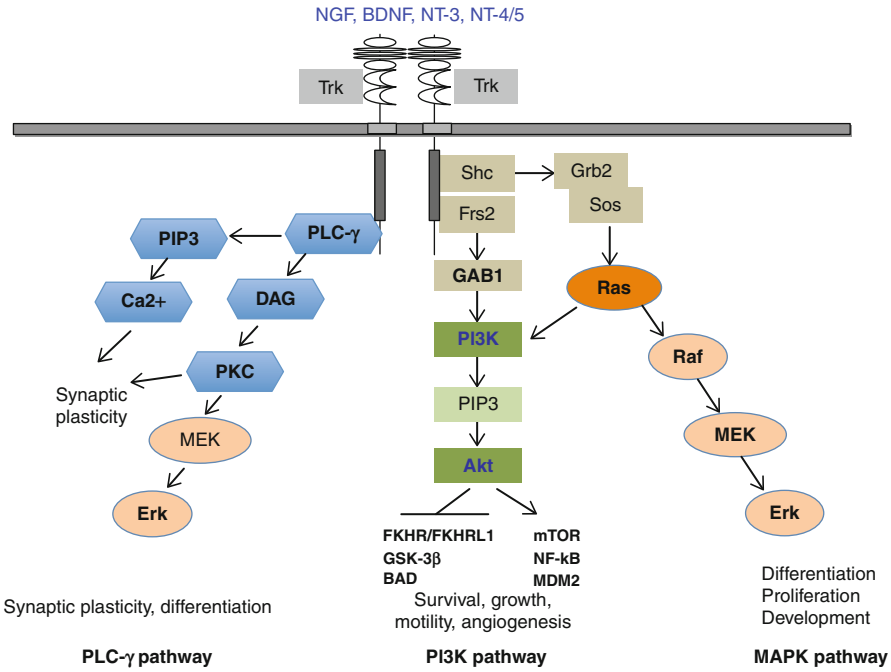

Fig. 4 Neurotrophin signaling pathways mediated by Trk receptors: Binding of neurotrophins to their Trk receptors leads to dimerization of the receptors, activation of tyrosine kinases, and the recruitment of different adaptors proteins and enzymes, resulting in activation of several signaling pathways, including Ras/MAPK pathway, PI3K/Akt pathway, and PLC-γ pathway. These signaling pathways are involved in the cell differentiation, proliferation, survival, motility, and synaptic plasticity

also be activated through Ras-independent pathways. Recruitment of Gab1 and Frs2 permits subsequent binding and activation of PI3K. Activated PI3-kinase generates phosphatidylinositol 3,4-bisphosphate (PIP2). PIP2 acts as a substrate to generate the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3), which recruits and binds AKT to the cell membrane via its pleckstrin homology domain. Akt is then phosphorylated and activated by PDK1 and PDK2. Phosphorylated Akt is translocated to the cytoplasm, nucleus, or mitochondria where it functions to phosphorylate downstream targets, leading to their activation or inactivation. Akt inactivates the FKHR/FKHRL1 (FOXO1) transcription factor, glycogen synthesis kinase (GSK-3β), and the Bcl-2 pro-apoptotic family member BAD known to play a pro-apoptotic role. Akt activates a number of proteins important for cell survival such as mTOR (mammalian target of rapamycin) which is important in protein synthesis, MDM2 which is an important negative regulator of the p53 tumor suppressor, and the transcription factor NF-κB (Fig. 4) (Arevalo and Wu 2006; Brodeur et al. 2009; Reichardt 2006; Thiele et al. 2009). Aside from a role in cell growth and survival, neurotrophin-mediated activation of the PI3K signaling pathway stimulates cell motility and increases production of VEGF. BDNF-mediated activation of TrkB in

cerebellar granule neurons, as well as NGF-mediated activation of TrkA in dorsal root ganglion neurons, stimulates an increased production of VEGF that are mediated by increases in HIF-α (Nakamura et al. 2011). Neurotrophin-stimulated increases in VEGF may mediate communication between the vasculature and neurons during development, cell stress, or hypoxia.

4.1.3 PLC-γ Pathway

Following Trk kinase activation, PLC-γ is recruited to a docking site surrounding phosphorylated Y785 on TrkA and to similar sites on TrkB and TrkC. Docked PLC-γ is activated through Trk-mediated phosphorylation and then hydrolyzes PIP2 to generate PIP3 and DAG. The presence of PIP3 results in Ca2+ release from cytoplasmic stores. DAG stimulates DAG-regulated isoforms of protein kinase C. Together, these signaling molecules activate a number of intracellular enzymes, including almost all PKC isoforms, Ca2+ -calmodulin-dependent protein kinases, and other Ca2+ -calmodulin-regulated targets. The proteins activated as a result of PLC-γ activation include PKC-δ, which is required for NGF-induced activation of MEK1 and Erk (Fig. 4). Signaling through this pathway controls expression and/or activity of many proteins, including ion channels and transcription factors (Arevalo and Wu 2006; Brodeur et al. 2009; Reichardt 2006; Thiele et al. 2009).

4.2 Neurotrophin Signaling Pathways Mediated by p75NTR Receptor

Binding of p75NTR to neurotrophins initiates opposing pathways, the pro-apoptotic signaling pathway and the pro-survival signaling pathway. The pro-survival effect of p75NTR/neurotrophins is mediated by the activation of the transcription factor, NF-κB (Karin and Lin 2002). The prototypic NF-κB molecule consists of a p65/p50 heterodimer, which is normally sequestered in the cytoplasm via binding to the inhibitory protein IκB. In response to neurotrophin binding to p75NTR, IκB is phosphorylated by the upstream IKK complex, leading to ubiquitination and degradation. Without the inhibition of IκB, NF-κB translocates into the nucleus where it regulates transcription of target genes (Fig. 5). Among the genes induced by NF-κB are several that promote survival, including members of the inhibitor of apoptosis family, IAP, and Bcl-2 homologs (Pahl 1999).

The pro-apoptotic effecter of activated neurotrophin/p75NTR is the JNK (c-Jun N-terminal kinase) signaling pathway. Activation of p75NTR induces activation of ASK1 and MEKK which in turn activates JNK. JNK either directly, or through the phosphorylation of c-jun, activates p53 which induces apoptosis by regulating its targets such as pro-apoptotic Bcl-2 family members Bax, Bad, Puma. In addition, activation of JNK induces expression of Fas ligand in neuronal cells, promoting apoptosis through activation of the Fas receptor (Fig. 5).

As mentioned early, p75NTR binds the unprocessed pro-neurotrophins with high affinity, stimulating an apoptotic signal. The pro-neurotrophins actually bind with high affinity to a complex of p75NTR with sortilin, a Vps10-domain

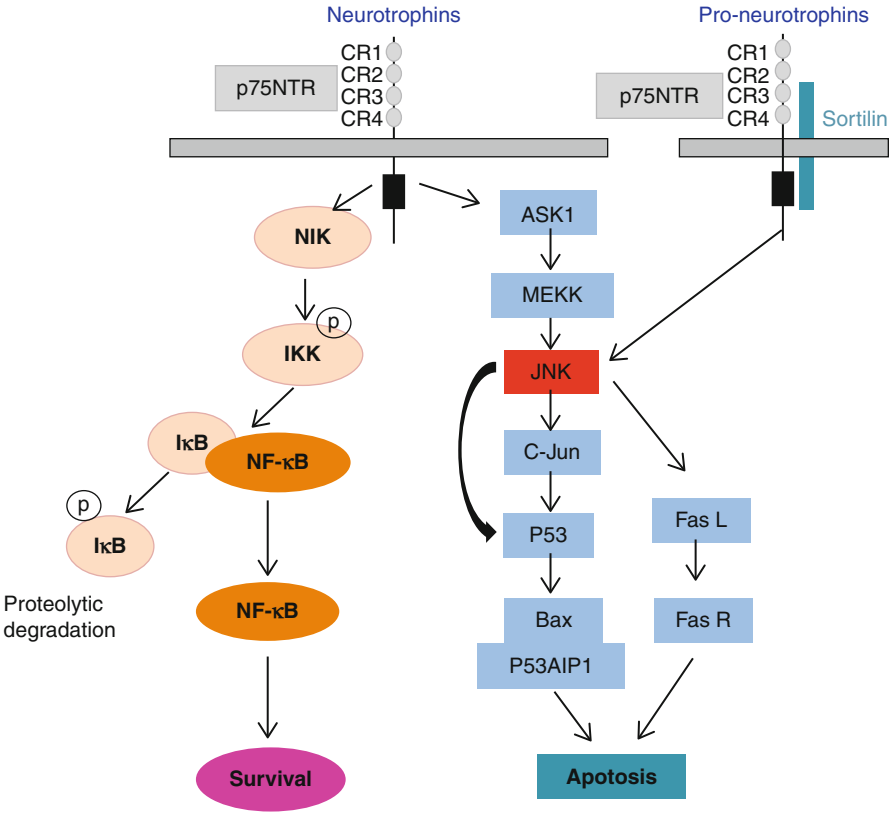

Fig. 5 Neurotrophin signaling pathways mediated by p75NTR receptor: Binding of neurotrophins to p75NTR either activates NF-κB signaling pathway to mediate cell survival, or activates JNK signaling pathway to induce cell apoptosis. Pro-neurotrophins bind to p75NTR to initiate cell apoptosis via the JNK signaling pathway

containing protein. Both sortilin and p75NTR participate directly in binding to the pro-neurotrophins. The presence of sortilin is required to generate apoptosis following engagement of p75NTR by the pro-neurotrophins. The JNK signaling pathway mediates the p75NTR/pro-neurotrophins-induced apoptosis.

5 Neurotrophins and Their Receptors in Cancers

5.1 Neurotrophins and Their Receptors in Neuronal Origin Cancers

Neuroblastoma is the most common extracranial solid tumor in children. It arises from neural crest precursor cells. The correlation between the expression levels of neurotrophins/neurotrophin receptors in tumors and the biological/clinical

characteristics of tumors has been extensively studied in neuroblastoma. The prognosis of neuroblastoma patients is varied. In some neuroblastoma patients, the tumors can regress or are very sensitive to the current cytotoxic regimens and they have a 90 % overall survival. In contrast, some neuroblastoma patients have tumors that are very resistant to chemotherapy, readily metastasize, and these patients have a poor prognosis. Differential Trk/neurotrophin expression has been used to correlate these clinical features.

TrkA (I, II) is expressed at high levels in biologically favorable neuroblastoma, and associated with favorable clinical features, so TrkA expression in tumors of neuroblastoma patients becomes a strong predictor of good prognosis (Nakagawara et al. 1993; Suzuki et al. 1993). Consistent with this observation, high TrkA expression in neuroblastoma cell lines is associated with decreased invasiveness, slow tumor cell growth, and low expression of angiogenic growth factors (Eggert et al. 2002; Lucarelli et al. 1997). Low-stage neuroblastomas usually express high TrkA and respond to NGF by enhanced survival and terminal differentiation (Nakagawara et al. 1993). TrkA expression is low or absent in most advanced stage tumors, and they do not undergo terminal differentiation in response to NGF, consistent with a model in which the NGF/TrkA pathway is responsible for differentiation and/or regression of favorable neuroblastoma. However, the recently identified isoform of TrkA (TrkA-III) was found to be more highly expressed in neuroblastoma tumors of patients with a poor prognosis (Tacconelli et al. 2004).

Most neuroblastomas express very low or undetectable levels of NGF. A limited amount of NGF may be supplied from the stromal-infiltrating cells such as schwannian cells and fibroblasts. One model posits that in regressing neuroblastoma, tumor cells expressing TrkA receptor are dependent on limiting amounts of NGF which would lead to differentiation in the presence of NGF or apoptosis in its absence. This model is based on the observation that normal developing sympathetic neurons survive and differentiate in the presence of target-derived supplement of neurotrophins or die in its absence (Nakagawara 1998a, b). Recently, a study found CCM2 as a key mediator of TrkA-dependent cell death. CCM2 is the protein product of the *cerebral cavernous malformation* 2 gene, it interacts with the juxtamembrane region of TrkA via its PTB (phosphotyrosine binding) domain. Coexpression with TrkA, CCM2 mediates TrkA-induced cell death, while CCM2 knockdown attenuates TrkA-dependent cell death. Furthermore, increased TrkA and CCM2 transcript expression correlates strikingly with positive clinical outcome in a large cohort of neuroblastoma patients (Harel et al. 2009).

Full-length TrkB and BDNF are expressed individually or co-expressed in more aggressive, unfavorable neuroblastoma (Nakagawara et al. 1994), Whereas truncated TrkB is expressed in more differentiated tumors, such as ganglioneuroblastoma. Addition of BDNF to the TrkB-expressing neuroblastoma cells enhanced the survival of cells exposed to serum-free media (Nakagawara et al. 1994), or chemotherapeutic drugs (etoposide, cisplatin, and vinblastin) (Jaboin et al. 2002; Scala et al. 1996). The survival of neuroblastoma tumor cells exposed to most chemotherapeutic drugs is mainly mediated by activation of the BDNF/TrkB

via activation of PI-3kinase/Akt and inactivation of GSK-3β(Jaboin et al. 2002; Li et al. 2005, 2007a). But for some chemotherapeutic drug (paclitaxel), the survival of neuroblastoma cells is mediated by activation of MAPK pathway and inhibition of Bim (Li et al. 2007b). Such studies also demonstrated that the effects of chemotherapeutic agents are attenuated even if a tumor cell with high levels of TrkB expression is in an environment with low levels of BDNF. More importantly, if tumor cells express low levels of TrkB receptor, and are in a BDNF-rich environment, the effects of cytotoxic agents were decreased (Jaboin et al. 2002). These findings indicate that sites of relatively high levels of TrkB ligands (BDNF and NT-4) may serve as sanctuary sites for TrkB-expressing tumor cells. BDNF also stimulates tumor cell disaggregation and increases the ability of TrkB-expressing neuroblastoma tumor cells to degrade and invade through extracellular matrix proteins. The BDNF/TrkB-mediated invasiveness in neuroblastoma cells may be due to upregulation of Hepatocyte Growth Factor (HGF), originally described as Scatter factor and its receptor, c-met. The siRNA-mediated silencing of c-met or antibodies to HGF diminishes BDNF/TrkB-induced invasiveness. Whether this is the generalized mechanism by which TrkB mediates motility/invasiveness awaits evaluation in other model systems. Additionally, BDNF activation of TrkB increases tumor cell production of VEGF and this is enhanced in hypoxic conditions (Nakamura et al. 2006). Thus, the expression of both BDNF and full-length TrkB may represent an autocrine or paracrine survival pathway that is important for the aggressive behavior of some neuroblastoma (Acheson et al. 1995).

TrkC was first reported to be expressed predominantly in biologically favorable neuroblastomas (Ryden et al. 1996). Full-length TrkC is expressed in about 25 % of primary neuroblastomas, and these tumors essentially represent a subset of the TrkA-expressing tumors (Svensson et al. 1997; Yamashiro et al. 1996). So TrkC expression in neuroblastoma has been associated with good prognosis. The TrkC ligand NT-3 is recently reported to be overexpressed in 38 % of stage 4 neuroblastoma and also in a fraction of neuroblastoma cell lines mainly derived from stage 4 neuroblastoma tumor materials. Tumors with high NT-3 levels showed a high NT-3/TrkC ratio (Bouzas-Rodriguez et al. 2010). This observation is consistent with the theories of TrkC being a dependence receptor, which can initiate two completely opposite signaling pathways, depending on ligand availability (Tauszig-Delamasure et al. 2007). In the presence of ligand, a positive differentiation, guidance, or survival signal is transduced, while in the absence of ligand, receptors mediate an active process of apoptotic cell death. TrkA may also function as a dependence receptor (Nikoletopoulou et al. 2010). So autocrine NT-3 expression in stage 4 neuroblastoma may be a mechanism developed by a large fraction of tumor cells to bypass TrkC-induced cell death that would occur in regions of limited NT-3 concentrations.

Medulloblastoma is a central nervous system tumor which arises from the external granule cell layer of the cerebellum. TrkC expression was detected in medulloblastoma, and elevated TrkC expression is associated with favorable clinical outcome (Segal et al. 1994). Studies show that overexpression of TrkC inhibits the growth of intracerebral xenografts of a medulloblastoma cell line in nude mice

(Muragaki et al. 1997). NT-3 mRNA was also detected in medulloblastoma specimens, and in vitro studies find that adding NT-3 to TrkC-expressing medulloblastoma cells induces apoptosis as well as activation of multiple parallel signaling pathways, like PI3K, ERK, JNK/SAPK, p38MAPK. Of these, p38MAPK activity appears necessary for NT-3-/TrkC-induced apoptosis, based on experiments using pharmacological inhibitors. BDNF and TrkB were also detected in some medulloblastoma specimens. Treatment with BDNF of TrkB-expressing medulloblastoma cells significantly inhibited the cell viability in vitro. Besides, NGF treatment of TrkA-expressing medulloblastoma cells results in massive apoptosis (Muragaki et al. 1997), and this NGF/TrkA-induced apoptosis is mediated through Ras/Raf signaling pathway. Thus, activation of the neurotrophin/Trk signaling pathways plays a pro-apoptotic role in medulloblastoma.

5.2 Neurotrophins and Their Receptors in Nonneuronal Origin Cancers

5.2.1 Colorectal Cancer

In colorectal cancer, BDNF and TrkB are overexpressed in the patients' tumors relative to levels in their adjacent normal tissues and this is more pronounced in advanced stages of colorectal cancer (Akil et al. 2011; Brunetto de Farias et al. 2010; Yu et al. 2010). In colorectal cancer cell lines, TrkB protein and BDNF mRNA expression are detected under basal (10 % serum) culture conditions, and BDNF mRNA levels and mature BDNF protein levels increase under stress condition (serum starvation) (Akil et al. 2011). Furthermore, in basal culture conditions, TrkB and BDNF have a cytosolic location in colorectal cell lines, but serum starvation induces a relocation of TrkB to the cell membrane and a colocalization of TrkB and BDNF on the membrane. BDNF induces cell proliferation and stimulates a TrkB-dependent anti-apoptotic effect under serum starvation condition. However, pro-BDNF, also detected in colorectal cancer cells, is co-expressed with p75NTR and sortillin at the cell membrane. In contrast to BDNF, exogenous pro-BDNF-induced colorectal cancer cell apoptosis, which suggests that a counterbalance mechanism is involved in the control of colorectal cancer cell survival (Akil et al. 2011). In human colorectal tumors, mutations in the TrkB kinase domain at two different sites (T695I, 751 N) have been found. The mutated TrkB displayed reduced activity compared to that of wild-type TrkB that indicated no gain-of-function of these mutations (Geiger et al. 2011). It is not known how these mutated TrkB receptors affect colorectal tumor cell biology. TrkA and TrkC were not detected in tumor tissues or in cell lines (Akil et al. 2011).

5.2.2 Breast Cancer

High levels of TrkA and phospho-TrkA are expressed in breast cancer tumors (compared to normal breast tissues) and in breast cancer cells (Davidson et al. 2004; Lagadec et al. 2009). Overexpression of TrkA induces constitutive activation of its tyrosine kinase activity. This promotes cell growth, migration, and

invasion in vitro via the activation of PI3K/Akt and MAPK signaling pathways which are required for the maintenance of a more aggressive cellular phenotype. TrkA overexpression enhances tumor growth, angiogenesis, and metastasis of breast cancer xenografts in immunodeficient mice (Lagadec et al. 2009).

NGF expression is detected in most breast tumors as well as in breast cancer cell lines, but no direct relationship between NGF overexpression and any known prognostic factor has been observed. Inhibition of NGF with neutralizing antibodies, or small interfering RNA, strongly reduces angiogenesis and tumor xenograft development in immunodeficient mice (Adriaenssens et al. 2008). Since NGF activation of TrkA has been shown to increase VEGF production by neuroblastoma tumor cells (Nakamura et al. 2011), it is possible that the decreased angiogenesis results from decreased tumor cell production of VEGF when NGF is inhibited. The mitogenic effect of NGF on breast cancer cells requires the tyrosine kinase activity of TrkA as well as the mitogen-activated protein kinase (MAPK) cascade, but is independent of p75NTR. In contrast, the anti-apoptotic effect of NGF is not dependent on TrkA but rather p75NTR and the activation of the transcription factor NF-κB (Descamps et al. 2001).

Truncated TrkB (TrkB.T1) and p75NTR, as well as BDNF and NT-4/5, are detected in breast cancer specimens and breast cancer cells. Full-length TrkB (TrkB.FL) has only rarely been reported (Vanhecke et al. 2011). Blocking endogenous NT-4/5 and BDNF using neutralizing anti-NT-4/5 or anti-BDNF antibodies inhibits the cell survival. Adding exogenous BDNF or NT-4/5 has an anti-apoptotic effect in breast cancer cells, but little effect on cell proliferation and invasion. Targeted decrease in p75NTR or TrkB-T1 using siRNAs abolishes the NT-4/5 and BDNF anti-apoptotic effect. Moreover, the Trk pharmacological inhibitor K252a has no impact on the BDNF- and NT-4/5-mediated survival, suggesting the involvement of p75NTR and TrkB-T1, and not Trk kinase activity. Treatment of mice bearing breast cancer xenografts with anti-BDNF, anti-NT-4/5, anti-p75NTR, or anti-TrkB-T1 inhibits tumor growth and this is accompanied by an increase in cell apoptosis and no changes in cell proliferation. A study showed that higher expression of BDNF is significantly associated with unfavorable pathologic parameters and poor prognosis (Patani et al. 2011). This indicates that an autocrine loop of BDNF and NT-4/5 stimulates breast cancer cell survival.

TrkC is also detected in breast cancers, and there is an inverse relationship between tumor grade and TrkC expression. The expression of TrkC is higher in Grade I tumors that have a good prognosis compared to that in Grade III tumors which have a poor prognosis. Rise in TrkC expression could indicate good prognosis (Blasco-Gutierrez et al. 2007).

5.2.3 Prostate Cancer

TrkA receptor is expressed in normal prostate, and expression is retained or significantly overexpressed in prostate cancer (MacGrogan et al. 1992; Mahadeo et al. 1994). TrkB and TrkC receptors are only expressed in malignant prostate cells. p75NTR is expressed in normal human prostate epithelial cells, and its expression is progressively lost during progression of prostate cancer. There is an

inverse relationship between low p75NTR expression and high prostate cancer growth and metastasis. So p75NTR may function as a prostate tumor-suppressor and metastasis-suppressor gene (Krygier and Djakiew 2002). TrkA activation increases cell proliferation, while p75NTR expression is associated with apoptosis. So an imbalance between p75NTR and TrkA-mediated signals may be involved in the progression of prostate cancer through increased proliferation and reduced apoptosis.

Human prostate is the second most abundant source of NGF after central nervous system. NGF is detected in prostate cancer specimens and cell lines. NGF secreted by the prostate stromal cells stimulates the growth of prostate cancer cells, and some specific prostate tumor cell lines secrete NGF in an autocrine manner (Djakiew 2000). Thus, NGF contributes to prostate cancer growth in both a paracrine and an autocrine manner.

5.2.4 Lung Cancer

Trk receptors but not p75NTR are readily detected in multiple types of human lung cancer (Koizumi et al. 1998; Ricci et al. 2001). High levels of TrkA are observed in adenocarcinomas and squamous cell carcinomas, while TrkB is detected primarily in small cell lung cancers (SCLC). Some tumors also express neurotrophin ligands. In particular, NGF is expressed in a high proportion of bronchioloalveolar and squamous cell carcinomas, while BDNF is expressed in SCLC. Approximately one third of bronchioloalveolar carcinomas express both NGF and TrkA, an observation that raises the possibility of autocrine effects of neurotrophins in this cancer type. Such autocrine loops may also exist as BDNF and TrkB expression is co-expressed in adenocarcinomas, squamous cell carcinomas, and small cell lung cancers. NT-3 and its corresponding TrkC receptor are found in a small number of squamous cell carcinomas (Ricci et al. 2001).

Studies with lung tumor cell lines indicate that NGF inhibits the growth of SCLC cells, and this is accompanied by an upregulation of TrkA, p75NTR, and NGF expression. NGF markedly inhibits cellular proliferation, colony formation, and invasiveness in vitro. NGF-treated cell lines do not form tumors in nude mice, whereas the untreated cell lines are highly tumorigenic. Administration of NGF to mice bearing established xenografts inhibits tumor growth.

In non-small cell lung cancers (NSCLC), TrkB enhances cell migration, invasion, and transformation (Harada et al. 2011), and a higher expression of TrkB in NSCLC primary tumors is closely correlated with lymph node metastasis (Zhang et al. 2010).

6 Clinical-Translational Advances

After the discovery of the involvement of neurotrophins and their receptors in the biological and clinical behaviors of cancers, it was appreciated that inhibition of neurotrophins, their receptors, or their targets might be beneficial in the clinical oncology setting. One well-studied strategy targets the Trk tyrosine kinase domain. Since there is a high degree of homology among Trk tyrosine kinase domains, there

are currently no inhibitors reported that selectively block TrkA or TrkB kinase activity. The earliest Trk inhibitors to reach clinical trials were Cephalon's CEP-751 and CEP-701 (lestaurtinib). These compounds were modeled on the indolocarbazole K252a from *Nocardiopsis* species and were relatively nonselective in inhibiting Trks, as well as Flt3, JAK2, and protein kinase C (PKC). CEP-751 and CEP-701 reduced tumor burden in xenograft models of prostate and pancreatic cancer, neuroblastoma, medulloblastoma, and medullary thyroid carcinoma (Camoratto et al. 1997; Evans et al. 1999, 2001; George et al. 1999; Miknyoczki et al. 1999). In prostate cancers, CEP-701 also inhibits the metastasis of both primary and metastatic sites of prostate cancer. A phase 1 study of CEP-701 established the maximum tolerated dose and identified that toxicities such as nausea, gastrointestinal events, and dyspepsia were relatively well tolerated (Marshall et al. 2005). Recently, a phase 1 study indicated that CEP-701 was well tolerated in patients with refractory neuroblastoma, and a dose level sufficient to inhibit TrkB activity was established (Minturn et al. 2011). CEP-701 phase 2 studies are in progress. Given the important role Trk signaling plays in normal neurons, it is encouraging that neural toxicity has not been a dose-limiting toxicity.

Another series of Trk inhibitors came from the optimization of 4-aminopyrazolylpyrimidines (AstraZeneca, Inc). One such compound, AZ-23, inhibits TrkA/B in the nanomolar range and has reasonable pharmacodynamics and oral bioavailability in toxicology studies. Phase 1 testing in adults was initiated but recently halted as a result of poor pharmacodynamic properties (Wang et al. 2008). Study on another compound named AZ623 showed that AZ623 inhibits BDNF-mediated signaling and neuroblastoma cell proliferation in vitro, as well as also inhibits the growth of human neuroblastoma xenograft tumors in vivo (Zage et al. 2011). Other congeners of 4-aminopyrazolylpyrimidines remain poised for clinical development.

Another strategy suggested by preclinical modeling is to use Trk kinase inhibitors in combination with standard chemotherapy. In a preclinical neuroblastoma xenograft model, CEP-701 (or the active precursor CEP-751) significantly enhanced the activity of cyclophosphamide, as well as other cytotoxic drug combinations currently used in the treatment of high-risk neuroblastoma (Evans et al. 1999). A clinical trial of CEP-701 with gemcitabine was undertaken assessing drug interactions in patients with advanced pancreatic cancer (Chan et al. 2008). The combination was well tolerated with no unexpected toxicities, but because the trial was limited and not designed to assess efficacy, further studies are planned. A study in a combination of AZ623 with topotecan, a topoisomerase inhibitor that is currently used for neuroblastoma treatment, indicated that a combination of AZ623 and topotecan has much higher effect on the inhibition of xenograft tumor growth compared to either individual agent, and inhibits the regrowth of tumors after cessation of the treatment where either individual agent alone is ineffective (Zage et al. 2011).

Additional therapeutic options to block the effects of activated Trk receptors can be developed by moving downstream and targeting the key intracellular mediators of activated Trk's pro-survival and prometastatic signals, PI3K and Akt. Pharmacologic inhibitors of these "nodal" kinases have been extensively pursued. Perifosine is an oral alkyl-phospholipid Akt inhibitor. Unlike most kinase

inhibitors, which target the adenosine triphosphate–binding region, perifosine targets the pleckstrin homology domain of Akt. Phase I and II clinical trials have indicated that the side effect of perifosine is limited to dose-limiting gastrointestinal toxicity, such as nausea, diarrhea, fatigue, and dehydration, which can be ameliorated with the use of prophylactic medicine and well tolerated (Gills and Dennis 2009). As Akt is a key downstream target in PI3K pathway initiated by many growth factors and their receptors, including neurotrophins/Trk, perifosine as an Akt inhibitor has been evaluated in many types of tumors with elevated activity of Akt, and has showed antitumor growth effect either alone or in combination with chemotherapeutic drugs/other small molecular inhibitors. The role of perifosine in tumors with BDNF/TrkB-initiating activation of Akt has been evaluated in neuroblastoma. Perifosine alone inhibits tumor growth, but is most effective in combination with cytotoxic drugs such as etoposide. Combinations of Akt inhibition and etoposide cause tumor regression of TrkB-expressing neuroblastoma tumor xenografts (Li et al. 2011). Strategies targeting Akt may offer a broader spectrum of inhibitory activity than Trk kinase–targeted agents and may avoid resistance due to feedback suppression that has been seen when targeting some receptors.

7 Conclusion

Neurotrophins and their receptors were originally found to play important roles in the survival, proliferation, differentiation, apoptosis, and synaptic plasticity of neurons, with aberrant expression being associated with neurodegenerative diseases. However, increasingly studies have indicated that the neurotrophin/neurotrophin receptors play an important role during the genesis of tumors where oncogenic events may subvert the normal functions of neurotrophins and their receptors and influence biologic features of tumors that may impact tumor cell aggressiveness. Neurotrophins activate different signaling pathways based on binding to their Trk receptors or p75NTR receptor, and they may exert pro- or anti-tumorigenic activity, depending on the activation status of other cellular receptors and the presence or absence of ligands in the environment. For most types of cancers, expression of TrkA or TrkC in tumor tissues is associated with good prognosis, such as neuroblastoma, medulloblastoma, lung cancer; expression of TrkB and/or its ligand BDNF is associated with poor prognosis, such as neuroblastoma and colorectal cancer. One exception is breast cancer where overexpression of TrkA and NGF in breast cancer cells stimulates cell proliferation and invasion but not cell differentiation or apoptosis. These findings provide support for the development of small molecular inhibitors that target on Trk or the downstream targets in the neurotrophin/neurotrophin receptor-initiating signaling pathways in the treatment of cancers. The preclinical and clinical evidence of efficacy as a single agent or in combination with chemotherapy, as well as the well-tolerated limited toxicities, suggests the usage of these small molecular inhibitors as a potential strategy for the treatment of cancers.

References

Acheson, A., Conover, J. C., Fandl, J. P., DeChiara, T. M., Russell, M., Thadani, A., Squinto, S. P., Yancopoulos, G. D., & Lindsay, R. M. (1995). A BDNF autocrine loop in adult sensory neurons prevents cell death. *Nature, 374*, 450–453.

Adriaenssens, E., Vanhecke, E., Saule, P., Mougel, A., Page, A., Romon, R., Nurcombe, V., Le Bourhis, X., & Hondermarck, H. (2008). Nerve growth factor is a potential therapeutic target in breast cancer. *Cancer Research, 68*, 346–351.

Akil, H., Perraud, A., Melin, C., Jauberteau, M. O., & Mathonnet, M. (2011). Fine-tuning roles of endogenous brain-derived neurotrophic factor, TrkB and sortilin in colorectal cancer cell survival. *PLoS One, 6*, e25097.

Arevalo, J. C., & Wu, S. H. (2006). Neurotrophin signaling: Many exciting surprises! *Cellular and Molecular Life Sciences, 63*, 1523–1537.

Benedetti, M., Levi, A., & Chao, M. V. (1993). Differential expression of nerve growth factor receptors leads to altered binding affinity and neurotrophin responsiveness. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 7859–7863.

Binder, D. K., & Scharfman, H. E. (2004). Brain-derived neurotrophic factor. *Growth Factors, 22*, 123–131.

Blasco-Gutierrez, M. J., Jose-Crespo, I. J., Zozaya-Alvarez, E., Ramos-Sanchez, R., & Garcia-Atares, N. (2007). TrkC: A new predictive marker in breast cancer? *Cancer Investigation, 25*, 405–410.

Bouzas-Rodriguez, J., Cabrera, J. R., Delloye-Bourgeois, C., Ichim, G., Delcros, J. G., Raquin, M. A., Rousseau, R., Combaret, V., Benard, J., Tauszig-Delamasure, S., et al. (2010). Neurotrophin-3 production promotes human neuroblastoma cell survival by inhibiting TrkC-induced apoptosis. *Journal of Clinical Investigation, 120*, 850–858.

Brodeur, G. M., Minturn, J. E., Ho, R., Simpson, A. M., Iyer, R., Varela, C. R., Light, J. E., Kolla, V., & Evans, A. E. (2009). Trk receptor expression and inhibition in neuroblastomas. *Clinical Cancer Research, 15*, 3244–3250.

Brunetto de Farias, C., Rosemberg, D. B., Heinen, T. E., Koehler-Santos, P., Abujamra, A. L., Kapczinski, F., Brunetto, A. L., Ashton-Prolla, P., Meurer, L., Reis Bogo, M., et al. (2010). BDNF/TrkB content and interaction with gastrin-releasing peptide receptor blockade in colorectal cancer. *Oncology, 79*, 430–439.

Butte, M. J. (2001). Neurotrophic factor structures reveal clues to evolution, binding, specificity, and receptor activation. *Cellular and Molecular Life Sciences, 58*, 1003–1013.

Camoratto, A. M., Jani, J. P., Angeles, T. S., Maroney, A. C., Sanders, C. Y., Murakata, C., Neff, N. T., Vaught, J. L., Isaacs, J. T., & Dionne, C. A. (1997). CEP-751 inhibits TRK receptor tyrosine kinase activity in vitro exhibits anti-tumor activity. *International Journal of Cancer, 72*, 673–679.

Chan, E., Mulkerin, D., Rothenberg, M., Holen, K. D., Lockhart, A. C., Thomas, J., & Berlin, J. (2008). A phase I trial of CEP-701 + gemcitabine in patients with advanced adenocarcinoma of the pancreas. *Investigational New Drugs, 26*, 241–247.

Chao, M. V., Bothwell, M. A., Ross, A. H., Koprowski, H., Lanahan, A. A., Buck, C. R., & Sehgal, A. (1986). Gene transfer and molecular cloning of the human NGF receptor. *Science, 232*, 518–521.

Clary, D. O., & Reichardt, L. F. (1994). An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 11133–11137.

Davidson, B., Reich, R., Lazarovici, P., Ann Florenes, V., Nielsen, S., & Nesland, J. M. (2004). Altered expression and activation of the nerve growth factor receptors TrkA and p75 provide the first evidence of tumor progression to effusion in breast carcinoma. *Breast Cancer Research and Treatment, 83*, 119–128.

Descamps, S., Toillon, R. A., Adriaenssens, E., Pawlowski, V., Cool, S. M., Nurcombe, V., Le Bourhis, X., Boilly, B., Peyrat, J. P., & Hondermarck, H. (2001). Nerve growth factor stimulates proliferation and survival of human breast cancer cells through two distinct signaling pathways. *Journal of Biological Chemistry, 276*, 17864–17870.

Djakiew, D. (2000). Dysregulated expression of growth factors and their receptors in the development of prostate cancer. *Prostate, 42*, 150–160.

Eggert, A., Grotzer, M. A., Ikegaki, N., Liu, X. G., Evans, A. E., & Brodeur, G. M. (2002). Expression of the neurotrophin receptor TrkA down-regulates expression and function of angiogenic stimulators in SH-SY5Y neuroblastoma cells. *Cancer Research, 62*, 1802–1808.

Evans, A. E., Kisselbach, K. D., Yamashiro, D. J., Ikegaki, N., Camoratto, A. M., Dionne, C. A., & Brodeur, G. M. (1999). Antitumor activity of CEP-751 (KT-6587) on human neuroblastoma and medulloblastoma xenografts. *Clinical Cancer Research, 5*, 3594–3602.

Evans, A. E., Kisselbach, K. D., Liu, X., Eggert, A., Ikegaki, N., Camoratto, A. M., Dionne, C., & Brodeur, G. M. (2001). Effect of CEP-751 (KT-6587) on neuroblastoma xenografts expressing TrkB. *Medical and Pediatric Oncology, 36*, 181–184.

Geiger, T. R., Song, J. Y., Rosado, A., & Peeper, D. S. (2011). Functional characterization of human cancer-derived TRKB mutations. *PLoS One, 6*, e16871.

George, D. J., Dionne, C. A., Jani, J., Angeles, T., Murakata, C., Lamb, J., & Isaacs, J. T. (1999). Sustained in vivo regression of Dunning H rat prostate cancers treated with combinations of androgen ablation and Trk tyrosine kinase inhibitors, CEP-751 (KT-6587) or CEP-701 (KT-5555). *Cancer Research, 59*, 2395–2401.

Gills, J. J., & Dennis, P. A. (2009). Perifosine: Update on a novel Akt inhibitor. *Current Oncology Reports, 11*, 102–110.

Gotz, R., Koster, R., Winkler, C., Raulf, F., Lottspeich, F., Schartl, M., & Thoenen, H. (1994). Neurotrophin-6 is a new member of the nerve growth factor family. *Nature, 372*, 266–269.

Harada, T., Yatabe, Y., Takeshita, M., Koga, T., Yano, T., Wang, Y., & Giaccone, G. (2011). Role and relevance of TrkB mutations and expression in non-small cell lung cancer. *Clinical Cancer Research, 17*, 2638–2645.

Harel, L., Costa, B., Tcherpakov, M., Zapatka, M., Oberthuer, A., Hansford, L. M., Vojvodic, M., Levy, Z., Chen, Z. Y., Lee, F. S., et al. (2009). CCM2 mediates death signaling by the TrkA receptor tyrosine kinase. *Neuron, 63*, 585–591.

Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R., Parada, L. F., & Chao, M. V. (1991). High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. *Nature, 350*, 678–683.

Jaboin, J., Kim, C. J., Kaplan, D. R., & Thiele, C. J. (2002). Brain-derived neurotrophic factor activation of TrkB protects neuroblastoma cells from chemotherapy-induced apoptosis via phosphatidylinositol 3'-kinase pathway. *Cancer Research, 62*, 6756–6763.

Kaplan, D. R., & Miller, F. D. (2000). Neurotrophin signal transduction in the nervous system. *Current Opinion in Neurobiology, 10*, 381–391.

Karin, M., & Lin, A. (2002). NF-kappaB at the crossroads of life and death. *Nature Immunology, 3*, 221–227.

Klein, R., Parada, L. F., Coulier, F., & Barbacid, M. (1989). trkB, a novel tyrosine protein kinase receptor expressed during mouse neural development. *EMBO Journal, 8*, 3701–3709.

Klein, R., Nanduri, V., Jing, S. A., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F., & Barbacid, M. (1991). The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell, 66*, 395–403.

Koizumi, H., Morita, M., Mikami, S., Shibayama, E., & Uchikoshi, T. (1998). Immunohistochemical analysis of TrkA neurotrophin receptor expression in human non-neuronal carcinomas. *Pathology International, 48*, 93–101.

Krygier, S., & Djakiew, D. (2002). Neurotrophin receptor p75(NTR) suppresses growth and nerve growth factor-mediated metastasis of human prostate cancer cells. *International Journal of Cancer, 98*, 1–7.

Lagadec, C., Meignan, S., Adriaenssens, E., Foveau, B., Vanhecke, E., Romon, R., Toillon, R. A., Oxombre, B., Hondermarck, H., & Le Bourhis, X. (2009). TrkA overexpression enhances growth and metastasis of breast cancer cells. *Oncogene, 28*, 1960–1970.

Lamballe, F., Klein, R., & Barbacid, M. (1991). trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. *Cell, 66*, 967–979.

Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*, 1945–1948.

Li, Z., Jaboin, J., Dennis, P. A., & Thiele, C. J. (2005). Genetic and pharmacologic identification of Akt as a mediator of brain-derived neurotrophic factor/TrkB rescue of neuroblastoma cells from chemotherapy-induced cell death. *Cancer Research, 65*, 2070–2075.

Li, Z., Tan, F., & Thiele, C. J. (2007a). Inactivation of glycogen synthase kinase-3beta contributes to brain-derived neutrophic factor/TrkB-induced resistance to chemotherapy in neuroblastoma cells. *Molecular Cancer Therapeutics, 6*, 3113–3121.

Li, Z., Zhang, J., Liu, Z., Woo, C. W., & Thiele, C. J. (2007b). Downregulation of Bim by brain-derived neurotrophic factor activation of TrkB protects neuroblastoma cells from paclitaxel but not etoposide or cisplatin-induced cell death. *Cell Death and Differentiation, 14*, 318–326.

Li, Z., Oh, D. Y., Nakamura, K., & Thiele, C. J. (2011). Perifosine-induced inhibition of Akt attenuates brain-derived neurotrophic factor/TrkB-induced chemoresistance in neuroblastoma in vivo. *Cancer, 117*, 5412–5422.

Lucarelli, E., Kaplan, D., & Thiele, C. J. (1997). Activation of trk-A but not trk-B signal transduction pathway inhibits growth of neuroblastoma cells. *European Journal of Cancer, 33*, 2068–2070.

MacGrogan, D., Saint-Andre, J. P., & Dicou, E. (1992). Expression of nerve growth factor and nerve growth factor receptor genes in human tissues and in prostatic adenocarcinoma cell lines. *Journal of Neurochemistry, 59*, 1381–1391.

Mahadeo, D., Kaplan, L., Chao, M. V., & Hempstead, B. L. (1994). High affinity nerve growth factor binding displays a faster rate of association than p140trk binding. Implications for multi-subunit polypeptide receptors. *J Biol Chem, 269*, 6884–6891.

Marshall, J. L., Kindler, H., Deeken, J., Bhargava, P., Vogelzang, N. J., Rizvi, N., Luhtala, T., Boylan, S., Dordal, M., Robertson, P., et al. (2005). Phase I trial of orally administered CEP-701, a novel neurotrophin receptor-linked tyrosine kinase inhibitor. *Investigational New Drugs, 23*, 31–37.

Miknyoczki, S. J., Dionne, C. A., Klein-Szanto, A. J., & Ruggeri, B. A. (1999). The novel Trk receptor tyrosine kinase inhibitor CEP-701 (KT-5555) exhibits antitumor efficacy against human pancreatic carcinoma (Panc1) xenograft growth and in vivo invasiveness. *Annals of the New York Academy of Sciences, 880*, 252–262.

Minturn, J. E., Evans, A. E., Villablanca, J. G., Yanik, G. A., Park, J. R., Shusterman, S., Groshen, S., Hellriegel, E. T., Bensen-Kennedy, D., Matthay, K. K., et al. (2011). Phase I trial of lestaurtinib for children with refractory neuroblastoma: A new approaches to neuroblastoma therapy consortium study. *Cancer Chemotherapy and Pharmacology, 68*, 1057–1065.

Muragaki, Y., Chou, T. T., Kaplan, D. R., Trojanowski, J. Q., & Lee, V. M. (1997). Nerve growth factor induces apoptosis in human medulloblastoma cell lines that express TrkA receptors. *Journal of Neuroscience, 17*, 530–542.

Nakagawara, A. (1998a). Molecular basis of spontaneous regression of neuroblastoma: Role of neurotrophic signals and genetic abnormalities. *Human Cell, 11*, 115–124.

Nakagawara, A. (1998b). The NGF story and neuroblastoma. *Medical and Pediatric Oncology, 31*, 113–115.

Nakagawara, A., Arima-Nakagawara, M., Scavarda, N. J., Azar, C. G., Cantor, A. B., & Brodeur, G. M. (1993). Association between high levels of expression of the TRK gene and favorable outcome in human neuroblastoma. *New England Journal of Medicine, 328*, 847–854.

Nakagawara, A., Azar, C. G., Scavarda, N. J., & Brodeur, G. M. (1994). Expression and function of TRK-B and BDNF in human neuroblastomas. *Molecular Cell Biology, 14*, 759–767.

Nakamura, K., Martin, K. C., Jackson, J. K., Beppu, K., Woo, C. W., & Thiele, C. J. (2006). Brain-derived neurotrophic factor activation of TrkB induces vascular endothelial growth factor expression via hypoxia-inducible factor-1alpha in neuroblastoma cells. *Cancer Research, 66*, 4249–4255.

Nakamura, K., Tan, F., Li, Z., & Thiele, C. J. (2011). NGF activation of TrkA induces vascular endothelial growth factor expression via induction of hypoxia-inducible factor-1alpha. *Molecular and Cellular Neuroscience, 46*, 498–506.

Nikoletopoulou, V., Lickert, H., Frade, J. M., Rencurel, C., Giallonardo, P., Zhang, L., Bibel, M., & Barde, Y. A. (2010). Neurotrophin receptors TrkA and TrkC cause neuronal death whereas TrkB does not. *Nature, 467*, 59–63.

Nilsson, A. S., Fainzilber, M., Falck, P., & Ibanez, C. F. (1998). Neurotrophin-7: A novel member of the neurotrophin family from the zebrafish. *FEBS Letters, 424*, 285–290.

Nykjaer, A., Lee, R., Teng, K. K., Jansen, P., Madsen, P., Nielsen, M. S., Jacobsen, C., Kliemannel, M., Schwarz, E., Willnow, T. E., et al. (2004). Sortilin is essential for proNGF-induced neuronal cell death. *Nature, 427*, 843–848.

Pahl, H. L. (1999). Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene, 18*, 6853–6866.

Patani, N., Jiang, W. G., & Mokbel, K. (2011). Brain-derived neurotrophic factor expression predicts adverse pathological & clinical outcomes in human breast cancer. *Cancer Cell International, 11*, 23.

Radeke, M. J., Misko, T. P., Hsu, C., Herzenberg, L. A., & Shooter, E. M. (1987). Gene transfer and molecular cloning of the rat nerve growth factor receptor. *Nature, 325*, 593–597.

Reichardt, L. F. (2006). Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 361*, 1545–1564.

Ricci, A., Greco, S., Mariotta, S., Felici, L., Bronzetti, E., Cavazzana, A., Cardillo, G., Amenta, F., Bisetti, A., & Barbolini, G. (2001). Neurotrophins and neurotrophin receptors in human lung cancer. *American Journal of Respiratory Cell and Molecular Biology, 25*, 439–446.

Ryden, M., Sehgal, R., Dominici, C., Schilling, F. H., Ibanez, C. F., & Kogner, P. (1996). Expression of mRNA for the neurotrophin receptor trkC in neuroblastomas with favourable tumour stage and good prognosis. *British Journal of Cancer, 74*, 773–779.

Scala, S., Wosikowski, K., Giannakakou, P., Valle, P., Biedler, J. L., Spengler, B. A., Lucarelli, E., Bates, S. E., & Thiele, C. J. (1996). Brain-derived neurotrophic factor protects neuroblastoma cells from vinblastine toxicity. *Cancer Research, 56*, 3737–3742.

Segal, R. A., Goumnerova, L. C., Kwon, Y. K., Stiles, C. D., & Pomeroy, S. L. (1994). Expression of the neurotrophin receptor TrkC is linked to a favorable outcome in medulloblastoma. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 12867–12871.

Snider, W. D. (1994). Functions of the neurotrophins during nervous system development: What the knockouts are teaching us. *Cell, 77*, 627–638.

Sofroniew, M. V., Howe, C. L., & Mobley, W. C. (2001). Nerve growth factor signaling, neuroprotection, and neural repair. *Annual Review of Neuroscience, 24*, 1217–1281.

Straub, J. A., Sholler, G. L., & Nishi, R. (2007). Embryonic sympathoblasts transiently express TrkB in vivo and proliferate in response to brain-derived neurotrophic factor in vitro. *BMC Developmental Biology, 7*, 10.

Strohmaier, C., Carter, B. D., Urfer, R., Barde, Y. A., & Dechant, G. (1996). A splice variant of the neurotrophin receptor trkB with increased specificity for brain-derived neurotrophic factor. *EMBO Journal, 15*, 3332–3337.

Suzuki, T., Bogenmann, E., Shimada, H., Stram, D., & Seeger, R. C. (1993). Lack of high-affinity nerve growth factor receptors in aggressive neuroblastomas. *Journal of the National Cancer Institute, 85*, 377–384.

Svensson, T., Ryden, M., Schilling, F. H., Dominici, C., Sehgal, R., Ibanez, C. F., & Kogner, P. (1997). Coexpression of mRNA for the full-length neurotrophin receptor trk-C and trk-A in favourable neuroblastoma. *European Journal of Cancer, 33*, 2058–2063.

Tacconelli, A., Farina, A. R., Cappabianca, L., Desantis, G., Tessitore, A., Vetuschi, A., Sferra, R., Rucci, N., Argenti, B., Screpanti, I., et al. (2004). TrkA alternative splicing: A regulated tumor-promoting switch in human neuroblastoma. *Cancer Cell, 6*, 347–360.

Tauszig-Delamasure, S., Yu, L. Y., Cabrera, J. R., Bouzas-Rodriguez, J., Mermet-Bouvier, C., Guix, C., Bordeaux, M. C., Arumae, U., & Mehlen, P. (2007). The TrkC receptor induces apoptosis when the dependence receptor notion meets the neurotrophin paradigm. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 13361–13366.

Teng, H. K., Teng, K. K., Lee, R., Wright, S., Tevar, S., Almeida, R. D., Kermani, P., Torkin, R., Chen, Z. Y., Lee, F. S., et al. (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *Journal of Neuroscience, 25*, 5455–5463.

Thiele, C. J., Li, Z., & McKee, A. E. (2009). On Trk–the TrkB signal transduction pathway is an increasingly important target in cancer biology. *Clinical Cancer Research, 15*, 5962–5967.

Valenzuela, D. M., Maisonpierre, P. C., Glass, D. J., Rojas, E., Nunez, L., Kong, Y., Gies, D. R., Stitt, T. N., Ip, N. Y., & Yancopoulos, G. D. (1993). Alternative forms of rat TrkC with different functional capabilities. *Neuron, 10*, 963–974.

Vanhecke, E., Adriaenssens, E., Verbeke, S., Meignan, S., Germain, E., Berteaux, N., Nurcombe, V., Le Bourhis, X., & Hondermarck, H. (2011). Brain-derived neurotrophic factor and neurotrophin-4/5 are expressed in breast cancer and can be targeted to inhibit tumor cell survival. *Clinical Cancer Research, 17*, 1741–1752.

Wang, T., Lamb, M. L., Scott, D. A., Wang, H., Block, M. H., Lyne, P. D., Lee, J. W., Davies, A. M., Zhang, H. J., Zhu, Y., et al. (2008). Identification of 4-aminopyrazolylpyrimidines as potent inhibitors of Trk kinases. *Journal of Medicinal Chemistry, 51*, 4672–4684.

Wiesmann, C., & de Vos, A. M. (2001). Nerve growth factor: Structure and function. *Cellular and Molecular Life Sciences, 58*, 748–759.

Yamashiro, D. J., Nakagawara, A., Ikegaki, N., Liu, X. G., & Brodeur, G. M. (1996). Expression of TrkC in favorable human neuroblastomas. *Oncogene, 12*, 37–41.

Yan, H., & Chao, M. V. (1991). Disruption of cysteine-rich repeats of the p75 nerve growth factor receptor leads to loss of ligand binding. *Journal of Biological Chemistry, 266*, 12099–12104.

Yu, Y., Zhang, S., Wang, X., Yang, Z., & Ou, G. (2010). Overexpression of TrkB promotes the progression of colon cancer. *APMIS, 118*, 188–195.

Zage, P. E., Graham, T. C., Zeng, L., Fang, W., Pien, C., Thress, K., Omer, C., Brown, J. L., & Zweidler-McKay, P. A. (2011). The selective Trk inhibitor AZ623 inhibits brain-derived neurotrophic factor-mediated neuroblastoma cell proliferation and signaling and is synergistic with topotecan. *Cancer, 117*, 1321–1391.

Zhang, S., Guo, D., Luo, W., Zhang, Q., Zhang, Y., Li, C., Lu, Y., Cui, Z., & Qiu, X. (2010). TrkB is highly expressed in NSCLC and mediates BDNF-induced the activation of Pyk2 signaling and the invasion of A549 cells. *BMC Cancer, 10*, 43.

Zupan, A. A., Osborne, P. A., Smith, C. E., Siegel, N. R., Leimgruber, R. M., & Johnson, E. M., Jr. (1989). Identification, purification, and characterization of truncated forms of the human nerve growth factor receptor. *Journal of Biological Chemistry, 264*, 11714–11720.

NGF and Immune Regulation

Luisa Bracci-Laudiero and Luigi Manni

Contents

Abstract

For some time after its discovery, the action of nerve growth factor (NGF) was considered restricted to the nervous system. Instead, a variety of experimental data indicate that NGF can influence the activity of both the nervous and immune systems. This should not be surprising since these two systems are responsible both for maintaining homeostasis and for adapting the body to the environment. To orchestrate strictly integrated responses, they need to have close anatomical connections and to share common chemical signals and specific receptors. The well-known effects of NGF on peripheral neuron survival and maintenance and dynamic control by NGF of innervation and neuropeptide synthesis, together with its direct effects on immune cell functions, indicate that NGF has a key role in the complex network of bidirectional signals between the nervous and immune systems. NGF receptors are expressed in immune organs and cell populations, allowing NGF to modulate cell differentiation and regulate immune response. NGF concentrations in tissues change during inflammation, and inflammatory mediators induce NGF

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synthesis in a variety of cell types. As a growing number of studies have shown, an enhanced production of NGF characterizes inflamed tissues of patients with inflammatory and autoimmune diseases. Unfortunately, although the dynamic regulation of NGF synthesis seems to be a common feature of chronic inflammatory diseases, the reasons why NGF concentrations are enhanced and how this can affect inflammatory responses and the course of the diseases are far from being understood.

List of Abbreviations

BBB	Blood–brain barrier
CGRP	Calcitonin gene-related peptide
CHS	Contact hypersensitivity
CIPA	Congenital insensitivity to pain with anhidrosis
CNS	Central nervous system
COX2	Cyclooxygenase 2
CSF	Cerebrospinal fluid
DRG	Dorsal root ganglion
EAE	Experimental autoimmune encephalomyelitis
Gal	Galanin
GM-CSF	Granulocyte-macrophage colony-stimulating factor
IFN	Interferon
IL	Interleukin
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MPB	Myelin basic protein
NGF	Nerve growth factor
NO	Nitric oxide
NPY	Neuropeptide Y
NT	Neurotrophin
NTR	Neurotrophin receptor
PI3K	Phosphatidylinositol 3-kinase
PNS	Peripheral nervous system
SP	Substance P
TH	Tyrosine hydroxylase
TNF	Tumor necrosis factor
TrkA	Tropomyosin kinase receptor A
UV	Ultraviolet
VIP	Vasoactive intestinal peptide

1 Introduction

The neurotrophin (NT) family of proteins is responsible for the growth and survival of neurons during development and for the maintenance and function of adult neurons (Rush et al. 1997; Ernfors 2001; McAllister 2001; Poo 2001). NTs also promote

regeneration of damaged axons after various peripheral and central nervous system injuries (Olson 1993; Connor and Dragunow 1998; Terenghi 1999; Chao et al. 2006).

Nerve growth factor (NGF) was the first NT to be discovered (Levi-Montalcini 1987). It is essential for the development and maintenance of neurons in the peripheral nervous system (PNS) and for the functional integrity of cholinergic neurons in the central nervous system (CNS) (Whittemore et al. 1987). For over 35 years, NGF has been considered a very powerful and selective growth factor for sympathetic and sensory neurons and for cells derived from the neuronal crest (Alleva et al. 1993; Rush et al. 1997; Cowan 2001). In the CNS, the phenotypic maintenance of cholinergic neurons is dependent on NGF, which is produced mainly in the cortex, the hippocampus, and the pituitary gland, although significant quantities of this NT are also produced in other brain areas, including the basal ganglia, thalamus, hypothalamus, and spinal cord, and in the retina (McAllister 2001).

NGF exerts its biological action by challenging the specific receptor tropomyosin kinase receptor A (TrkA), a typical tyrosine kinase receptor (Huang and Reichardt 2003). The major cytosolic/endosomal pathways activated by TrkA are Ras-mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K)-Akt, and PLC-γ (Klesse and Parada 1999; Chao et al. 2006; Reichardt 2006). NGF also binds to and activates the nonselective p75 pan-neurotrophin receptor ($p75^{NTR}$). This receptor is a transmembrane glycoprotein that regulates signaling through TrkA (Friedman and Greene 1999; Schor 2005; Reichardt 2006); the binding of NGF to $p75^{NTR}$ activates additional signaling pathways that, in the absence of co-expressed TrkA, may signal cell death via apoptosis (Friedman and Greene 1999; Miller and Kaplan 2001; Schor 2005). Signaling pathways activated by $p75^{NTR}$ are the Jun kinase signaling cascade, NF-κB, and ceramide generation (Reichardt 2006).

The pioneering studies of Levi-Montalcini showed that, during development, limited amounts of NGF produced in target organs are responsible for the proliferation, differentiation, and survival of peripheral autonomic and sensory neurons (Levi-Montalcini 1987). Moreover, neutralizing endogenous NGF with anti-NGF antibodies during fetal life causes permanent damage to the sympathetic ganglia and a marked decrease in sensory neuron numbers (Levi-Montalcini 1987). In adult life, while sympathetic neurons continue to depend on NGF for their survival (Ruit et al. 1990), there is a change in the role of NGF in the sensory neurons. NGF is no longer essential for their survival but remains crucial for the maintenance of a differentiated phenotype (McMahon et al. 1995; Verge et al. 1995). In these mature sensory neurons, as well as in sympathetic neurons, NGF dynamically controls neurotransmitter and neuropeptide synthesis. In sympathetic neurons, the levels of norepinephrine are regulated by NGF through the selective induction of tyrosine hydroxylase (TH) (Otten et al. 1977). In dorsal root ganglia (DRG), the expression of neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) in primary sensory neurons is under NGF control (Lindsay and Harmar 1989), and in vivo deprivation of NGF, following nerve transection or anti-NGF treatment, causes a marked decrease in SP and CGRP synthesis (Verge et al. 1995).

The NGF supplied from the innervation field influences the neuronal plasticity that allows the adult nervous system to modify its structure and functions in response to stimuli. Indeed, the constitutive synthesis of NGF in adult tissues correlates with the phenotypic features of PNS neurons, such as innervation density, cell body size, axonal terminal sprouting, dendritic arborization, and induction or inhibition of neuropeptides and neurotransmitters or transmitter-producing enzymes (Otten et al. 1977; Lindsay and Harmar 1989; Mearow and Kril 1995; Verge et al. 1995).

The spectrum of NGF action was once considered limited to functions related to specific CNS and PNS neuronal classes; however, more recent studies have revealed that the effects of NGF also extend to several kinds of nonneuronal cells. Indeed, NGF can influence the functioning of the immune-hematopoietic system (Aloe et al. 1997a; Bonini et al. 1999; Aloe 2001) and the neuro-endocrine system (Aloe et al. 1997a). Thus, the current view is that *NGF can affect the maintenance of a balanced interplay between the nervous*, *endocrine*, *and immune systems* (Alleva et al. 1993; Aloe et al. 1999, 2002) (Fig. 1).

2 NGF and the Immune System

2.1 NGF Influences Development and Differentiation of the Immune System

The release of neuropeptides from sensory nerve endings or of neurotransmitters from autonomic terminals represents a direct means for the nervous system to regulate immune response. Indeed, neurotransmitters and/or neuropeptide receptors are expressed by immune organs and cells, and their challenge has immune-modulatory value (Elenkov 2008; Tausk et al. 2008). NGF regulates neuropeptides and neurotransmitters in peripheral neurons by acting on their promoters or on neurotransmitter-producing enzymes (Otten et al. 1977; Lindsay and Harmar 1989; Verge et al. 1995). Thus, a possible mechanism by which NGF can affect the immune response is through the regulation of neurotransmitter and neuropeptide production. But NGF can also influence immune cell functions directly, as both NGF and NGF receptors are expressed in lymphoid organs and in purified immune cell populations. The expression of NGF receptors has been found in primary (thymus, bursa of Fabricius, bone marrow) and secondary (spleen, tonsils, lymph nodes) lymphoid organs (Laurenzi et al. 1994; Ciriaco et al. 1996; Garcia-Suarez et al. 2001), being raised during lymphocyte precursor migration but declining during postnatal life. NGF is produced in primary lymphoid organs (Laurenzi et al. 1994; Aloe et al. 1997b), and it is possible that localized synthesis of NGF may contribute to create the microenvironment for the differentiation of hematopoietic stem cells (Pezzati et al. 1992; Cattoretti et al. 1993). In vitro NGF stimulates the functional activity of thymic epithelial cells, inducing the expression of adhesion molecules important for thymocyte-thymic epithelia interaction, and upregulates the expression of thymopoietic factors such as IL-7, granulocyte-macrophage colony-stimulating factor (GM-CSF), and stromal cell-derived

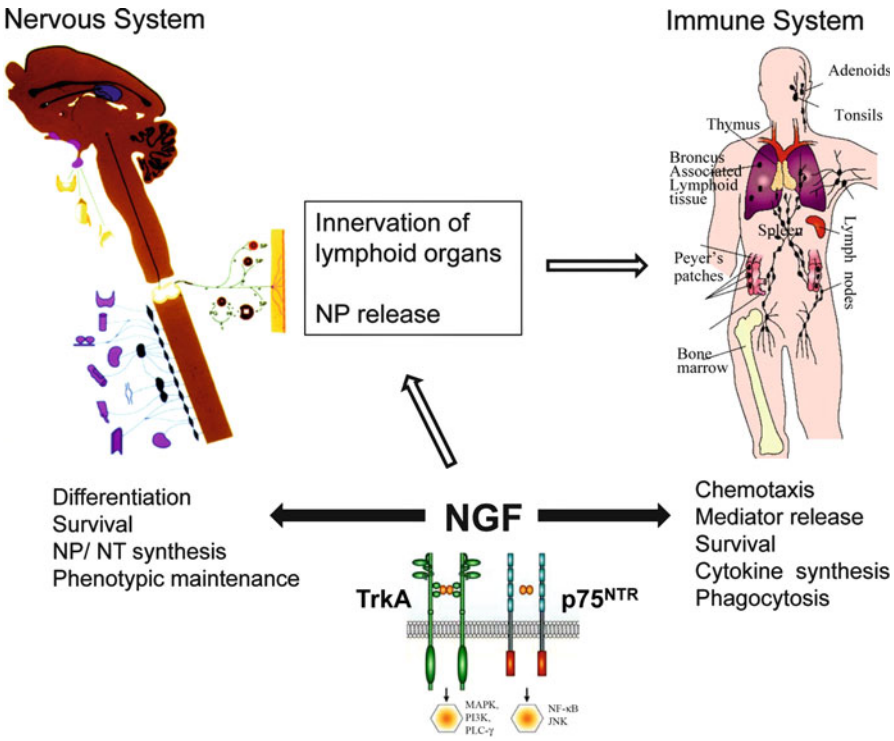


Fig. 1 *Nervous-immune systems interplay*. The NGF is at the interface of the nervous and immune system interactions. By activation of the specific TrkA receptor and/or the pan-neurotrophin p75 receptor, it acts on nervous system (*left side*) regulating survival, differentiation, and phenotypic features such as the synthesis of neurotransmitters and neuropeptides or the shape of cells and terminals. NGF influences immune system development and activity directly, by challenging NGF receptors expressed on the surface of immune cells. NGF also indirectly modulates immune system functions by regulation of the activity of nerve terminals innervating lymphoid organs and of the synthesis and release of neurotransmitters and neuropeptides that in turn activate specific receptors expressed by immune cells/organs. Abbreviations: *NP* neuropeptides, *NT* neurotransmitters, *TrkA* tyrosine kinase A receptor, $p75^{NTR}$ p75 neurotrophin receptor

factor-1 (Lee et al. 2008). TrkA expression has been described in hematopoietic precursors (Chevalier et al. 1994) and NGF functions as a survival factor (Bracci-Laudiero et al. 1993b; Auffray et al. 1996) and a colony-stimulating factor (Matsuda et al. 1988, 1991; Welker et al. 2000) for myeloid progenitors. Human CD34+ hematopoietic stem cells are characterized by the concomitant expression of TrkA and production of their own NGF, which is maintained during lineage differentiation but declines with the acquisition of a mature phenotype (Bracci-Laudiero et al. 2003). Moreover, the changes in TrkA expression observed in immature cord blood cells and mature immune cells may indicate a different requirement for NGF in these cells that depends on the state of maturity and functional activity of the immune cell (Bracci-Laudiero et al. 2003) (Fig. 2).

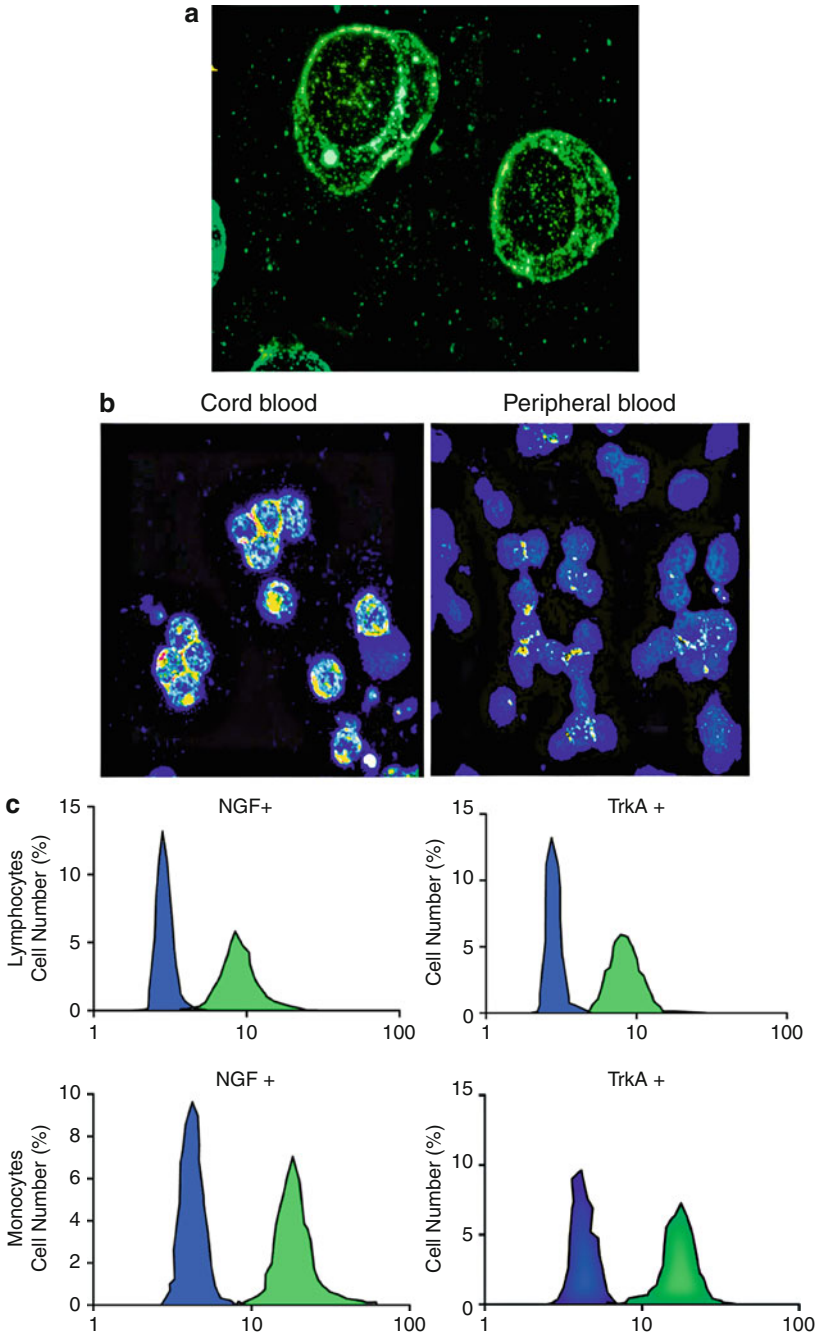


Fig. 2 (continued)

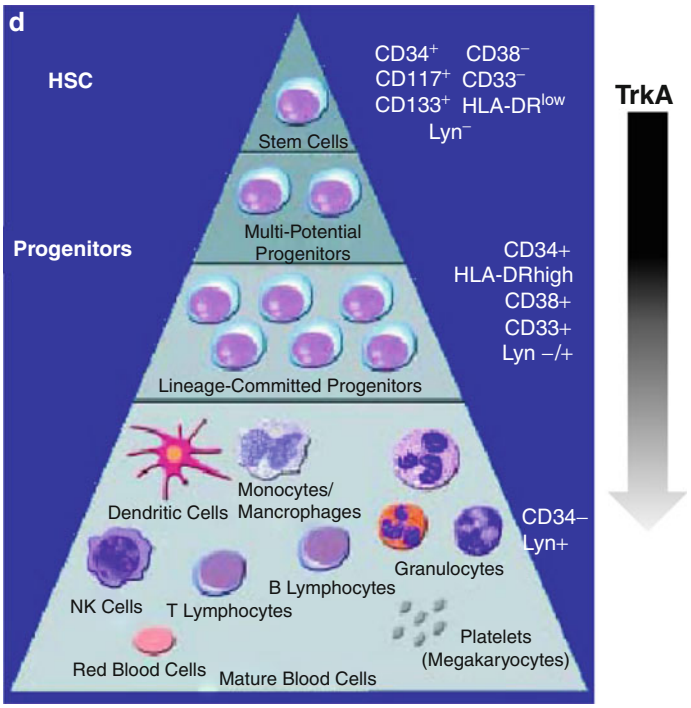


Fig. 2 *NGF influences the differentiation of hemopoietic stem cell*. TrkA (**a**) and NGF (**b**) expression is detected by immunofluorescence in CD34+ stem cells purified from human umbilical cord blood (HCB). Flow cytometry analysis (**c**) reveals that NGF and TrkA are co-expressed in the lymphocytic and monocytic components of HCB. As a clue of the importance of NGF in the development of immune cell lineage, the expression of TrkA is decreasing on immune cell surface as they become more differentiated (**d**)

2.2 NGF and Immune Cells

NGF receptor expression has been studied in purified immune cell populations. TrkA expression has been demonstrated in mononuclear cells (Thorpe et al. 1987a), thymocytes (Laurenzi et al. 1994), human B and T lymphocytes (Brodie and Gelfand 1992; Ehrhard et al. 1993; Torcia et al. 1996; Lambiase et al. 1997), monocytes (Ehrhard et al. 1993; Caroleo et al. 2001), mast cells (Nilsson et al. 1997), basophils (Burgi et al. 1996), and eosinophils (Noga et al. 2002). These cells have a basal expression of TrkA in the resting condition and upregulate it after antigenic or mitogenic stimulation, when functional activation is necessary (Ehrhard et al. 1993a, b; Torcia et al. 1996; Lambiase et al. 1997; Caroleo et al. 2001). The evidence that almost all immune cells express NGF receptors provided great impulse to studies on the influence of NGF on immune functions. The study demonstrating that the injection of NGF in newborn rats caused an increase in the number and size of mast cells (Aloe and Levi-Montalcini 1977) was the earliest

evidence of an effect of NGF on immune cells and outside the nervous system. After this, numerous studies have shown that NGF is able to exert a wide spectrum of effects on immune cells (Table 1). One of the effects of NGF that emerges as common to the greater part of cell populations analyzed is its dose-dependent influence on the survival of hematopoietic stem cells and mature, differentiated immune cells (Auffray et al. 1996; Bullock and Johnson 1996; Torcia et al. 1996; Bracci-Laudiero et al. 2003). The viability-sustaining activity of NGF has been attributed to its effect on the induction of anti-apoptotic proteins such as Bcl-2 (Bullock and Johnson 1996).

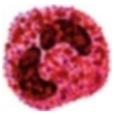
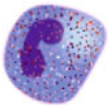
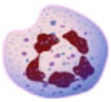
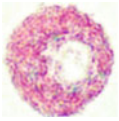
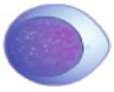
2.2.1 Granulocytes and Mast Cells

In general, in vitro studies of granulocytes and mast cells have shown that *NGF can potentiate their responses to inflammatory stimuli*, but its effects differ depending on the cell type analyzed. In activated basophils, NGF enhances the synthesis of lipid mediators (leukotrienes) in response to inflammatory stimuli (Bischoff and Dahinden 1992; Takafuji et al. 1992) and, together with IL-3 and IL-33, acts as cofactor to induce the release of histamine and enhance the response to IgE in both basophils and mast cells (Mazurek et al. 1986; Horigome et al. 1993; Sin et al. 2001; Gibbs et al. 2005). In contrast to its effect on basophils, NGF tends to suppress leukotriene formation in eosinophils (Takafuji et al. 1992). However, NGF seems to stimulate the production of IL-4 (Noga et al. 2002); it influences the release of peroxidase in eosinophils and enhances cytotoxic activity (Hamada et al. 1996). It also enhances, in a dose-dependent way, the survival of eosinophils (Hamada et al. 1996) and neutrophils (Kannan et al. 1991) by suppressing apoptosis, an effect abolished by anti-NGF antibody treatment. In neutrophils, in addition to its survival effect, NGF enhances superoxide production after cell activation with zymosan and stimulates phagocytosis (Kannan et al. 1991).

As for granulocytes, a pro-survival effect of NGF has also been demonstrated in mast cells (Kawamoto et al. 1995; Bullock and Johnson 1996). NGF also influences mast cell activation in various stages of cell differentiation: it causes a dose-dependent upregulation of tryptase and IgE receptors in immature mast cells (Welker et al. 1998), while the addition of NGF after stimulation of mature mast cells with lysophosphatidylserine induces and enhances cyclooxygenase2 (COX2) expression and the generation of prostaglandin D2 (Murakami et al. 1997).

Several studies have shown that NGF is a chemoattractant factor for both granulocytes (Gee et al. 1983; Boyle et al. 1985) and mast cells (Sawada et al. 2000). The migration of eosinophils towards the site of inflammation in the airways is influenced by the presence of NGF, and this effect seems to be regulated by neuropeptides (Quarcoo et al. 2004). Circulating neutrophils also seem to be involved in NGF-induced hyperalgesia in the skin of rat paw (Bennett et al. 1998). Indeed, local administration of NGF in rat skin induced hyperalgesia and neutrophil accumulation, an effect that appears to be mediated by the induction by NGF of adhesion molecules (i.e., ICAM-1) in endothelial cells (Foster et al. 2003). The use of anti-ICAM-1 antibodies blocked neutrophil accumulation in

Table 1 Summary of main effects of NGF on immune cells

	Cell type	NGF-/TrkA-regulated functions	References
	Hematopoietic stem cell	Survival, differentiation	Matsuda et al. (1991), Bischoff and Dahinden (1992), Takafuji et al. (1992), Cattoretti et al. (1993), Chevalier et al. (1994), Auffray et al. (1996), Bracci-Laudiero et al. (2003), Sato et al. (2004)
	Eosinophils	Differentiation, mediator release	Takafuji et al. (1992), Hamada et al. (1996), Noga et al. (2002), Quarcoo et al. (2004)
	Basophils	Differentiation, mediator release	Bischoff and Dahinden (1992), Takafuji et al. (1992), Burgi et al. (1996), Sin et al. (2001), Gibbs et al. (2005)
	Neutrophils	Chemotaxis, phagocytosis, mediator release, survival	Gee et al. (1983), Boyle et al. (1985), Kannan et al. (1991), Bennett et al. (1998), Beigelman et al. (2009)
	Mast cells	Degranulation, mediator release, survival	Mazurek et al. (1986), Matsuda et al. (1991), Kawamoto et al. (1995), Lambiase et al. (1995), Bullock and Johnson (1996), Lowe et al. (1997), Murakami et al. (1997), Nilsson et al. (1997), Welker et al. (1998), di Mola et al. (2000), Sawada et al. (2000), Welker et al. (2000), Xiang and Nilsson (2000), Stanzel et al. (2008)
	B lymphocytes	Proliferation, differentiation, IgG synthesis, survival, CGRP synthesis	Otten et al. (1989), Kimata et al. (1991), Brodie and Gelfand (1992), Franklin et al. (1995), Brodie et al. (1996), Melamed et al. (1996), Torcia et al. (1996), Bracci-Laudiero et al. (2002), Coppola et al. (2004), Sato et al. (2004), Abram et al. (2009)
	T lymphocytes	Proliferation, NPY synthesis	Thorpe et al. (1987b), Ehrhard et al. (1993), Bracci-Laudiero et al. (1996a), Lambiase et al. (1997)
	Monocytes	Oxidative burst, cytokine release, survival, CGRP release	Ehrhard et al. (1993), Susaki et al. (1996), la Sala et al. (2000), Barouch et al. (2001), Caroleo et al. (2001), Bracci-Laudiero et al. (2002), Bracci-Laudiero et al. (2005), Noga et al. (2007), Samah et al. (2009), Ma et al. (2010)

NGF-treated skin, suggesting that NGF has primarily an activating effect on endothelial cells, resulting in the accumulation of neutrophils (Foster et al. 2003). Abnormally decreased migration of neutrophils has been reported in patients with congenital insensitivity to pain with anhidrosis (CIPA), a rare genetic disease characterized by mutation of the TrkA gene (Beigelman et al. 2009). All these data suggest that the presence of *NGF is pivotal to cell recruitment at the inflammation site*, though the mechanisms and mediators involved still remain to be clarified.

2.2.2 B and T Lymphocytes

Experimental evidence produced in the 1980s indicated that NGF receptors are expressed on lymphocytes and that NGF is able to potentiate the proliferative response of B- and T-cells to mitogens (Thorpe and Perez-Polo 1987; Thorpe et al. 1987a, b). The addition of NGF to culture media induces interleukin-2 receptors in mature lymphoid cells, and the proliferative response is augmented in B-cells when NGF is added together with IL-2 (Brodie and Gelfand 1992). Although CD4+ lymphocytes also express TrkA receptors (Ehrhard et al. 1993a), few data on these cells are available, while more numerous studies have focused on B lymphocytes. B-cells express TrkA (Torcia et al. 1996), and the binding of NGF to this receptor activates intracellular pathways and nuclear factors in a manner similar to that described in neurons (Franklin et al. 1995; Melamed et al. 1996). Abolition of TrkA function in knockout mice models (Coppola et al. 2004) or in CIPA patients (Sato et al. 2004) results in altered differentiation and survival of B lymphocytes. Moreover, NGF can influence the production of IgM and IgG (especially IgG4) (Otten et al. 1989; Kimata et al. 1991). Overall these results support the conclusion that *NGF stimulates B-cell immune responses*.

However, other reports describe evident contradictions to those cited above, with findings showing that NGF is a survival factor for memory B-cells (Torcia et al. 1996), promotes the differentiation of B-cells into immunoglobulin-secreting plasma cells (Otten et al. 1989), and influences plasma cell survival (Abram et al. 2009). Activation of B-cells with anti-CD40 plus NGF decreases instead of increasing immunoglobulin secretion in a dose-dependent manner (Brodie and Gelfand 1994; Brodie et al. 1996). Moreover, the finding that TrkA knockout mice have increased IgM, IgG1, and IgG2 levels (Coppola et al. 2004) suggests that a clear understanding of the physiological effects of NGF on B-cells is still lacking.

2.2.3 Monocytes/Macrophages and Dendritic Cells

Monocytes and macrophages express TrkA and $p75^{NTR}$, while differentiated dendritic cells lose TrkA and maintain $p75^{NTR}$ expression only (Ehrhard et al. 1993b; la Sala et al. 2000; Caroleo et al. 2001). Inflammatory stimuli enhance (Ehrhard et al. 1993b; Caroleo et al. 2001) or induce a reexpression (Noga et al. 2007) of TrkA in all these cells. NGF protects monocytes from apoptosis

induced by UVB radiation or gliotoxin, regulating the expression of anti-apoptotic proteins Bcl-2, Bcl-xl, and Bfl-1 (la Sala et al. 2000). In vitro migratory properties are also affected by NGF, which increases CXCR4 expression and chemotactic response to suboptimal CXCL-12 concentrations in macrophages (Samah et al. 2009). Inflammatory macrophages isolated from the peritoneum after stimulation and then treated with NGF show increased phagocytosis, enhanced parasite-killing activity, and increased production of IL-1beta (Susaki et al. 1996). NGF can also induce TNF-alpha secretion and increase nitric oxide (NO) production in macrophages, this latter effect being strongly amplified by pretreatment with interferon-gamma (Barouch et al. 2001). NGF promotes LPS-induced maturation and secretion of inflammatory cytokines in dendritic cells (Noga et al. 2007). Dendritic cells derived from allergic donors and stimulated with LPS and CD40 ligand increase IL-6 production when challenged with NGF, while the effect of NGF on dendritic cells generated from healthy donors is to induce more IL-10 production (Noga et al. 2007). Consistent with this finding is the fact that NGF deprivation in LPS-treated monocytes results in a significant decrease in IL-10 synthesis (Bracci-Laudiero et al. 2005).

2.3 NGF Induces Neuropeptide Production in Immune Cells

Besides its ability to induce inflammatory mediators and cytokines, NGF can also modulate immune response by regulating the synthesis of neuropeptides in immune cells. Similarly to its effect on neuronal cells, NGF induces NPY expression in T lymphocytes (Bracci-Laudiero et al. 1996a) and regulates the expression and release of CGRP in human B lymphocytes (Bracci-Laudiero et al. 2002) and monocytes (Bracci-Laudiero et al. 2005; Ma et al. 2010) and macrophages. By inducing CGRP and IL-10 production, NGF can reduce the antigen-presenting capacity and co-stimulatory function of monocytes and may contribute to the downregulation of T-cell responses. Indeed, using CGRP receptor antagonists in LPS-treated monocytes, the expression of membrane molecules involved in antigen presentation, such as HLA-DR and CD86, is increased (Bracci-Laudiero et al. 2005). These data suggest that at least some of the NGF effects described to date are not directly dependent on NGF itself but rather are regulated by intermediate mediators whose synthesis is under NGF control. Neuropeptides, such as SP, CGRP, NPY, and VIP, have all been indicated as possible mediators of NGF effects on immune system response, in regulating inflammatory and anti-inflammatory responses and in maintaining homeostasis (Brain 1997; Delgado et al. 2004; O'Connor et al. 2004; Prod'homme et al. 2006). The point that deserves underlining is that *NGF can act on immune cells*, *stimulating neuropeptide synthesis in both the neuronal and immune systems* (Fig. 3).

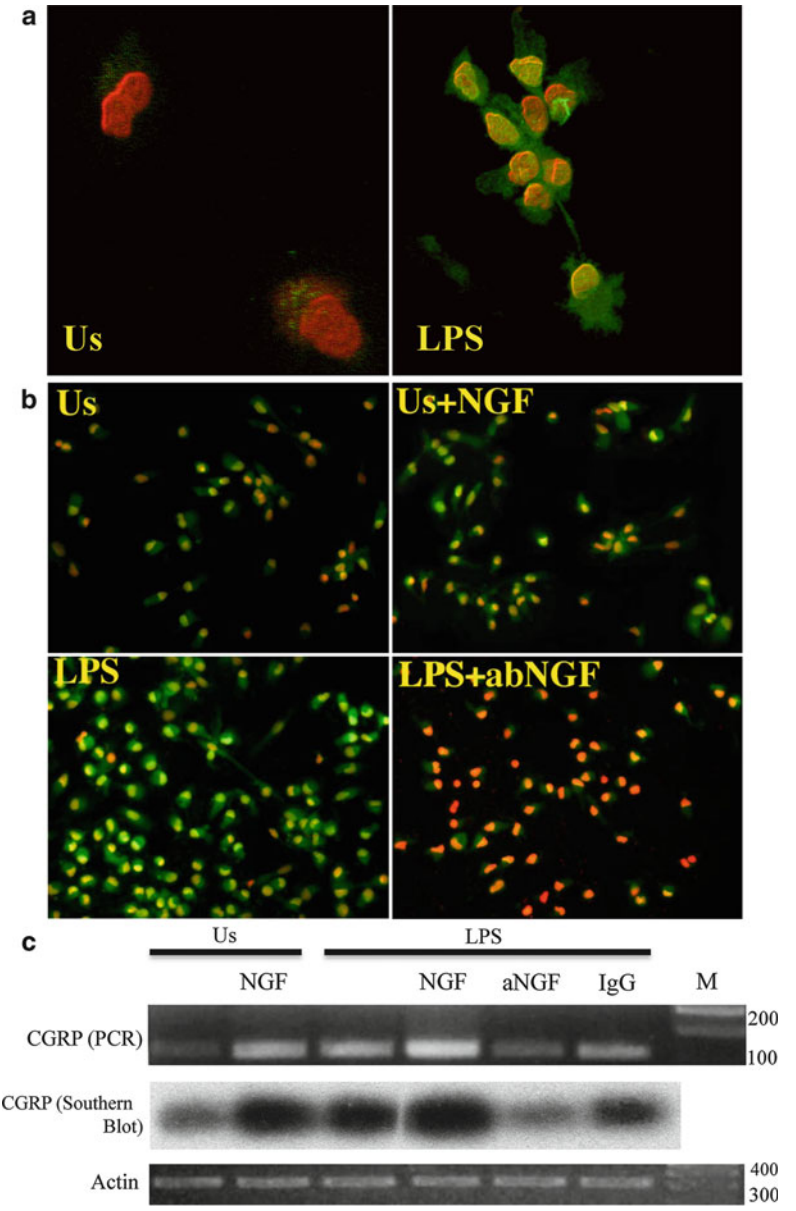


Fig. 3 *NGF induces CGRP in cultured macrophages*. Purified macrophages express calcitonin gene-related peptide (CGRP), as revealed by green fluorescence, if challenged with lipopolysaccharide (LPS) (**a**). Neutralizing NGF by specific anti-NGF antibody counteracted the CGRP expression in macrophages activated by exposure to LPS (**b**). Gene expression analysis (**c**) by PCR and Southern blot reveals that both unstimulated (Us) and LPS-challenged macrophages increase CGRP gene expression when exposed to NGF in culture, while anti-NGF decreases CGRP mRNA in stimulated cells

3 NGF and Inflammation

3.1 In Vivo Evidence and Experimental Models

An increase in NGF in tissues at the site of inflammation is a characteristic of several inflammatory diseases. Increased NGF concentrations, which closely follow the course of the disease, were initially found in the cerebrospinal fluid of multiple sclerosis patients (Bracci-Laudiero et al. 1992), in the synovial fluids of rheumatoid arthritis patients (Aloe et al. 1992), and in the sera of systemic lupus erythematosus (SLE) patients (Bracci-Laudiero et al. 1993a; Aalto et al. 2002). NGF mRNA expression and protein levels increase in animal models of inflammation such as Freund's adjuvant-induced and carrageenan-induced arthritis (Safieh-Garabedian et al. 1995; Manni and Aloe 1998), in a model of human SLE (Bracci-Laudiero et al. 1996b), and in experimental autoimmune encephalomyelitis (EAE) (De Simone et al. 1996). Many other inflammatory diseases, such as interstitial cystitis (Lowe et al. 1997; Jacobs et al. 2010), allergic asthma (Bonini et al. 1996; Braun et al. 1998), vernal keratoconjunctivitis (Lambiase et al. 1995), Crohn's disease and colitis (di Mola et al. 2000; Stanzel et al. 2008), psoriasis (Fantini et al. 1995; Raychaudhuri et al. 1998), and atopic dermatitis (Toyoda et al. 2002), are characterized by increased tissue and/or circulating NGF. Taken together, these data suggested that inflammation and tissue damage generate mediators that control the local concentration of NGF. Inflammatory cytokines such as IL-1beta, TNF-alpha, and IL-6 are able to modify the basal production of NGF in the organism and induce the synthesis of NGF in a variety of cell types and tissues (Lindholm et al. 1987; Friedman et al. 1990; Gadient et al. 1990; Steiner et al. 1991; Hattori et al. 1994; Kossmann et al. 1996; Marz et al. 1999; Olgart and Frossard 2001; Manni et al. 2003; Moser et al. 2004; Ryan et al. 2008; Seidel et al. 2010). Other inflammatory mediators such as prostaglandins and histamine have also been shown to induce NGF production in certain cell types (Lipnik-Stangelj and Carman-Krzan 2004a, b; Toyomoto et al. 2004; Bullo et al. 2005). In addition, stimulation with specific antigens and cytokines of immune cells involved in innate and acquired immunity modulates their basal expression of NGF (Torcia et al. 1996; Lambiase et al. 1997; Xiang and Nilsson 2000; Caroleo et al. 2001; Kobayashi et al. 2002). Activation of the NF-κB pathway seems to be essential in regulating NGF expression in B lymphocytes (Heese et al. 2006). The endogenous levels of NGF are substantially increased in inflamed tissues, dynamically regulating innervations and neural activities during the inflammatory process (Carr and Undem 2001). During inflammation, the neuronal phenotype can be modified, altering the amount or type of neurotransmitters produced and stored in nerve terminals. As a result, pain sensitivity is increased during inflammation: the properties of the somatosensory neurons change so that the pain threshold decreases, noxious stimuli evoke more acute and prolonged pain (hyperalgesia), and normally innocuous stimuli became painful (allodynia) (Woolf and Salter 2000). This peripheral sensitization increases the firing of nociceptors, and the intense stimulation induces

the release from nerve endings of neuropeptides that can influence a variety of immune cell functions. One of the best-known neuroimmune mechanisms involving sensory neuropeptides is neurogenic inflammation: this is characterized by a release of neuropeptides by sensory nerves that in turn induces vasodilatation and plasma extravasation, promotes leukocyte chemotaxis and phagocytosis (Zegarska et al. 2006), and directly affects the release of inflammatory mediators from mast cells and macrophages (Tore and Tuncel 2009). In the mucosae and in lymphoid organs, sensory fibers are in close contact with the cell membranes of mast cells, macrophages, Langerhans cells, and endothelial cells in what has been defined a "neuroimmune junction" (Straub 2004). Neuropeptides released from nerve terminals induce synthesis of inflammatory mediators and cytokines in these cells and affect their functions. While SP has a demonstrated pro-inflammatory role (O'Connor et al. 2004), CGRP, which is often co-localized with SP in nerve endings, can also have an inhibitory action. CGRP is a potent inhibitor of mitogen- and antigen-stimulated proliferation of T-cells (Carucci et al. 2000), antigen presentation by antigen-presenting cells (Hosoi et al. 1993), and cytokine synthesis (Gomes et al. 2005). The localized increased production of NGF in inflammatory conditions induces modification of gene expression in dorsal root ganglia that can be prevented using anti-NGF antibodies (Donnerer et al. 1992). The neuronal phenotypic changes driven by NGF during inflammation include elevation of neuropeptides, which amplify sensory input signals in the spinal cord and augment neurogenic inflammation in the peripheral tissues.

3.2 In Vivo Anti-inflammatory Action

The previously cited evidence indicates that *NGF maintains immune cells in a* "*state of alert*" to potential danger signals. NGF by itself does not seem to induce inflammatory and immune responses efficiently, but, in the presence of specific stimuli, it enhances mediator release and cytokine production and activates the functions of innate immune cells. In other words, *NGF helps to mount a faster and stronger inflammatory response*. However, at present, some of the data obtained in vivo are not consistent with this conclusion. The reasons why NGF production is increased during inflammation and how this enhanced concentration affects the immune and inflammatory responses are still far from being understood. The notion that NGF plays a much more complex role in the scenario of immune response has emerged from studies on experimental models of EAE. In marmoset EAE, intracerebroventricular administration of NGF delays the onset of clinical signs and prevents the full development of histological lesions in the CNS (Villoslada et al. 2000). NGF reduces the number and size of inflammatory infiltrates, and demyelination is minimal in the brain of NGF-treated animals (Villoslada et al. 2000). Moreover, the production of interferon-gamma by the T-cells infiltrating the brain is reduced, while production of IL-10 is enhanced in NGF-treated animals (Villoslada et al. 2000). In a murine model of EAE, intraperitoneal injections of NGF starting one day after immunization with myelin basic

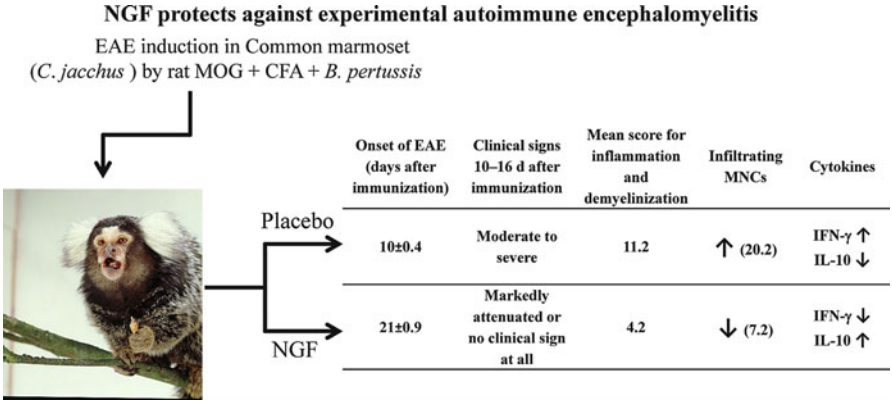


Fig. 4 *NGF protects common marmosets against autoimmune encephalomyelitis*. The figure is a summary of results published by Villoslada and collaborators (Villoslada et al. 2000). In this paper, the authors demonstrated that intracerebroventricular (icv) NGF was able to revert clinical manifestations of the experimental autoimmune encephalomyelitis (EAE), an animal model for human multiple sclerosis. In such a model, the immunization with myelin oligodendrocyte glycoprotein (MOG) induces a massive autoimmune response in the central nervous system, characterized by infiltration of activated T-cells and activation of glial cells. Brain-delivered NGF was able to delay the onset of clinical signs and prevent the full development of histological lesions, reducing the number and size of inflammatory infiltrates and the demyelination. Moreover, the production of interferon-gamma by the T-cells infiltrating the brain was reduced, while production of IL-10 by glial cells was enhanced in NGF-treated animals

protein (MBP) or after adoptive transfer of encephalitogenic T-cells led to a delayed onset, decreased clinical signs of the disease, and enhanced survival of the animals (Arredondo et al. 2001). In a T-cell transfer model of EAE, myelin basic protein-specific CD4+ T-cell clones were transfected with recombinant retrovirus encoding for NGF and used to induce EAE in rats (Flugel et al. 2001). The clones producing NGF did not induce EAE, while the pathogenic wild-type clones injected together with the NGF-transfected clones induced a very mild disease with no or only minor clinical signs (Flugel et al. 2001). A reduction in inflammatory cells crossing the endothelial blood–brain barrier (BBB), with the number of activated macrophages being especially decreased, was also found (Flugel et al. 2001). Complementary effects are observed in another EAE rat model, in which NGF deprivation results in exacerbated brain inflammation and more severe clinical signs (Micera et al. 2000). Thus, the overall data obtained from EAE models point to an anti-inflammatory role of NGF in CNS immune-mediated inflammation. NGF seems to exert its effects directly on immune cell activity: inhibition of monocyte migration and antigen presentation, altered T-helper balance, downregulation of IFN-gamma synthesis, and upregulation of the anti-inflammatory cytokine IL-10 (Fig. 4). Similarly, in experimental models of colitis, it has been shown that NGF production remains elevated in the tissue until colon inflammation subsides (Barada et al. 2007), while neutralizing NGF by anti-NGF antibodies causes a significant increase in the severity of clinical signs with more

extended lesions and ulcers, together with an increase in the number of infiltrating neutrophils and macrophages (Reinshagen et al. 2000). Also in this experimental model, the increase in NGF correlates with the enhancement of IL-10 (Barada et al. 2007), possibly because NGF and IL-10 are able to regulate each other's expression in intestinal epithelial cells (Ma et al. 2003). In addition, functional sensory neurons and their neuropeptides (i.e., CGRP) are probably involved in the in vivo action of NGF. In the colitis model, enhanced tissue inflammation caused by NGF neutralization is associated with a marked reduction in CGRP content in the gut (Reinshagen et al. 1994). Moreover, the ablation of sensory fibers results in a marked severity of inflammation in acute and chronic models of experimental colitis, and in both cases the decrease in CGRP content or its inhibition in the colon using CGRP receptor antagonist aggravates experimental colitis (Reinshagen et al. 1998).

The NGF produced in vivo *at the site of inflammation thus represents a critical link between nervous and immune cells*. This hypothesis is supported by studies on a mouse model in which the animal's skin received ultraviolet B (UVB) irradiation. UVB induces systemic suppression of contact hypersensitivity (CHS) responses, and NGF, together with nerves and neuropeptides, seems to play a relevant role in this phenomenon. In mouse epidermis, UVB irradiation upregulates NGF expression, which peaks after 12 h (Gillardon et al. 1995). Anti-NGF antibodies administered prior to UVB irradiation abrogate the systemic suppression of CHS in mice (Townley et al. 2002). The CHS response in healthy mice was also inhibited by NGF treatment of the skin prior to trinitrochlorobenzene sensitization (Townley et al. 2002). Moreover, when mice were treated with capsaicin, which depletes nerve endings of sensory neuropeptides, preincubation with NGF was not effective in suppressing CHS response (Townley et al. 2002). Also in this model, *NGF-induced synthesis of CGRP seems to represent a key mediator for the control of immune cell activity and for balancing pro- and anti-inflammatory responses*. In UV-induced tolerance, the induction of CGRP synthesis in skin after UV radiation (Legat et al. 2004) is regulated by keratinocyte-derived NGF (Townley et al. 2002). In vivo CGRP administration dampens the immune response essentially by affecting antigen presentation in a variety of antigen-presenting cells (Raud et al. 1991; Hosoi et al. 1993; Asahina et al. 1995; Carucci et al. 2000), through inhibition of the NF-kappaB pathway (Millet et al. 2000) and a rapid upregulation of inducible cAMP early repressor (Harzenetter et al. 2007), which causes premature repression of inflammation-induced transcriptional activity. Thus, the ability of NGF in vivo to regulate production and release of sensory neuropeptides seems to be a key factor not only in *keeping the defense system active* (i.e., through SP) but also in *activating inhibitory pathways that limit inflammation*.

Data from in vivo models of inflammation show that integrity of the peripheral nervous system is essential for the activation of an anti-inflammatory feedback circuit (Rook et al. 2002; Holzer 2007; Dirmeier et al. 2008; Boisse et al. 2009; Kroeger et al. 2009). Cutaneous infections and altered immune responses in diabetes represent a characteristic example of nerve damage leading to uncontrolled

tissue inflammation. Impaired production of NGF is responsible for the reduction in skin innervations in diabetes (Hellweg and Hartung 1990; Anand 1996). In animal models, the exogenous administration of NGF, while improving nerve functions and restoring innervation in the skin (Christianson et al. 2003), normalizes the diabetes-impaired response to wound healing by decreasing neutrophil accumulation and increasing reepithelialization and matrix density (Matsuda et al. 1998).

4 Conclusion

Since its discovery in the 1950s, NGF has been extensively studied for its trophic, pro-survival, and differentiative effects in both neuronal and nonneuronal cell populations. Several studies have shown that the neurotrophin can display contradictory behavior, acting either as a survival or as an apoptotic factor in neuronal cells (Lee et al. 2001). In the last decade, the apparently controversial effects of NGF on neuronal cells have been better elucidated, in light of the discovery and characterization of the precursor form of NGF, named proNGF. ProNGF can be released by cells and cleaved to form mature NGF (Bruno and Cuello 2006) or can act as an active molecule, being a specific and selective ligand for a receptor complex made by $p75^{NTR}$ and sortilin (Fahnestock et al. 2004), a protein that shuttles other proteins within cells and acts as a receptor for neurotensin (Mazella 2001). In several injury models in the central nervous system, proNGF expression is detectable for several days to weeks following injury. Surprisingly, little conversion of proNGF to mature NGF is observed in these in vivo settings (Beattie et al. 2002; Harrington et al. 2004; Jansen et al. 2007). This finding suggests that the mature and pro-forms of NGF may perform opposing actions. Expression of the $p75^{NTR}$ receptor has emerged as a key regulatory element in proNGF-induced cell death. In most adult tissues, $p75^{NTR}$ is expressed at low levels, in contrast to its stronger and more widespread distribution in development (Yang et al. 2009; Roux and Barker 2002). However, in pathologic states, including seizure, brain injury, ischemia, and excitotoxicity, $p75^{NTR}$ expression is induced. The significant reduction in injury-induced apoptosis observed in $p75^{NTR}$-deficient mice (Troy et al. 2002; Harrington et al. 2004) clearly indicates the importance of $p75^{NTR}$ induction in determining cell loss following injury.

Until now, very few studies have addressed the function of proNGF in the immune system, but this promises to be an exciting field of research for the near future. Only very recently was the action of proNGF on immune cells addressed in a work investigating the effects of $p75^{NTR}$/sortilin activation in NK cells in vitro (Rogers et al. 2010). Freshly isolated NK and B-cells, but not T lymphocytes, expressed significant amounts of sortilin but did not express significant amounts of $p75^{NTR}$ in unstimulated conditions. The expression of $p75^{NTR}$ is induced in NK cells by IL-12. Addition of proNGF in the presence of IL-12 induced NK cell death (Rogers et al. 2010). Immune cells, such as mast cells, have been indicated as a possible source of proNGF. Indeed, proNGF

immunoreactivity was increased in skin-homed mast cells in an in vivo model of neuropathic pain (Peleshok and Ribeiro-da-Silva 2012). Mast cells may also have a role as key players in the extracellular maturation of NGF from its precursor. Tryptase-secreting mast cells have been detected in close proximity to NGF-secreting cells in the testis of infertile patients, and it is likely that they are able to alter the ratios of proNGF/NGF by increasing tryptase enzymatic cleavage of proNGF (Spinnler et al. 2011). There is also evidence that neuroimmune modulators may be involved in regulating the proNGF/NGF balance. In an in vitro model of inflamed skin, the addition of neuropeptides such as SP, CGRP, VIP, and GAL to normal human keratinocytes results in an increased expression of IL-1β, IL-8, and TNF-α; release of proNGF; and its increased conversion into mature NGF in the culture medium (Dallos et al. 2006).

In view of the apparent discrepancies in the effects of NGF on immune functions, it is to be hoped that future studies of NGF and proNGF will help to clarify the role of this neurotrophin in regulating immune response and inflammation. A better understanding of proNGF/NGF actions could be of immense value, with translational implications for human diseases.

References

Aalto, K., Korhonen, L., Lahdenne, P., Pelkonen, P., & Lindholm, D. (2002). Nerve growth factor in serum of children with systemic lupus erythematosus is correlated with disease activity. *Cytokine, 20*(3), 136–139.

Abram, M., Wegmann, M., Fokuhl, V., Sonar, S., Luger, E. O., Kerzel, S., Radbruch, A., Renz, H., & Zemlin, M. (2009). Nerve growth factor and neurotrophin-3 mediate survival of pulmonary plasma cells during the allergic airway inflammation. *Journal of Immunology, 182*(8), 4705–4712.

Alleva, E., Aloe, L., & Bigi, S. (1993). An updated role for nerve growth factor in neurobehavioural regulation of adult vertebrates. *Reviews in the Neurosciences, 4*(1), 41–62.

Aloe, L. (2001). Nerve growth factor and neuroimmune responses: Basic and clinical observations. *Archives of Physiology and Biochemistry, 109*(4), 354–356.

Aloe, L., & Levi-Montalcini, R. (1977). Mast cells increase in tissues of neonatal rats injected with the nerve growth factor. *Brain Research, 133*(2), 358–366.

Aloe, L., Tuveri, M. A., Carcassi, U., & Levi-Montalcini, R. (1992). Nerve growth factor in the synovial fluid of patients with chronic arthritis. *Arthritis and Rheumatism, 35*(3), 351–355.

Aloe, L., Bracci-Laudiero, L., Bonini, S., & Manni, L. (1997a). The expanding role of nerve growth factor: From neurotrophic activity to immunologic diseases. *Allergy, 52*(9), 883–894.

Aloe, L., Micera, A., Bracci-Laudiero, L., Vigneti, E., & Turrini, P. (1997b). Presence of nerve growth factor in the thymus of prenatal, postnatal and pregnant rats. *Thymus, 24*(4), 221–231.

Aloe, L., Simone, M. D., & Properzi, F. (1999). Nerve growth factor: A neurotrophin with activity on cells of the immune system. *Microscopy Research and Technique, 45*(4–5), 285–291.

Aloe, L., Alleva, E., & Fiore, M. (2002). Stress and nerve growth factor: Findings in animal models and humans. *Pharmacology, Biochemistry, and Behavior, 73*(1), 159–166.

Anand, P. (1996). Neurotrophins and peripheral neuropathy. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 351*(1338), 449–454.

Arredondo, L. R., Deng, C., Ratts, R. B., Lovett-Racke, A. E., Holtzman, D. M., & Racke, M. K. (2001). Role of nerve growth factor in experimental autoimmune encephalomyelitis. *European Journal of Immunology, 31*(2), 625–633.

Asahina, A., Hosoi, J., Murphy, G. F., & Granstein, R. D. (1995). Calcitonin gene-related peptide modulates Langerhans cell antigen-presenting function. *Proceedings of the Association of American Physicians, 107*(2), 242–244.

Auffray, I., Chevalier, S., Froger, J., Izac, B., Vainchenker, W., Gascan, H., & Coulombel, L. (1996). Nerve growth factor is involved in the supportive effect by bone marrow–derived stromal cells of the factor-dependent human cell line UT-7. *Blood, 88*(5), 1608–1618.

Barada, K. A., Mourad, F. H., Sawah, S. I., Khoury, C., Safieh-Garabedian, B., Nassar, C. F., Tawil, A., Jurjus, A., & Saade, N. E. (2007). Up-regulation of nerve growth factor and interleukin-10 in inflamed and non-inflamed intestinal segments in rats with experimental colitis. *Cytokine, 37*(3), 236–245.

Barouch, R., Kazimirsky, G., Appel, E., & Brodie, C. (2001). Nerve growth factor regulates TNF-alpha production in mouse macrophages via MAP kinase activation. *Journal of Leukocyte Biology, 69*(6), 1019–1026.

Beattie, M. S., Harrington, A. W., Lee, R., Kim, J. Y., Boyce, S. L., Longo, F. M., Bresnahan, J. C., Hempstead, B. L., & Yoon, S. O. (2002). ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron, 36*, 375–386.

Beigelman, A., Levy, J., Hadad, N., Pinsk, V., Haim, A., Fruchtman, Y., & Levy, R. (2009). Abnormal neutrophil chemotactic activity in children with congenital insensitivity to pain with anhidrosis (CIPA): The role of nerve growth factor. *Clinical Immunology, 130*(3), 365–372.

Bennett, G., al-Rashed, S., Hoult, J. R., & Brain, S. D. (1998). Nerve growth factor induced hyperalgesia in the rat hind paw is dependent on circulating neutrophils. *Pain, 77*(3), 315–322.

Bischoff, S. C., & Dahinden, C. A. (1992). Effect of nerve growth factor on the release of inflammatory mediators by mature human basophils. *Blood, 79*(10), 2662–2669.

Boisse, L., Chisholm, S. P., Lukewich, M. K., & Lomax, A. E. (2009). Clinical and experimental evidence of sympathetic neural dysfunction during inflammatory bowel disease. *Clinical and Experimental Pharmacology & Physiology, 36*(10), 1026–1033.

Bonini, S., Lambiase, A., Angelucci, F., Magrini, L., Manni, L., & Aloe, L. (1996). Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proceedings of the National Academy of Sciences of the United States of America, 93*(20), 10955–10960.

Bonini, S., Lambiase, A., Bonini, S., Levi-Schaffer, F., & Aloe, L. (1999). Nerve growth factor: An important molecule in allergic inflammation and tissue remodelling. *International Archives of Allergy and Immunology, 118*(2–4), 159–162.

Boyle, M. D., Lawman, M. J., Gee, A. P., & Young, M. (1985). Nerve growth factor: A chemotactic factor for polymorphonuclear leukocytes in vivo. *Journal of Immunology, 134*(1), 564–568.

Bracci-Laudiero, L., Aloe, L., Levi-Montalcini, R., Buttinelli, C., Schilter, D., Gillessen, S., & Otten, U. (1992). Multiple sclerosis patients express increased levels of beta-nerve growth factor in cerebrospinal fluid. *Neuroscience Letters, 147*(1), 9–12.

Bracci-Laudiero, L., Aloe, L., Levi-Montalcini, R., Galeazzi, M., Schilter, D., Scully, J. L., & Otten, U. (1993a). Increased levels of NGF in sera of systemic lupus erythematosus patients. *Neuroreport, 4*(5), 563–565.

Bracci-Laudiero, L., Vigneti, E., Iannicola, C., & Aloe, L. (1993b). NGF retards apoptosis in chick embryo bursal cell in vitro. *Differentiation, 53*(2), 61–66.

Bracci-Laudiero, L., Aloe, L., Stenfors, C., Tirassa, P., Theodorsson, E., & Lundberg, T. (1996a). Nerve growth factor stimulates production of neuropeptide Y in human lymphocytes. *Neuroreport, 7*(2), 485–488.

Bracci-Laudiero, L., Lundeberg, T., Stenfors, C., Theodorsson, E., Tirassa, P., & Aloe, L. (1996b). Modification of lymphoid and brain nerve growth factor levels in systemic lupus erythematosus mice. *Neuroscience Letters, 204*(1–2), 13–16.

Bracci-Laudiero, L., Aloe, L., Buanne, P., Finn, A., Stenfors, C., Vigneti, E., Theodorsson, E., & Lundeberg, T. (2002). NGF modulates CGRP synthesis in human B-lymphocytes:

A possible anti-inflammatory action of NGF? *Journal of Neuroimmunology, 123*(1–2), 58–65.

Bracci-Laudiero, L., Celestino, D., Starace, G., Antonelli, A., Lambiase, A., Procoli, A., Rumi, C., Lai, M., Picardi, A., Ballatore, G., Bonini, S., & Aloe, L. (2003). CD34-positive cells in human umbilical cord blood express nerve growth factor and its specific receptor TrkA. *Journal of Neuroimmunology, 136*(1–2), 130–139.

Bracci-Laudiero, L., Aloe, L., Caroleo, M. C., Buanne, P., Costa, N., Starace, G., & Lundeberg, T. (2005). Endogenous NGF regulates CGRP expression in human monocytes, and affects HLA-DR and CD86 expression and IL-10 production. *Blood, 106*(10), 3507–3514.

Brain, S. D. (1997). Sensory neuropeptides: Their role in inflammation and wound healing. *Immunopharmacology, 37*(2–3), 133–152.

Braun, A., Appel, E., Baruch, R., Herz, U., Botchkarev, V., Paus, R., Brodie, C., & Renz, H. (1998). Role of nerve growth factor in a mouse model of allergic airway inflammation and asthma. *European Journal of Immunology, 28*(10), 3240–3251.

Brodie, C., & Gelfand, E. W. (1992). Functional nerve growth factor receptors on human B lymphocytes. Interaction with IL-2. *Journal of Immunology, 148*(11), 3492–3497.

Brodie, C., & Gelfand, E. W. (1994). Regulation of immunoglobulin production by nerve growth factor: Comparison with anti-CD40. *Journal of Neuroimmunology, 52*(1), 87–96.

Brodie, C., Oshiba, A., Renz, H., Bradley, K., & Gelfand, E. W. (1996). Nerve growth-factor and anti-CD40 provide opposite signals for the production of IgE in interleukin-4-treated lymphocytes. *European Journal of Immunology, 26*(1), 171–178.

Bruno, M. A., & Cuello, A. C. (2006). Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor, and its degradation by a protease cascade. *Proceedings of the National Academy of Sciences of the United States of America, 103*(17), 6735–6740.

Bullo, M., Peeraully, M. R., & Trayhurn, P. (2005). Stimulation of NGF expression and secretion in 3T3-L1 adipocytes by prostaglandins PGD2, PGJ2, and Delta12-PGJ2. *American Journal of Physiology. Endocrinology and Metabolism, 289*(1), E62–E67.

Bullock, E. D., & Johnson, E. M. (1996). Nerve growth factor induces the expression of certain cytokine genes and bcl-2 in mast cells. Potential role in survival promotion. *Journal of Biological Chemistry, 271*(44), 27500–27508.

Burgi, B., Otten, U. H., Ochensberger, B., Rihs, S., Heese, K., Ehrhard, P. B., Ibanez, C. F., & Dahinden, C. A. (1996). Basophil priming by neurotrophic factors. Activation through the trk receptor. *Journal of Immunology, 157*(12), 5582–5588.

Caroleo, M. C., Costa, N., Bracci-Laudiero, L., & Aloe, L. (2001). Human monocyte/macrophages activate by exposure to LPS overexpress NGF and NGF receptors. *Journal of Neuroimmunology, 113*(2), 193–201.

Carr, M. J., & Undem, B. J. (2001). Inflammation-induced plasticity of the afferent innervation of the airways. *Environmental Health Perspectives, 109*(Suppl 4), 567–571.

Carucci, J. A., Ignatius, R., Wei, Y., Cypess, A. M., Schaer, D. A., Pope, M., Steinman, R. M., & Mojsov, S. (2000). Calcitonin gene-related peptide decreases expression of HLA-DR and CD86 by human dendritic cells and dampens dendritic cell-driven T cell-proliferative responses via the type I calcitonin gene-related peptide receptor. *Journal of Immunology, 164*(7), 3494–3499.

Cattoretti, G., Schiro, R., Orazi, A., Soligo, D., & Colombo, M. P. (1993). Bone marrow stroma in humans: Anti-nerve growth factor receptor antibodies selectively stain reticular cells in vivo and in vitro. *Blood, 81*(7), 1726–1738.

Chao, M. V., Rajagopal, R., & Lee, F. S. (2006). Neurotrophin signalling in health and disease. *Clinical Science (London, England), 110*(2), 167–173.

Chevalier, S., Praloran, V., Smith, C., MacGrogan, D., Ip, N. Y., Yancopoulos, G. D., Brachet, P., Pouplard, A., & Gascan, H. (1994). Expression and functionality of the trkA proto-oncogene product/NGF receptor in undifferentiated hematopoietic cells. *Blood, 83*(6), 1479–1485.

Christianson, J. A., Riekhof, J. T., & Wright, D. E. (2003). Restorative effects of neurotrophin treatment on diabetes-induced cutaneous axon loss in mice. *Experimental Neurology, 179*(2), 188–199.

Ciriaco, E., Dall'Aglio, C., Hannestad, J., Huerta, J. J., Laura, R., Germana, G., & Vega, J. A. (1996). Localization of Trk neurotrophin receptor-like proteins in avian primary lymphoid organs (thymus and bursa of Fabricius). *Journal of Neuroimmunology, 69* (1–2), 73–83.

Connor, B., & Dragunow, M. (1998). The role of neuronal growth factors in neurodegenerative disorders of the human brain. *Brain Research. Brain Research Reviews, 27*(1), 1–39.

Coppola, V., Barrick, C. A., Southon, E. A., Celeste, A., Wang, K., Chen, B., Haddad el, B., Yin, J., Nussenzweig, A., Subramaniam, A., & Tessarollo, L. (2004). Ablation of TrkA function in the immune system causes B cell abnormalities. *Development, 131*(20), 5185–5195.

Cowan, W. M. (2001). Viktor Hamburger and Rita Levi-Montalcini: The path to the discovery of nerve growth factor. *Annual Review of Neuroscience, 24*, 551–600.

Dallos, A., Kiss, M., Polyanka, H., Dobozy, A., Kemeny, L., & Husz, S. (2006). Effects of the neuropeptides substance P, calcitonin gene-related peptide, vasoactive intestinal polypeptide and galanin on the production of nerve growth factor and inflammatory cytokines in cultured human keratinocytes. *Neuropeptides, 40*(4), 251–263.

De Simone, R., Micera, A., Tirassa, P., & Aloe, L. (1996). mRNA for NGF and p75 in the central nervous system of rats affected by experimental allergic encephalomyelitis. *Neuropathology and Applied Neurobiology, 22*(1), 54–59.

Delgado, M., Pozo, D., & Ganea, D. (2004). The significance of vasoactive intestinal peptide in immunomodulation. *Pharmacological Reviews, 56*(2), 249–290.

di Mola, F. F., Friess, H., Zhu, Z. W., Koliopanos, A., Bley, T., Di Sebastiano, P., Innocenti, P., Zimmermann, A., & Buchler, M. W. (2000). Nerve growth factor and Trk high affinity receptor (TrkA) gene expression in inflammatory bowel disease. *Gut, 46*(5), 670–679.

Dirmeier, M., Capellino, S., Schubert, T., Angele, P., Anders, S., & Straub, R. H. (2008). Lower density of synovial nerve fibres positive for calcitonin gene-related peptide relative to substance P in rheumatoid arthritis but not in osteoarthritis. *Rheumatology (Oxford, England), 47*(1), 36–40.

Donnerer, J., Schuligoi, R., & Stein, C. (1992). Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: Evidencefor a regulatory function of nerve growth factor in vivo. *Neuroscience, 49*(3), 693–698.

Ehrhard, P. B., Erb, P., Graumann, U., & Otten, U. (1993a). Expression of nerve growth factor and nerve growth factor receptor tyrosine kinase Trk in activated CD4-positive T-cell clones. *Proceedings of the National Academy of Sciences of the United States of America, 90*(23), 10984–10988.

Ehrhard, P. B., Ganter, U., Stalder, A., Bauer, J., & Otten, U. (1993b). Expression of functional trk protooncogene in human monocytes. *Proceedings of the National Academy of Sciences of the United States of America, 90*(12), 5423–5427.

Elenkov, I. J. (2008). Neurohormonal-cytokine interactions: Implications for inflammation, common human diseases and well-being. *Neurochemistry International, 52*(1–2), 40–51.

Ernfors, P. (2001). Local and target-derived actions of neurotrophins during peripheral nervous system development. *Cellular and Molecular Life Sciences, 58*(8), 1036–1044.

Fahnestock, M., Yu, G., & Coughlin, M. D. (2004). ProNGF: A neurotrophic or an apoptotic molecule? *Progress in Brain Research, 146*, 101–110.

Fantini, F., Magnoni, C., Bracci-Laudiero, L., & Pincelli, C. T. (1995). Nerve growth factor is increased in psoriatic skin. *Journal of Investigative Dermatology, 105*(6), 854–855.

Flugel, A., Matsumuro, K., Neumann, H., Klinkert, W. E., Birnbacher, R., Lassmann, H., Otten, U., & Wekerle, H. (2001). Anti-inflammatory activity of nerve growth factor in experimental

autoimmune encephalomyelitis: Inhibition of monocyte transendothelial migration. *European Journal of Immunology, 31*(1), 11–22.

Foster, P. A., Costa, S. K., Poston, R., Hoult, J. R., & Brain, S. D. (2003). Endothelial cells play an essential role in the thermal hyperalgesia induced by nerve growth factor. *The FASEB Journal, 17*(12), 1703–1705.

Franklin, R. A., Brodie, C., Melamed, I., Terada, N., Lucas, J. J., & Gelfand, E. W. (1995). Nerve growth factor induces activation of MAP-kinase and p90rsk in human B lymphocytes. *Journal of Immunology, 154*(10), 4965–4972.

Friedman, W. J., & Greene, L. A. (1999). Neurotrophin signaling via Trks and p75. *Experimental Cell Research, 253*(1), 131–142.

Friedman, W. J., Larkfors, L., Ayer-LeLievre, C., Ebendal, T., Olson, L., & Persson, H. (1990). Regulation of beta-nerve growth factor expression by inflammatory mediators in hippocampal cultures. *Journal of Neuroscience Research, 27*(3), 374–382.

Gadient, R. A., Cron, K. C., & Otten, U. (1990). Interleukin-1 beta and tumor necrosis factor-alpha synergistically stimulate nerve growth factor (NGF) release from cultured rat astrocytes. *Neuroscience Letters, 117*(3), 335–340.

Garcia-Suarez, O., Germana, A., Hannestad, J., Ciriaco, E., Silos-Santiago, I., Germana, G., & Vega, J. A. (2001). Involvement of the NGF receptors (Trka and p75lngfr) in the development and maintenance of the thymus. *Italian Journal of Anatomy and Embryology, 106*(2 Suppl 1), 279–285.

Gee, A. P., Boyle, M. D., Munger, K. L., Lawman, M. J., & Young, M. (1983). Nerve growth factor: Stimulation of polymorphonuclear leukocyte chemotaxis in vitro. *Proceedings of the National Academy of Sciences of the United States of America, 80*(23), 7215–7218.

Gibbs, B. F., Zillikens, D., & Grabbe, J. (2005). Nerve growth factor influences IgE-mediated human basophil activation: Functional properties and intracellular mechanisms compared with IL-3. *International Immunopharmacology, 5*(4), 735–747.

Gillardon, F., Eschenfelder, C., Rush, R. A., & Zimmerman, M. (1995). Increase in neuronal Jun immunoreactivity and epidermal NGF levels following UV exposure of rat skin. *Neuroreport, 6*(9), 1322–1324.

Gomes, R. N., Castro-Faria-Neto, H. C., Bozza, P. T., Soares, M. B., Shoemaker, C. B., David, J. R., & Bozza, M. T. (2005). Calcitonin gene-related peptide inhibits local acute inflammation and protects mice against lethal endotoxemia. *Shock, 24*(6), 590–594.

Hamada, A., Watanabe, N., Ohtomo, H., & Matsuda, H. (1996). Nerve growth factor enhances survival and cytotoxic activity of human eosinophils. *British Journal of Haematology, 93*(2), 299–302.

Harrington, A. W., Leiner, B., Blechschmitt, C., Arevalo, J. C., Lee, R., Morl, K., Meyer, M., Hempstead, B. L., Yoon, S. O., & Giehl, K. M. (2004). Secreted proNGF is a pathophysiological death-inducing ligand after adult CNS injury. *Procedings of National Acadamy of Science USA, 101*, 6226–6230.

Harzenetter, M. D., Novotny, A. R., Gais, P., Molina, C. A., Altmayr, F., & Holzmann, B. (2007). Negative regulation of TLR responses by the neuropeptide CGRP is mediated by the transcriptional repressor ICER. *Journal of Immunology, 179*(1), 607–615.

Hattori, A., Iwasaki, S., Murase, K., Tsujimoto, M., Sato, M., Hayashi, K., & Kohno, M. (1994). Tumor necrosis factor is markedly synergistic with interleukin 1 and interferon-gamma in stimulating the production of nerve growth factor in fibroblasts. *FEBS Letters, 340*(3), 177–180.

Heese, K., Inoue, N., & Sawada, T. (2006). NF-kappaB regulates B-cell-derived nerve growth factor expression. *Cellular and molecular immunology, 3*(1), 63–66.

Hellweg, R., & Hartung, H. D. (1990). Endogenous levels of nerve growth factor (NGF) are altered in experimental diabetes mellitus: A possible role for NGF in the pathogenesis of diabetic neuropathy. *Journal of Neuroscience Research, 26*(2), 258–267.

Holzer, P. (2007). Role of visceral afferent neurons in mucosal inflammation and defense. *Current Opinion in Pharmacology, 7*(6), 563–569.

Horigome, K., Pryor, J. C., Bullock, E. D., & Johnson, E. M., Jr. (1993). Mediator release from mast cells by nerve growth factor. Neurotrophin specificity and receptor mediation. *Journal of Biological Chemistry, 268*(20), 14881–14887.

Hosoi, J., Murphy, G. F., Egan, C. L., Lerner, E. A., Grabbe, S., Asahina, A., & Granstein, R. D. (1993). Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. *Nature, 363*(6425), 159–163.

Huang, E. J., & Reichardt, L. F. (2003). Trk receptors: Roles in neuronal signal transduction. *Annual Review of Biochemistry, 72*, 609–642.

Jacobs, B. L., Smaldone, M. C., Tyagi, V., Philips, B. J., Jackman, S. V., Leng, W. W., & Tyagi, P. (2010). Increased nerve growth factor in neurogenic overactive bladder and interstitial cystitis patients. *The Canadian Journal of Urology, 17*(1), 4989–4994.

Jansen, P., Giehl, K., Nyengaard, J. R., Teng, K., Lioubinski, O., Sjoegaard, S. S., Breiderhoff, T., Gotthardt, M., Lin, F., Eilers, A., Petersen, C. M., Lewin, G. R., Hempstead, B. L., Willnow, T. E., & Nykjaer, A. (2007). Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. *Nature Neuroscience*, *10*, 1449–1457.

Kannan, Y., Ushio, H., Koyama, H., Okada, M., Oikawa, M., Yoshihara, T., Kaneko, M., & Matsuda, H. (1991). 2.5S nerve growth factor enhances survival, phagocytosis, and superoxide production of murine neutrophils. *Blood, 77*(6), 1320–1325.

Kawamoto, K., Okada, T., Kannan, Y., Ushio, H., Matsumoto, M., & Matsuda, H. (1995). Nerve growth factor prevents apoptosis of rat peritoneal mast cells through the trk proto-oncogene receptor. *Blood, 86*(12), 4638–4644.

Kimata, H., Yoshida, A., Ishioka, C., Kusunoki, T., Hosoi, S., & Mikawa, H. (1991). Nerve growth factor specifically induces human IgG4 production. *European Journal of Immunology, 21*(1), 137–141.

Klesse, L. J., & Parada, L. F. (1999). Trks: Signal transduction and intracellular pathways. *Microscopy Research and Technique, 45*(4–5), 210–216.

Kobayashi, H., Gleich, G. J., Butterfield, J. H., & Kita, H. (2002). Human eosinophils produce neurotrophins and secrete nerve growth factor on immunologic stimuli. *Blood, 99*(6), 2214–2220.

Kossmann, T., Hans, V., Imhof, H. G., Trentz, O., & Morganti-Kossmann, M. C. (1996). Interleukin-6 released in human cerebrospinal fluid following traumatic brain injury may trigger nerve growth factor production in astrocytes. *Brain Research, 713*(1–2), 143–152.

Kroeger, I., Erhardt, A., Abt, D., Fischer, M., Biburger, M., Rau, T., Neuhuber, W. L., & Tiegs, G. (2009). The neuropeptide calcitonin gene-related peptide (CGRP) prevents inflammatory liver injury in mice. *Journal of Hepatology, 51*(2), 342–353.

la Sala, A., Corinti, S., Federici, M., Saragovi, H. U., & Girolomoni, G. (2000). Ligand activation of nerve growth factor receptor TrkA protects monocytes from apoptosis. *Journal of Leukocyte Biology, 68*(1), 104–110.

Lambiase, A., Bonini, S., Bonini, S., Micera, A., Magrini, L., Bracci-Laudiero, L., & Aloe, L. (1995). Increased plasma levels of nerve growth factor in vernal keratoconjunctivitis and relationship to conjunctival mast cells. *Investigative Ophthalmology & Visual Science, 36*(10), 2127–2132.

Lambiase, A., Bracci-Laudiero, L., Bonini, S., Bonini, S., Starace, G., D'Elios, M. M., De Carli, M., & Aloe, L. (1997). Human CD4+ T cell clones produce and release nerve growth factor and express high-affinity nerve growth factor receptors. *The Journal of Allergy and Clinical Immunology, 100*(3), 408–414.

Laurenzi, M. A., Barbany, G., Timmusk, T., Lindgren, J. A., & Persson, H. (1994). Expression of mRNA encoding neurotrophins and neurotrophin receptors in rat thymus, spleen tissue and immunocompetent cells. Regulation of neurotrophin-4 mRNA expression by mitogens and leukotriene B4. *European Journal of Biochemistry, 223*(3), 733–741.

Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*(5548), 1945–1948.

Lee, H. W., Na, Y. J., Jung, P. K., Kim, M. N., Kim, S. M., Chung, J. S., Kim, B. S., Kim, J. B., Moon, J. O., & Yoon, S. (2008). Nerve growth factor stimulates proliferation, adhesion and thymopoietic cytokine expression in mouse thymic epithelial cells in vitro. *Regulatory Peptides, 147*(1–3), 72–81.

Legat, F. J., Jaiani, L. T., Wolf, P., Wang, M., Lang, R., Abraham, T., Solomon, A. R., Armstrong, C. A., Glass, J. D., & Ansel, J. C. (2004). The role of calcitonin gene-related peptide in cutaneous immunosuppression induced by repeated subinflammatory ultraviolet irradiation exposure. *Experimental Dermatology, 13*(4), 242–250.

Levi-Montalcini, R. (1987). The nerve growth factor: Thirty-five years later. *Science, 237*, 1154–1162.

Lindholm, D., Heumann, R., Meyer, M., & Thoenen, H. (1987). Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature, 330*(6149), 658–659.

Lindsay, R. M., & Harmar, A. J. (1989). Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. *Nature, 337*(6205), 362–364.

Lipnik-Stangelj, M., & Carman-Krzan, M. (2004a). Activation of histamine H1-receptor enhances neurotrophic factor secretion from cultured astrocytes. *Inflammation Research, 53*(6), 245–252.

Lipnik-Stangelj, M., & Carman-Krzan, M. (2004b). Histamine-stimulated nerve growth factor secretion from cultured astrocyctes is blocked by protein kinase C inhibitors. *Inflammation Research, 53*(Suppl 1), S57–S58.

Lowe, E. M., Anand, P., Terenghi, G., Williams-Chestnut, R. E., Sinicropi, D. V., & Osborne, J. L. (1997). Increased nerve growth factor levels in the urinary bladder of women with idiopathic sensory urgency and interstitial cystitis. *British Journal of Urology, 79*(4), 572–577.

Ma, D., Wolvers, D., Stanisz, A. M., & Bienenstock, J. (2003). Interleukin-10 and nerve growth factor have reciprocal upregulatory effects on intestinal epithelial cells. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology, 284*(5), R1323–R1329.

Ma, W., Dumont, Y., Vercauteren, F., & Quirion, R. (2010). Lipopolysaccharide induces calcitonin gene-related peptide in the RAW264.7 macrophage cell line. *Immunology, 130*(3), 399–409.

Manni, L., & Aloe, L. (1998). Role of IL-1 beta and TNF-alpha in the regulation of NGF in experimentally induced arthritis in mice. *Rheumatology International, 18*(3), 97–102.

Manni, L., Lundeberg, T., Fiorito, S., Bonini, S., Vigneti, E., & Aloe, L. (2003). Nerve growth factor release by human synovial fibroblasts prior to and following exposure to tumor necrosis factor-alpha, interleukin-1 beta and cholecystokinin-8: The possible role of NGF in the inflammatory response. *Clinical and Experimental Rheumatology, 21*(5), 617–624.

Marz, P., Heese, K., Dimitriades-Schmutz, B., Rose-John, S., & Otten, U. (1999). Role of interleukin-6 and soluble IL-6 receptor in region-specific induction of astrocytic differentiation and neurotrophin expression. *Glia, 26*(3), 191–200.

Matsuda, H., Coughlin, M. D., Bienenstock, J., & Denburg, J. A. (1988). Nerve growth factor promotes human hemopoietic colony growth and differentiation. *Proceedings of the National Academy of Sciences of the United States of America, 85*(17), 6508–6512.

Matsuda, H., Kannan, Y., Ushio, H., Kiso, Y., Kanemoto, T., Suzuki, H., & Kitamura, Y. (1991). Nerve growth factor induces development of connective tissue-type mast cells in vitro from murine bone marrow cells. *Journal of Experimental Medicine, 174*(1), 7–14.

Matsuda, H., Koyama, H., Sato, H., Sawada, J., Itakura, A., Tanaka, A., Matsumoto, M., Konno, K., Ushio, H., & Matsuda, K. (1998). Role of nerve growth factor in cutaneous wound healing: Accelerating effects in normal and healing-impaired diabetic mice. *Journal of Experimental Medicine, 187*(3), 297–306.

Mazella, J. (2001). Sortilin/neurotensin receptor-3: A new tool to investigate neurotensin signaling and cellular trafficking? *Cellular Signalling, 13*(1), 1–6.

Mazurek, N., Weskamp, G., Erne, P., & Otten, U. (1986). Nerve growth factor induces mast cell degranulation without changing intracellular calcium levels. *FEBS Letters, 198*(2), 315–320.

McAllister, A. K. (2001). Neurotrophins and neuronal differentiation in the central nervous system. *Cellular and Molecular Life Sciences, 58*(8), 1054–1060.

McMahon, S. B., Bennett, D. L., Priestley, J. V., & Shelton, D. L. (1995). The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule. *Nature Medicine, 1*(8), 774–780.

Mearow, K. M., & Kril, Y. (1995). Anti-NGF treatment blocks the upregulation of NGF receptor mRNA expression associated with collateral sprouting of rat dorsal root ganglion neurons. *Neuroscience Letters, 184*(1), 55–58.

Mearowa, K. M., & Kril, Y. (1995). Anti-NGF treatment blocks the upregulation of NGF receptor mRNA expression associated with collateral sprouting of rat dorsal root ganglion neurons. *Neuroscience Letters, 184*(1), 55–58.

Melamed, I., Kelleher, C. A., Franklin, R. A., Brodie, C., Hempstead, B., Kaplan, D., & Gelfand, E. W. (1996). Nerve growth factor signal transduction in human B lymphocytes is mediated by gp140trk. *European Journal of Immunology, 26*(9), 1985–1992.

Micera, A., Properzi, F., Triaca, V., & Aloe, L. (2000). Nerve growth factor antibody exacerbates neuropathological signs of experimental allergic encephalomyelitis in adult lewis rats. *Journal of Neuroimmunology, 104*(2), 116–123.

Miller, F. D., & Kaplan, D. R. (2001). Neurotrophin signalling pathways regulating neuronal apoptosis. *Cellular and Molecular Life Sciences, 58*(8), 1045–1053.

Millet, I., Phillips, R. J., Sherwin, R. S., Ghosh, S., Voll, R. E., Flavell, R. A., Vignery, A., & Rincon, M. (2000). Inhibition of NF-kappaB activity and enhancement of apoptosis by the neuropeptide calcitonin gene-related peptide. *Journal of Biological Chemistry, 275*(20), 15114–15121.

Moser, K. V., Reindl, M., Blasig, I., & Humpel, C. (2004). Brain capillary endothelial cells proliferate in response to NGF, express NGF receptors and secrete NGF after inflammation. *Brain Research, 1017*(1–2), 53–60.

Murakami, M., Tada, K., Nakajima, K., & Kudo, I. (1997). Cyclooxygenase-2-dependent delayed prostaglandin D2 generation is initiated by nerve growth factor in rat peritoneal mast cells: Its augmentation by extracellular type II secretory phospholipase A2. *Journal of Immunology, 159*(1), 439–446.

Nilsson, G., Forsberg-Nilsson, K., Xiang, Z., Hallbook, F., Nilsson, K., & Metcalfe, D. D. (1997). Human mast cells express functional TrkA and are a source of nerve growth factor. *European Journal of Immunology, 27*(9), 2295–2301.

Noga, O., Englmann, C., Hanf, G., Grutzkau, A., Guhl, S., & Kunkel, G. (2002). Activation of the specific neurotrophin receptors TrkA, TrkB and TrkC influences the function of eosinophils. *Clinical and Experimental Allergy, 32*(9), 1348–1354.

Noga, O., Peiser, M., Altenahr, M., Knieling, H., Wanner, R., Hanf, G., Grosse, R., & Suttorp, N. (2007). Differential activation of dendritic cells by nerve growth factor and brain-derived neurotrophic factor. *Clinical and Experimental Allergy, 37*(11), 1701–1708.

O'Connor, T. M., O'Connell, J., O'Brien, D. I., Goode, T., Bredin, C. P., & Shanahan, F. (2004). The role of substance P in inflammatory disease. *Journal of Cellular Physiology, 201*(2), 167–180.

Olgart, C., & Frossard, N. (2001). Human lung fibroblasts secrete nerve growth factor: Effect of inflammatory cytokines and glucocorticoids. *European Respiratory Journal, 18*(1), 115–121.

Olson, L. (1993). Reparative strategies in the brain: Treatment strategies based on trophic factors and cell transfer techniques. *Acta Neurochir Suppl (Wien), 58*, 3–7.

Otten, U., Schwab, M., Gagnon, C., & Thoenen, H. (1977). Selective induction of tyrosine hydroxylase and dopamine beta-hydroxylase by nerve growth factor: Comparison between adrenal medulla and sympathetic ganglia of adult and newborn rats. *Brain Research, 133*(2), 291–303.

Otten, U., Ehrhard, P., & Peck, R. (1989). Nerve growth factor induces growth and differentiation of human B lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America, 86*(24), 10059–10063.

Peleshok, J. C., & Ribeiro-da-Silva, A. (2012). Neurotrophic factor changes in the rat thick skin following chronic constriction injury of the sciatic nerve. *Molecular Pain, 8*, 1.

Pezzati, P., Stanisz, A. M., Marshall, J. S., Bienenstock, J., & Stead, R. H. (1992). Expression of nerve growth factor receptor immunoreactivity on follicular dendritic cells from human mucosa associated lymphoid tissues. *Immunology, 76*(3), 485–490.

Poo, M. M. (2001). Neurotrophins as synaptic modulators. *Nature Reviews. Neuroscience, 2*(1), 24–32.

Prod'homme, T., Weber, M. S., Steinman, L., & Zamvil, S. S. (2006). A neuropeptide in immune-mediated inflammation, Y? *Trends in Immunology, 27*(4), 164–167.

Quarcoo, D., Schulte-Herbruggen, O., Lommatzsch, M., Schierhorn, K., Hoyle, G. W., Renz, H., & Braun, A. (2004). Nerve growth factor induces increased airway inflammation via a neuropeptide-dependent mechanism in a transgenic animal model of allergic airway inflammation. *Clinical and Experimental Allergy, 34*(7), 1146–1151.

Raud, J., Lundeberg, T., Brodda-Jansen, G., Theodorsson, E., & Hedqvist, P. (1991). Potent anti-inflammatory action of calcitonin gene-related peptide. *Biochemical and Biophysical Research Communications, 180*(3), 1429–1435.

Raychaudhuri, S. P., Jiang, W. Y., & Farber, E. M. (1998). Psoriatic keratinocytes express high levels of nerve growth factor. *Acta Dermato-Venereologica, 78*(2), 84–86.

Reichardt, L. F. (2006). Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 361*(1473), 1545–1564.

Reinshagen, M., Patel, A., Sottili, M., Nast, C., Davis, W., Mueller, K., & Eysselein, V. (1994). Protective function of extrinsic sensory neurons in acute rabbit experimental colitis. *Gastroenterology, 106*(5), 1208–1214.

Reinshagen, M., Flamig, G., Ernst, S., Geerling, I., Wong, H., Walsh, J. H., Eysselein, V. E., & Adler, G. (1998). Calcitonin gene-related peptide mediates the protective effect of sensory nerves in a model of colonic injury. *Journal of Pharmacology and Experimental Therapeutics, 286*(2), 657–661.

Reinshagen, M., Rohm, H., Steinkamp, M., Lieb, K., Geerling, I., Von Herbay, A., Flamig, G., Eysselein, V. E., & Adler, G. (2000). Protective role of neurotrophins in experimental inflammation of the rat gut. *Gastroenterology, 119*(2), 368–376.

Rogers, M. L., Bailey, S., Matusica, D., Nicholson, I., Muyderman, H., Pagadala, P. C., Neet, K. E., Zola, H., Macardle, P., & Rush, R. A. (2010). ProNGF mediates death of natural killer cells through activation of the p75NTR-sortilin complex. *Journal of Neuroimmunology, 226* (1–2), 93–103.

Rook, G. A., Lightman, S. L., & Heijnen, C. J. (2002). Can nerve damage disrupt neuroendocrine immune homeostasis? Leprosy as a case in point. *Trends in Immunology, 23*(1), 18–22.

Roux, P. P., & Barker, P. A. (2002). Neurotrophin signaling through the p75 neurotrophin receptor. *Progress in Neurobiology*, *67*, 203–233.

Ruit, K. G., Osborne, P. A., Schmidt, R. E., Johnson, E. M., Jr., & Snider, W. D. (1990). Nerve growth factor regulates sympathetic ganglion cell morphology and survival in the adult mouse. *Journal of Neuroscience, 10*(7), 2412–2419.

Rush, R. A., Chie, E., Liu, D., Tafreshi, A., Zettler, C., & Zhou, X. F. (1997). Neurotrophic factors are required by mature sympathetic neurons for survival, transmission and connectivity. *Clinical and Experimental Pharmacology & Physiology, 24*(8), 549–555.

Ryan, V. H., German, A. J., Wood, I. S., Hunter, L., Morris, P., & Trayhurn, P. (2008). NGF gene expression and secretion by canine adipocytes in primary culture: Upregulation by the inflammatory mediators LPS and TNFalpha. *Hormone and Metabolic Research, 40*(12), 861–868.

Safieh-Garabedian, B., Poole, S., Allchorne, A., Winter, J., & Woolf, C. J. (1995). Contribution of interleukin-1 beta to the inflammation-induced increase in nerve growth factor levels and inflammatory hyperalgesia. *British Journal of Pharmacology, 115*(7), 1265–1275.

Samah, B., Porcheray, F., Dereuddre-Bosquet, N., & Gras, G. (2009). Nerve growth factor stimulation promotes CXCL-12 attraction of monocytes but decreases human immunodeficiency virus replication in attracted population. *Journal of Neurovirology, 15*(1), 71–80.

Sato, Y., Tsuboi, Y., Kurosawa, H., Sugita, K., & Eguchi, M. (2004). Anti-apoptotic effect of nerve growth factor is lost in congenital insensitivity to pain with anhidrosis (CIPA) B lymphocytes. *Journal of Clinical Immunology, 24*(3), 302–308.

Sawada, J., Itakura, A., Tanaka, A., Furusaka, T., & Matsuda, H. (2000). Nerve growth factor functions as a chemoattractant for mast cells through both mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling pathways. *Blood, 95*(6), 2052–2058.

Schor, N. F. (2005). The p75 neurotrophin receptor in human development and disease. *Progress in Neurobiology, 77*(3), 201–214.

Seidel, M. F., Herguijuela, M., Forkert, R., & Otten, U. (2010). Nerve growth factor in rheumatic diseases. *Seminars in Arthritis and Rheumatism, 40*(2), 109–126.

Sin, A. Z., Roche, E. M., Togias, A., Lichtenstein, L. M., & Schroeder, J. T. (2001). Nerve growth factor or IL-3 induces more IL-13 production from basophils of allergic subjects than from basophils of nonallergic subjects. *The Journal of Allergy and Clinical Immunology, 108*(3), 387–393.

Spinnler, K., Frohlich, T., Arnold, G. J., Kunz, L., & Mayerhofer, A. (2011). Human tryptase cleaves pro-nerve growth factor (pro-NGF): Hints of local, mast cell-dependent regulation of NGF/pro-NGF action. *Journal of Biological Chemistry, 286*(36), 31707–31713.

Stanzel, R. D., Lourenssen, S., & Blennerhassett, M. G. (2008). Inflammation causes expression of NGF in epithelial cells of the rat colon. *Experimental Neurology, 211*(1), 203–213.

Steiner, P., Pfeilschifter, J., Boeckh, C., Radeke, H., & Otten, U. (1991). Interleukin-1 beta and tumor necrosis factor-alpha synergistically stimulate nerve growth factor synthesis in rat mesangial cells. *American Journal of Physiology, 261*(5 Pt 2), F792–F798.

Straub, R. H. (2004). Complexity of the bi-directional neuroimmune junction in the spleen. *Trends in Pharmacological Sciences, 25*(12), 640–646.

Susaki, Y., Shimizu, S., Katakura, K., Watanabe, N., Kawamoto, K., Matsumoto, M., Tsudzuki, M., Furusaka, T., Kitamura, Y., & Matsuda, H. (1996). Functional properties of murine macrophages promoted by nerve growth factor. *Blood, 88*(12), 4630–4637.

Takafuji, S., Bischoff, S. C., De Weck, A. L., & Dahinden, C. A. (1992). Opposing effects of tumor necrosis factor-alpha and nerve growth factor upon leukotriene C4 production by human eosinophils triggered with N-formyl-methionyl-leucyl-phenylalanine. *European Journal of Immunology, 22*(4), 969–974.

Tausk, F., Elenkov, I., & Moynihan, J. (2008). Psychoneuroimmunology. *Dermatologic Therapy, 21*(1), 22–31.

Terenghi, G. (1999). Peripheral nerve regeneration and neurotrophic factors. *Journal of Anatomy, 194*(Pt 1), 1–14.

Thorpe, L. W., & Perez-Polo, J. R. (1987). The influence of nerve growth factor on the in vitro proliferative response of rat spleen lymphocytes. *Journal of Neuroscience Research, 18*(1), 134–139.

Thorpe, L. W., Stach, R. W., Hashim, G. A., Marchetti, D., & Perez-Polo, J. R. (1987a). Receptors for nerve growth factor on rat spleen mononuclear cells. *Journal of Neuroscience Research, 17*(2), 128–134.

Thorpe, L. W., Werrbach-Perez, K., & Perez-Polo, J. R. (1987b). Effects of nerve growth factor on the expression of interleukin-2 receptors on cultured human lymphocytes. *Annals of the New York Academy of Sciences, 496*, 310–311.

Torcia, M., Bracci-Laudiero, L., Lucibello, M., Nencioni, L., Labardi, D., Rubartelli, A., Cozzolino, F., Aloe, L., & Garaci, E. (1996). Nerve growth factor is an autocrine survival factor for memory B lymphocytes. *Cell, 85*(3), 345–356.

Tore, F., & Tuncel, N. (2009). Mast cells: Target and source of neuropeptides. *Current Pharmaceutical Design, 15*(29), 3433–3445.

Townley, S. L., Grimbaldeston, M. A., Ferguson, I., Rush, R. A., Zhang, S. H., Zhou, X. F., Conner, J. M., Finlay-Jones, J. J., & Hart, P. H. (2002). Nerve growth factor, neuropeptides,

and mast cells in ultraviolet-B-induced systemic suppression of contact hypersensitivity responses in mice. *Journal of Investigative Dermatology, 118*(3), 396–401.

Toyoda, M., Nakamura, M., Makino, T., Hino, T., Kagoura, M., & Morohashi, M. (2002). Nerve growth factor and substance P are useful plasma markers of disease activity in atopic dermatitis. *British Journal of Dermatology, 147*(1), 71–79.

Toyomoto, M., Ohta, M., Okumura, K., Yano, H., Matsumoto, K., Inoue, S., Hayashi, K., & Ikeda, K. (2004). Prostaglandins are powerful inducers of NGF and BDNF production in mouse astrocyte cultures. *FEBS Letters, 562*(1–3), 211–215.

Troy, C. M., Friedman, J. E., & Friedman, W. J. (2002). Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. *Journal of Biological Chemistry*, *277*, 34295–34302.

Verge, V. M., Richardson, P. M., Wiesenfeld-Hallin, Z., & Hokfelt, T. (1995). Differential influence of nerve growth factor on neuropeptide expression in vivo: A novel role in peptide suppression in adult sensory neurons. *Journal of Neuroscience, 15*(3 Pt 1), 2081–2096.

Villoslada, P., Hauser, S. L., Bartke, I., Unger, J., Heald, N., Rosenberg, D., Cheung, S. W., Mobley, W. C., Fisher, S., & Genain, C. P. (2000). Human nerve growth factor protects common marmosets against autoimmune encephalomyelitis by switching the balance of T helper cell type 1 and 2 cytokines within the central nervous system. *Journal of Experimental Medicine, 191*(10), 1799–1806.

Welker, P., Grabbe, J., Grutzkau, A., & Henz, B. M. (1998). Effects of nerve growth factor (NGF) and other fibroblast-derived growth factors on immature human mast cells (HMC-1). *Immunology, 94*(3), 310–317.

Welker, P., Grabbe, J., Gibbs, B., Zuberbier, T., & Henz, B. M. (2000). Nerve growth factor-beta induces mast-cell marker expression during in vitro culture of human umbilical cord blood cells. *Immunology, 99*(3), 418–426.

Whittemore, S. R., Larkfors, L., Ebendal, T., Holets, V. R., Ericsson, A., & Persson, H. (1987). Increased beta-nerve growth factor messenger RNA and protein levels in neonatal rat hippocampus following specific cholinergic lesions. *Journal of Neuroscience, 7*(1), 244–251.

Woolf, C. J., & Salter, M. W. (2000). Neuronal plasticity: Increasing the gain in pain. *Science, 288*(5472), 1765–1769.

Xiang, Z., & Nilsson, G. (2000). IgE receptor-mediated release of nerve growth factor by mast cells. *Clinical and Experimental Allergy, 30*(10), 1379–1386.

Yang, J., Siao, C. J., Nagappan, G., Marinic, T., Jing, D., McGrath, K., Chen, Z. Y., Mark, W., Tessarollo, L., Lee, F. S., Lu, B., & Hempstead, B. L. (2009). Neuronal release of proBDNF. *Nature Neuroscience*, *12*, 113–115.

Zegarska, B., Lelinska, A., & Tyrakowski, T. (2006). Clinical and experimental aspects of cutaneous neurogenic inflammation. *Pharmacological Reports, 58*(1), 13–21.

NGF/P75 in Cell Cycle and Tetraploidy

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Contents

Abstract

Neurotoxicity, neurodegeneration, and other disorders affecting neuron survival are often related to cell cycle reentry in neurons. Traditionally, cell cycle reentry of these postmitotic cells has been thought to be associated with apoptosis. Nevertheless, cell cycle reentry and DNA synthesis in neurons could also lead to tetraploidy which may explain long-lasting neurodegenerative processes. During development, a subpopulation of newborn neurons reactivates the cell cycle and becomes tetraploid in response to $p75^{NTR}$ activation. These neurons enlarge their cell bodies and increase their dendritic trees, thus generating specific neuronal populations that innervate particular areas. Pathological states in the nervous system could also lead to $p75^{NTR}$-dependent neuronal tetraploidy.

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De novo tetraploid neurons might become structurally and functionally altered, thus leading to neurodegeneration at late stages of the disease. This chapter describes what is currently known about the interplay between $p75^{NTR}$ and the cell cycle, stressing the role played by different $p75^{NTR}$ interactors, including the MAGE and Bex1/NADE adaptor proteins and the transcription factors SC1, NRIF, and Sall2, in cell cycle regulation. The chapter also discusses on the effects of $p75^{NTR}$, as a cell cycle regulator, in neural stem cell proliferation, neurogenesis, and neuronal tetraploidization, as well as on the connection of $p75^{NTR}$ in pathology, including its putative effects in neurodegeneration.

List of Abbreviations

Aβ	Amyloid-β
BDNF	Brain-derived neurotrophic factor
Bex-1	Brain-expressed X-linked 1
BrdU	Bromodeoxyuridine
cdk	Cyclin-dependent kinase
CMAGE	Chicken MAGE
CNS	Central nervous system
Dlxin-1	Dlx/Msx-interacting MAGE/Necdin family protein
E2F1	E2 promoter-binding factor-1
EGFP	Enhanced green fluorescent protein
ERK	Extracellular signal-regulated kinase
JNK	c-Jun N-terminal kinase
MAGE	Melanoma antigen
MAPK	Mitogen-activated protein kinase
NADE	$p75^{NTR}$-associated cell death executor
NGF	Nerve growth factor
NRAGE	Neurotrophin receptor-interacting MAGE homolog
NRIF	Neurotrophin receptor-interacting factor
NT3	Neurotrophin-3
$p75^{ICD}$	$p75^{NTR}$ intracellular domain
$p75^{NTR}$	p75 neurotrophin receptor
PCNA	Proliferating cell nuclear marker
PNS	Peripheral nervous system
PR/SET	Positive regulatory/suppressor of variegation, enhancer of zeste, trithorax
Rb	Retinoblastoma
RGCs	Retinal ganglion cells
Sall2	Sal-like protein 2
SC1	Schwann cell factor 1
SGZ	Subgranular zone
SVZ	Subventricular zone
TNF	Tumor necrosis factor
Trk	Tropomyosin-related kinase

1 Introduction

Neurotrophins are best known as neuronal survival factors with multiple effects in the structure and function of the adult vertebrate nervous system (Huang and Reichardt 2001). The first neurotrophin to be described was NGF in the early 1950s (Levi-Montalcini and Hamburger 1951), being initially purified from sarcoma tumor cells (Cohen et al. 1954). Several decades later, other members of the neurotrophin family were characterized, including BDNF (Barde et al. 1982), NT3 (Hohn et al. 1990; Maisonpierre et al. 1990; Rosenthal et al. 1990; Jones and Reichardt 1990), and NT4/5 (Hallböök et al. 1991; Berkemeier et al. 1991). In the brain, mature neurotrophins coexist with their precursor pro-forms, namely, proNGF, proBDNF, and proNT3 (Fahnestock et al. 2001; Michalski and Fahnestock 2003). The neurotrophin pro-forms fulfill specific functions, usually opposed to that of the mature neurotrophins, including induction of cell death (Lee et al. 2001; Teng et al. 2005; Yano et al. 2009) and, in the case of proBDNF, synapse modulation (Lu 2003; Woo et al. 2005; Greenberg et al. 2009). These opposed effects can be explained due to the capacity of neurotrophins to interact with two different types of receptors, $p75^{NTR}$ and the members of the Trk tyrosine kinase receptor family. $p75^{NTR}$ was the first member of the TNF receptor family to be described (Chao et al. 1986; Johnson et al. 1986; Radeke et al. 1987). Unlike the Trk receptors, known to interact with specific neurotrophins (Kaplan et al. 1991; Klein et al. 1991a, b, 1992; Lamballe et al. 1991), $p75^{NTR}$ can bind to, and be activated by, all neurotrophins (Rodríguez-Tébar et al. 1990, 1992). Although $p75^{NTR}$ was initially thought to be a mere Trk co-receptor, it is now clear that this receptor is at the core of at least two other signaling networks, including the $p75^{NTR}$/sortilin platform that induces apoptosis in response to the pro-forms of the neurotrophins (Nykjaer et al. 2004; Teng et al. 2005; Yano et al. 2009) and the $p75^{NTR}$/NgR/LINGO-1 complex that regulates axonal outgrowth (Wang et al. 2002; Mi et al. 2004). In some cases, signaling through $p75^{NTR}$ requires the release of its intracellular domain ($p75^{ICD}$), which can be translocated to the nucleus (Frade 2005; Parkhurst et al. 2010). This mechanism has been shown to lead to apoptosis (Majdan et al. 1997; Kenchappa et al. 2006, 2010; Podlesniy et al. 2006), and it can also participate in Trk signaling (Skeldal et al. 2011).

Mounting evidence indicates that neurotrophins can regulate cell cycle progression through both $p75^{NTR}$ and the Trk receptors (López-Sánchez and Frade 2002). This chapter will firstly describe what is known about the interplay between $p75^{NTR}$ and the cell cycle machinery. Then, the chapter will be focused on the role played by this receptor in the normal nervous system in terms of cell cycle regulation (proliferation of neural stem cells, embryonic and adult neurogenesis, and neuronal tetraploidization). Finally, a discussion on the possible involvement of $p75^{NTR}$ as a cell cycle regulator in neurodegeneration will be presented.

2 Interplay Between $p75^{NTR}$ and the Cell Cycle

$p75^{NTR}$ is the founder member of the tumor necrosis factor receptor family. This receptor, characterized by the presence of one transmembrane domain and a type II death domain within its intracellular domain, is expressed by a variety of neuronal and non-neuronal cell types, frequently showing proliferative capacity. In this regard, $p75^{NTR}$ was initially described in proliferating cells derived from pheochromocytoma, neuroblastoma, and melanoma tumors (Yankner and Shooter 1982). More recently, this receptor is often used as a marker of neural progenitor cells in the adult brain (Giuliani et al. 2004; Young et al. 2007; Bernabeu and Longo 2010) and as a tumor suppressor gene in a number of tumor cells (Krygier and Djakiew 2001; Yuanlong et al. 2008; Jin et al. 2007; Khwaja and Djakiew 2003; Khwaja et al. 2006). $p75^{NTR}$ has opposing effects on the cell cycle, inducing cell cycle arrest in some instances and cell cycle progression in other circumstances. For instance, $p75^{NTR}$-dependent cell cycle arrest, associated with reduced expression of cyclin D1, cyclin E, cdk2, E2F1, PCNA, and the cdk inhibitor $p16^{INK4a}$, decreased cdk2 activity, and hyper-phosphorylation of Rb protein, has been shown to occur in bladder tumor epithelial cells (Khwaja and Djakiew 2003). In human gastric cancer cells, $p75^{NTR}$ expression suppresses proliferation by downregulation of cyclin A, cyclin D1, cyclin E, cdk2, and phospho-Rb and upregulation of the cell cycle inhibitors Rb and $p27^{KIP1}$. This antiproliferative activity in human gastric cancer cells is dependent on the presence of the death domain of $p75^{NTR}$ (Jin et al. 2007). $p75^{NTR}$ expression in hepatocellular carcinoma cells has also been shown to downregulate the expression of cyclin A, cyclin D1, cyclin E, cdk2, p-Rb, and PCNA and upregulate the expression of the cell cycle inhibitor Rb (Yuanlong et al. 2008). Examples of $p75^{NTR}$-dependent cell cycle progression are also available in the literature. For instance, $p75^{NTR}$ has been shown to be expressed by human oral keratinocyte stem cells showing high in vitro proliferative capacity and clonal growth potential (Nakamura et al. 2007) as well as by human esophageal keratinocyte stem cells able to undergo self-renewal and self-amplification (Okumura et al. 2003). Moreover, $p75^{NTR}$ can be detected in cycling cells from the subventricular zone of adult male rats (Giuliani et al. 2004), and the addition of NGF has been shown to revert $p75^{NTR}$-dependent cell cycle arrest in bladder tumor epithelial cells (Khwaja and Djakiew 2003).

The mechanisms used by $p75^{NTR}$ to regulate cell cycle progression are beginning to be elucidated. In this regard, $p75^{NTR}$ is known to activate different members of the MAPK family able to regulate the cell cycle (Ambrosino and Nebreda 2001; Zhang and Liu 2002), including JNK, $p38^{MAPK}$, and ERK (Casaccia-Bonnefil et al. 1996; Susen et al. 1999; Costantini et al. 2005; Jiang et al. 2007). Moreover, the opposing effects of $p75^{NTR}$ on cell cycle regulation can be explained by the different molecules with which this receptor interacts (see Fig. 1). $p75^{NTR}$ can modulate the cell cycle through the interaction of its intracellular domain with a number of proteins known to regulate the cell cycle, some of which are transcription factors. In some instances, $p75^{NTR}$ has been shown to sequester these molecules at the plasma membrane.

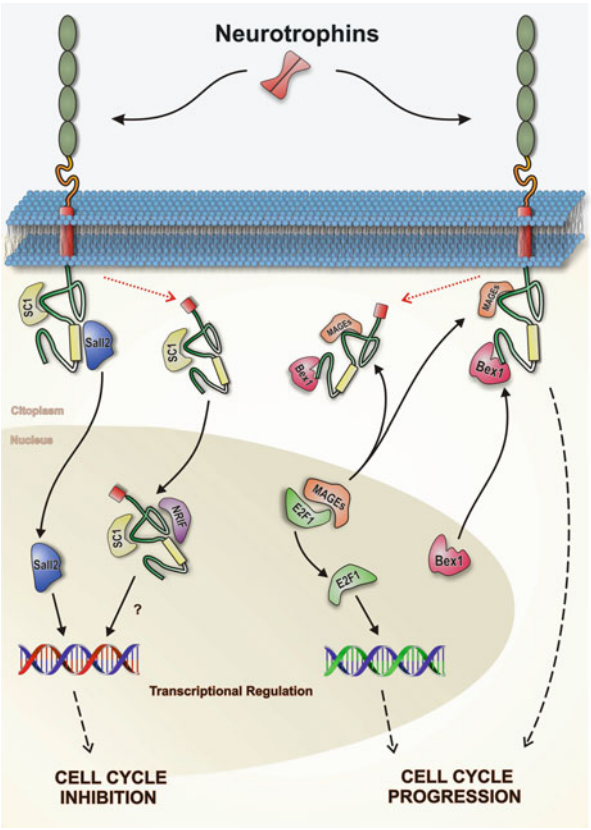

Fig. 1 Scheme of the signaling pathways used by $p75^{NTR}$ to regulate the cell cycle. *Dotted arrows* represent the release of $p75^{ICD}$ as a consequence of regulated γ-secretase-dependent cleavage of $p75^{NTR}$. *Solid arrows* describe the dynamics of the different proteins involved in the $p75^{NTR}$ signaling. *Dashed arrows* represent the final biological output of the corresponding pathways. The *question mark* represents the putative transcriptional activity of $p75^{ICD}$ complexed with NRIF and/or SC1. See main text for further details

In addition, $p75^{NTR}$ is susceptible to undergo γ-secretase-dependent cleavage and release its intracellular domain ($p75^{ICD}$) which may subsequently translocate to the nucleus (Kanning et al. 2003; Frade 2005). The interaction of $p75^{ICD}$ with transcription factors led to the suggestion that it can regulate gene expression when located in the nucleus. This notion has recently been confirmed as $p75^{ICD}$ has been shown to translocate to the nucleus and activate a green fluorescent protein reporter (Parkhurst et al. 2010). Furthermore, NGF can induce the interaction of $p75^{ICD}$ with the *cyclin E* promoter, thus inhibiting its activity in HeLa cells (Parkhurst et al. 2010), a result consistent with the observation that NGF-dependent nuclear translocation of $p75^{ICD}$ in schwannoma cells (Frade 2005) correlates with cell cycle repression in these cells (Morillo and Frade 2008). The expression of $p75^{ICD}$ in $p75^{NTR}$-negative PC12 cells downregulates cyclin D2 expression as well (Fritz et al. 2006). $p75^{NTR}$ can regulate the cell cycle by means of recruitment of adaptor proteins such as different MAGE proteins or Bex1, known to be involved in the cell cycle regulation, and also by regulating the expression of cell cycle-regulating genes through transcription factors such as SC1, NRIF, and Sall2, all of them able to interact with $p75^{ICD}$.

2.1 MAGE Proteins

The MAGE protein family comprises more than 60 adaptor proteins in mammals, containing a conserved MAGE homology domain. Originally, MAGE proteins were discovered because peptides derived from MAGE gene products are presented on the cell surface of human melanoma cells by the major histocompatibility complex (Barker and Salehi 2002). MAGE proteins can be divided into two major groups: type I and type II MAGE proteins. The former are only expressed in tumors, male germinal cells, and placenta. In contrast, the latter group, defined by a phylogenetically distinct MAGE homology domain, comprises around ten different proteins expressed in both differentiating and adult cells (Barker and Salehi 2002). Most of the MAGE proteins are encoded by genes containing a single exon, suggesting that the whole MAGE protein family evolved from a retrotransposition event that occurred in an ancestral gene orthologue of the gene encoding NRAGE/Dlxin-1/MAGE-D1, the only MAGE gene containing exons in its sequence, followed by extensive gene duplication. This hypothesis is consistent with the existence of a single MAGE gene in all nonmammalian species including unicellular, plants, fungi, invertebrates, and nonmammalian vertebrates (López-Sánchez et al. 2007).

The mammalian MAGE II proteins Necdin, NRAGE/Dlxin-1/MAGE-D1, MAGE-H1, and MAGE-G1 as well as CMAGE are known to interact with $p75^{NTR}$ through its intracellular domain (Salehi et al. 2000; Tcherpakov et al. 2002; Kuwako et al. 2004; López-Sánchez et al. 2007), and they have effects on cell cycle regulation. Therefore, these proteins are candidates for the effects of $p75^{NTR}$ on the cell cycle. Necdin can bind to the transcription factor E2F1, thus blocking its capacity to trigger G1/S transition (Taniura et al. 1998; Kuwako et al. 2004). The activation of $p75^{NTR}$ by NGF can recruit Necdin to the cell membrane, thus favoring E2F1-dependent cell cycle progression and subsequent death of differentiated neurons (Kuwako et al. 2004) (see Fig. 1). Alternatively, the presence of the $p75^{ICD}$ fragment can displace both Necdin and CMAGE from their interaction with E2F1 in differentiating neurons, thus leading to cell E2F1-dependent cycle reentry and apoptosis (López-Sánchez et al. 2007) (see Fig. 1). NRAGE/Dlxin-1/MAGE-D1 can also block cell cycle progression (Salehi et al. 2000), likely by inducing the expression of the cell cycle-inhibiting protein $p21^{Waf1}$ in a p53-dependent manner (Wen et al. 2004). NRAGE/Dlxin-1/MAGE-D1 associates with the plasma membrane when NGF is bound to $p75^{NTR}$ (Salehi et al. 2000), as it occurs with Necdin (see above). Therefore, sequestering of MAGE proteins by $p75^{NTR}$ seems to be a general mechanism for enhancement of cell cycle progression in different cellular systems.

2.2 Bex/NADE

$p75^{NTR}$ is also known to interact through its death domain with the small adaptor-like proteins from the Bex/NADE family (Mukai et al. 2000; Vilar et al. 2006), including Bex1 and Bex3/NADE. Among them, Bex1 can constitutively

interact with $p75^{NTR}$ (see Fig. 1) and favor cell cycle progression when overexpressed in differentiating PC12 cells (Vilar et al. 2006). This is consistent with the increase of Bex1 during S phase in PC12 cells, as it occurs with the presence of $p75^{NTR}$ on the surface of these same cells (Urdiales et al. 1998). Upon NGF treatment, Bex1 translocates from the nucleus to the cytoplasm (see Fig. 1), suggesting that this mechanism is required for the effect of this protein on cell cycle regulation. Interestingly, in other cell systems Bex1/2 seems to act as a putative tumor suppressor gene in malignant glioma (Foltz et al. 2006), although in this case the participation of $p75^{NTR}$ is currently unknown.

2.3 SC1

The $p75^{NTR}$-interacting protein SC1 is a member of the PR/SET domain-containing zinc finger protein family, which can be detected in both the nucleus and cytoplasm when overexpressed (Chittka and Chao 1999). SC1 is redistributed from the cytoplasm to the nucleus after NGF treatment in a $p75^{NTR}$-dependent manner, acting as a transcriptional repressor (see Fig. 1). Nuclear SC1 correlates with the loss of BrdU incorporation, likely due to its ability to repress cyclin E expression (Chittka et al. 2004). Interestingly, NGF has been shown to induce the association of endogenous $p75^{ICD}$ with the cyclin E promoter (Parkhurst et al. 2010), suggesting that a complex formed by $p75^{ICD}$ and SC1 can be formed in the promoter of different genes encoding cell cycle-regulating proteins (see Fig. 1).

2.4 Sall2

Sall2 is another transcription factor able to regulate cell cycle progression which can interact with $p75^{NTR}$ through its intracellular domain (see Fig. 1). This protein is a tumor suppressor that becomes dissociated from its binding to $p75^{NTR}$ upon interaction of the latter with NGF (Pincheira et al. 2009). Sall2 is known to activate $p21^{Waf1}$ and promote growth arrest in neurons. This observation is consistent with the $p75^{NTR}$-dependent increase of $p21^{Waf1}$ expression and decrease of Rb phosphorylation in breast cancer cells (Verbeke et al. 2010).

2.5 NRIF

The zinc finger proteins of the Krüppel family NRIF1 and NRIF2 are able to interact with the intracellular domain of $p75^{NTR}$ through its juxtamembrane and death domains (Casademunt et al. 1999; Benzel et al. 2001) (see Fig. 1). These proteins have been suggested to be transcription factors based on structural characteristics and their ability to translocate to the nucleus (Gentry et al. 2004), and they prevent cell cycle progression when expressed in 293T cells. Interestingly, the release of $p75^{ICD}$ correlates with the translocation of NRIF to the nucleus

(Kenchappa et al. 2006; Volosin et al. 2008), suggesting the complex NRIF/$p75^{ICD}$ may participate in the regulation of the cell cycle, as it occurs with $p75^{ICD}$ and SC1 (see above).

3 The Role of $p75^{NTR}$ as a Cell Cycle Regulator in Nervous System Physiology

A critical feature of the nervous system is the requirement of neurons to be postmitotic. The avoidance of cell division in these cells is likely derived from their complex morphology, required for their function, which is incompatible with the reorganization of the cytoskeleton necessary for the mitotic process. Therefore, at certain point of neural development, proliferating neuronal precursors stop dividing and differentiate as neurons in a process referred to as neurogenesis. During development, $p75^{NTR}$ can be detected in the proliferating epithelium of the retina (Frade and Barde 1999; Morillo et al. 2010; López-Sánchez et al. 2011), spinal cord (Frade and Barde 1999; López-Sánchez et al. 2011), and other structures of the CNS including the dorsal telencephalon in the chick (Fig. 2), suggesting that it can participate in the neurogenic process. Furthermore, $p75^{NTR}$ is expressed by neural crest cells (Bannerman and Pleasure 1993), a proliferating cell population giving rise to the PNS, among other structures. Some evidence exists for the participation of $p75^{NTR}$ in cell cycle withdrawal of precursor cells and neuronal differentiation during embryonic development (Hapner et al. 1998; Hosomi et al. 2003; Zhang et al. 2009). Much more evidence exists for the participation of $p75^{NTR}$ in adult neurogenesis and neuronal tetraploidization during embryonic development. These two processes are further described below.

3.1 Adult Neurogenesis

For many decades it was assumed that the generation of functional neurons from precursors (i.e., neurogenesis) only occurs during embryonic and perinatal stages of development in mammals. This view has been ruled out, and nowadays it is widely accepted that neural stem cells can be present in the adult nervous system, being able to proliferate in vitro giving rise to neurospheres (Reynolds and Weiss 1992). Neurons are known to be produced during adulthood in two main brain regions, in the SVZ of the lateral ventricle (giving rise to the rostral migratory stream that colonizes the olfactory bulb) and the SGZ from the dentate gyrus of the hippocampus (producing dentate granule neurons).

Interestingly, $p75^{NTR}$ is expressed by cycling cells in the SVZ of adult male rats (Giuliani et al. 2004). This $p75^{NTR}$-positive cell population integrates all of the neurosphere-producing SVZ precursors, which are known to enhance neuronal production in response to NGF or BDNF (Young et al. 2007). The importance of $p75^{NTR}$ in SVZ adult neurogenesis is further supported by the observation that the neurogenic potential is reduced in neurospheres derived from $p75^{NTR}$ knockout

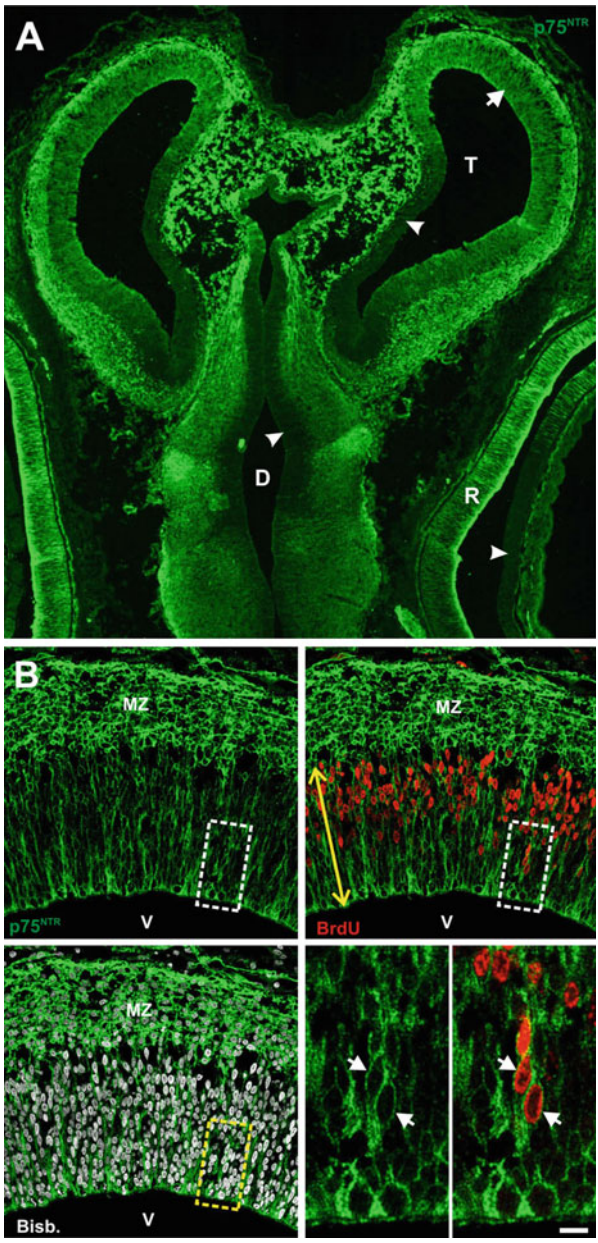

Fig. 2 $p75^{NTR}$ expression in the chick brain at developmental day 6 (E6). (**A**) Horizontal section through the E6 chick brain, immunostained with the ChEX anti-$p75^{NTR}$ antibody (kindly provided by L. F. Reichardt, University of California San Francisco). $p75^{NTR}$ is expressed in specific regions of the neuroepithelium (*arrow*), while this protein cannot be detected in other areas of the neuroepithelium (arrowheads). *T* telencephalic derivatives, *D* diencephalic derivatives, *R* retina. (**B**) A high magnification from the telencephalic wall (dorsal hyperpallium) from E6 chick embryos treated with BrdU for 20 min. BrdU is incorporated by nuclei of progenitors undergoing S phase (*red*) which, due to the interkinetic nuclear movement, are mostly located far from the ventricle (*V*). Nevertheless, some nuclei from $p75^{NTR}$-expressing precursors incorporate BrdU when located close to the ventricle (*dashed box* and *arrows* in the magnification of the *dashed box* shown in the *bottom right panels*). Nuclei were labeled with bisbenzimide (*Bisb.*). Abbreviation: *MZ* mantle zone. Bar – 65 μm (**A**), 25 μm (**B**)

mice (Young et al. 2007). $p75^{NTR}$ might also regulate proliferation of SVZ precursors in pathological states. For instance, $p75^{NTR}$ activation with $A\beta_{1\text{-}42}$, the pathological form of Aβ in Alzheimer's disease, has been shown to favor proliferation and neuronal differentiation in neurosphere-forming adult progenitors,

an effect that is not observed in $p75^{NTR}$ knockout mice (Sotthibundhu et al. 2009). Moreover, an increase in the proliferation activity of $p75^{NTR}$-positive SVZ precursors has been described in rats subjected to experimental allergic encephalomyelitis, a demyelinating disease widely used as an experimental model for human multiple sclerosis (Calzà et al. 1998). In the SGZ, $p75^{NTR}$ is expressed in dividing SGZ cells (Okano et al. 1996; Bernabeu and Longo 2010), and it seems to participate in adult hippocampal neurogenesis as neurogenic potential is reduced in the SGZ of $p75^{NTR}$ knockout mice (Bernabeu and Longo 2010; Colditz et al. 2010), maybe due to reduced survival of neuroblasts (Catts et al. 2008). Neurogenesis can also take place in different regions of the nervous system in response to injury. This is the case of the adult dorsal root ganglia, in which a subpopulation of glia may play a role in neurogenesis after peripheral nerve injury. These cells express $p75^{NTR}$, and in vitro they have been reported to form neurospheres (Li et al. 2007). Neurogenesis in the striatum in response to focal cerebral ischemia can be also potentiated by $p75^{NTR}$ since the proportion of neurons incorporating BrdU was increased in this paradigm (Zhu et al. 2011).

3.2 Neuronal Tetraploidy

The acquisition of multicellularity in evolution has allowed the emergence of the somatic lineage, in which genomic modifications may occur without transmission to the descendants. These genomic alterations, including somatic polyploidy, may participate in the differentiation of specific tissues. This is the case, for instance, of the liver and heart in vertebrates, which have both been shown to contain polyploid cells (Anatskaya and Vinogradov 2007).

Examples of somatic polyploidy in neurons have been described in some invertebrates (Coggeshall et al. 1970: Manfredi Romanini et al. 1973). In contrast, the presence of polyploid neurons in the vertebrate nervous system has been under controversy for decades (see Swartz and Bhatnagar 1981). It was not until the advent of modern techniques such as fluorescent in situ hybridization, flow cytometry, slide-based cytometry, and quantitative PCR analysis of nuclear DNA that the classical belief that all vertebrate neurons contain a diploid DNA amount (Swift 1953) was challenged. Recent analyses, for instance, have proved that 10 % of cortical neurons display a more than diploid DNA content in humans (Mosch et al. 2007). In this regard, tetraploid neurons have been shown to exist in different neural structures (López-Sánchez et al. 2011) including the normal vertebrate retina. In this latter tissue, tetraploid neurons constitute a subpopulation of RGCs that, in the chick, innervate lamina F in the stratum-griseum-et-fibrosum-superficiale of the tectal cortex (Morillo et al. 2010). In the retina, tetraploid RGCs are generated as they migrate to the ganglion cell layer, soon after they undergo their final mitosis and acquire neuronal markers (Morillo et al. 2010). During this stage differentiating RGCs express $p75^{NTR}$ and a subpopulation of these neurons incorporates BrdU, thus becoming tetraploid

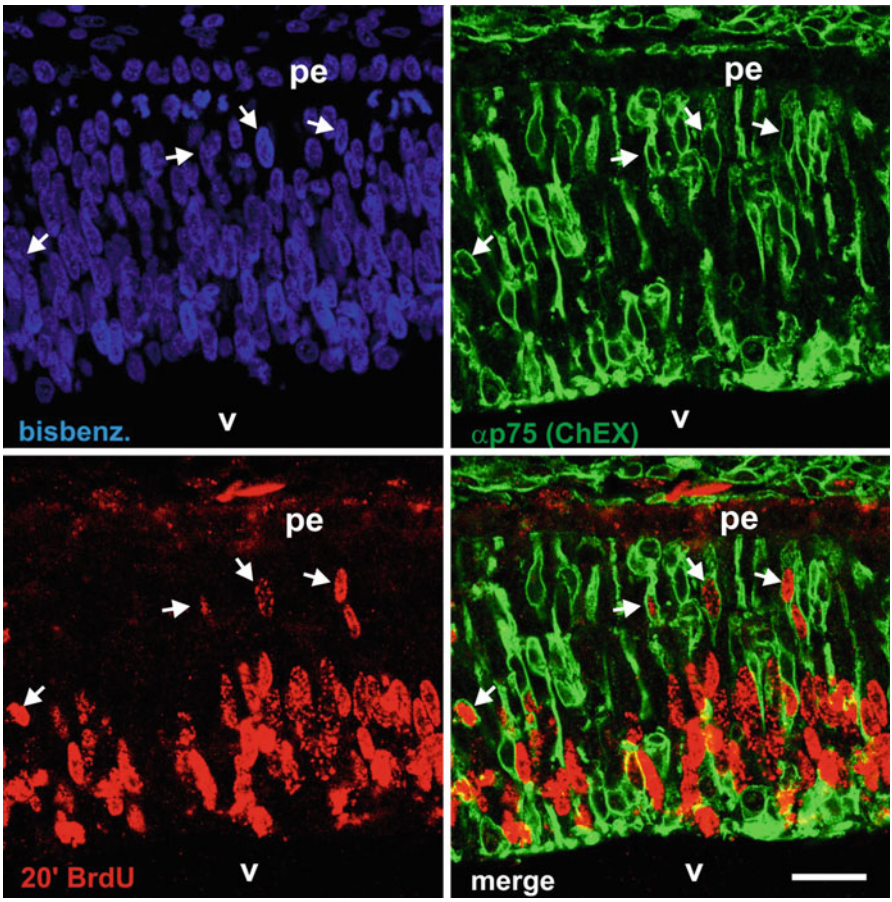

Fig. 3 Colocalization of $p75^{NTR}$ and BrdU in apically located cells from the E6 chick retina. Embryos were treated for 15 min with 40 μl of a solution containing 10 μg/ml BrdU and then fixed. Sagittal sections through the E6 chick retina were obtained, and they were subsequently immunostained with the ChEX anti-$p75^{NTR}$ antibody (*green*) and an anti-BrdU antibody (*red*) as previously described (López-Sánchez et al. 2011). $p75^{NTR}$ is expressed by BrdU-positive cells with nuclei located at the apical portion of the neuroepithelium (*arrows*). Nuclei were labeled with bisbenzimide (*Bisb.*). Abbreviations: *pe* pigment epithelium, *v* vitreous body. Bar, 18 μm

throughout an endoreduplicative mechanism (Morillo et al. 2010; see also Fig. 3). Our work has demonstrated that DNA duplication in differentiating RGCs is triggered by NGF through $p75^{NTR}$, since blocking antibodies against these molecules were able to prevent cell cycle reentry (Morillo et al. 2010). BrdU incorporation in neural progenitor cells expressing $p75^{NTR}$ can also be observed in other neural structures of the chick embryo such as the spinal cord (López-Sánchez et al. 2011) or the telencephalon (Fig. 2b). Although further analyses are still needed to demonstrate that these $p75^{NTR}$-positive cells are equivalent to those neurons

becoming tetraploid in the retina (Morillo et al. 2010), these results suggest that the participation of $p75^{NTR}$ in neuronal tetraploidization is a general feature of the developing vertebrate nervous system.

Somatic polyploidy can be generated by different mechanisms usually linked to alterations of the mitotic cycle, including endoreduplication (i.e., a modified version of the cell cycle characterized by S phase without mitosis) and endomitosis (i.e., a mitotic cycle in the absence of anaphase/cytokinesis thus resulting in genomic DNA duplication) (Edgar and Orr-Weaver 2001; Ullah et al. 2009). Tetraploid RGCs are originated in the developing retina through an endoreduplicative cycle since they remain in a G2-like state after replicating their nuclear DNA (Morillo et al. 2010). Evidence exists that the neurotrophin BDNF, through its receptor TrkB, fulfills a critical role in preventing G2/M transition in retinal tetraploid neurons. Indeed, blockade of endogenous BDNF resulted in an increase of differentiating RGCs undergoing mitosis (Morillo et al. 2010). Therefore, neurotrophins, including NGF and BDNF, seem to play a critical role in neuronal tetraploidization in vertebrates. Tetraploid neurons that undergo mitosis finally die (Frade 2000; Morillo et al. 2010; López-Sánchez et al. 2011), and the regulation of the G2/M transition in these cells is likely crucial for removal of specific neuronal types during development (Frade et al. 1997), thus adjusting the ratio between tetraploid and diploid neurons in specific areas. Importantly, this process may be involved in neuronal death associated to neurodegenerative diseases (see below).

Polyploidy is usually linked to enlarged cell size (Edgar and Orr-Weaver 2001; Ullah et al. 2009), a concept that can be extrapolated to the vertebrate nervous system. For instance, neuronal hypertrophy has been shown to occur in tetraploid strains of Xenopus laevis, whose neurons contain significantly enlarged cell somas and dendrites (Szaro and Tompkins 1987). As expected, our studies have revealed that tetraploid RGCs show enlarged cell somas and dendritic arbors (Morillo et al. 2010), suggesting that somatic tetraploidy is associated with morphological and functional diversity in the nervous system.

4 $p75^{NTR}$ in Neurodegeneration: The Tetraploid Connection

Cell cycle reentry in neurons represents a critical feature of both acute neuronal damage and chronic neurodegenerative diseases (Wang et al. 2009). In this regard, cell cycle regulators have been proposed as therapeutic targets for stroke (Osuga et al. 2000), excitotoxicity (Verdaguer et al. 2004), trauma (Di Giovanni et al. 2005), and Alzheimer's disease (Woods et al. 2007).

In cases of acute injury to the CNS such as kainic acid excitotoxicity and trauma, cell cycle reentry is usually stopped at the G1/S phase and it leads to rapid apoptosis (Kuan et al. 2004; Byrnes et al. 2007). In contrast, chronic neurodegenerative diseases are normally associated with DNA synthesis in particular neuronal populations, which can be arrested at the G2/M transition and survive for some time before dying by apoptosis. This is the case, for instance, of neurons subjected to ischemia-hypoxia (Byrnes et al. 2007; Burns et al. 2007).

These neurons incorporate BrdU and remain alive for days, before they die. Examples of cell cycle reentry and hyperploidization have also been shown to occur in Alzheimer's disease (Yang et al. 2001; Mosch et al. 2007; Arendt et al. 2010). Furthermore, increased expression in neurons of cell cycle regulators involved in G1/S transition has been described in patients suffering amyotrophic lateral sclerosis (Ranganathan and Bowser 2003, 2010; Ferraiuolo et al. 2007), Parkinson's disease (Höglinger et al. 2007), Huntington's disease (Pelegrí et al. 2008; Fernandez-Fernandez et al. 2011), and different tauopathies (Stone et al. 2011). Furthermore, the Parkin2 gene, known to be a frequent cause of early-onset Parkinson's disease, is a tumor suppressor gene whose inactivation results in an increase in cyclin E levels (Veeriah et al. 2010). Neuronal cell cycle reentry in pathological states has often been interpreted as de novo adult neurogenesis (Fallon et al. 2000; Arvidsson et al. 2002; Curtis et al. 2003; Becker et al. 2007). Nevertheless, this conclusion has been proved to be erroneous in some cases. For instance, the use of conditional transgenic mice expressing EGFP under the control of the nestin promoter allowed Burns et al. (2007) to demonstrate that ischemia-hypoxia-dependent BrdU incorporation mostly occurs in differentiated neurons.

In the adult nervous system, $p75^{NTR}$ is reexpressed in various neuropathological conditions, including Alzheimer's disease (Hu et al. 2002), amyotrophic lateral sclerosis (Lowry et al. 2001), Parkinson's disease (Chen et al. 2008), and Huntington's disease (Zuccato et al. 2008), likely participating in the etiology of neurodegeneration (Dechant and Barde 2002). $p75^{NTR}$ is therefore a candidate for the induction of cell cycle reentry leading to tetraploidy in the affected neurons (Frade and López-Sánchez 2010). Since developmentally regulated tetraploidy in neurons correlates with cell body and dendritic tree enlargement (see above), it is conceivable that a similar effect may occur in adult neurons undergoing de novo tetraploidization, thus explaining, at least partially, the pathological signs of the disease. Structural alterations in neurons becoming tetraploid may correlate with functional changes, including alterations of metabolic activity, higher rate of membrane biosynthesis, changes in neuronal circuits, and alterations in electrical signal propagation (see Frade and López-Sánchez 2010). Furthermore, genome duplication may result in changes of gene expression in particular loci, as those observed in liver and heart cells (Anatskaya and Vinogradov 2007) and tetraploid mouse embryos (Kawaguchi et al. 2009). It is likely that all these alterations associated with tetraploidy in neurons could functionally compromise the affected neurons. Further insights on the molecular mechanisms triggering tetraploidy in neurons will probably be crucial for the design of appropriate therapeutic tools for patients that suffer from neurodegenerative diseases.

5 Conclusion

$p75^{NTR}$ is a pleiotropic receptor that fulfills several critical functions in the nervous system, including the regulation of the cell cycle in both embryonic and adult nervous system. Indeed, $p75^{NTR}$ seems to be involved in stem cell

maintenance, neurogenesis, and neuronal tetraploidization. The molecular mechanisms used by $p75^{NTR}$ to regulate the cell cycle are far from being fully understood, but they are likely based on its capacity to interact with a number of cell cycle regulators, as well as its ability to translocate its intracellular domain to the nucleus to act as a transcriptional regulator. The capacity of $p75^{NTR}$ to regulate the cell cycle has opened new research avenues not only focused on the normal development of the vertebrate nervous system but also related with neuropathological conditions, since deregulation of this receptor in the adult brain could participate in neurodegenerative diseases. It is likely that future research in this field will yield hints of possible therapeutic approaches for these devastating diseases.

References

Ambrosino, C., & Nebreda, A. R. (2001). Cell cycle regulation by p38 MAP kinases. *Biology of the Cell, 93*, 47–51.

Anatskaya, O. V., & Vinogradov, A. E. (2007). Genome multiplication as adaptation to tissue survival: evidence from gene expression in mammalian heart and liver. *Genomics, 89*, 70–80.

Arendt, T., Brückner, M. K., Mosch, B., & Lösche, A. (2010). Selective cell death of hyperploid neurons in Alzheimer's disease. *The American Journal of Pathology, 177*, 15–20.

Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z., & Lindvall, O. (2002). Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nature Medicine, 8*, 963–970.

Bannerman, P. G., & Pleasure, D. (1993). Protein growth factor requirements of rat neural crest cells. *Journal of Neuroscience Research, 36*, 46–57.

Barde, Y. A., Edgar, D., & Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *The EMBO Journal, 1*, 549–553.

Barker, P. A., & Salehi, A. (2002). The MAGE proteins: emerging roles in cell cycle progression, apoptosis, and neurogenetic disease. *Journal of Neuroscience Research, 67*, 705–712.

Becker, M., Lavie, V., & Solomon, B. (2007). Stimulation of endogenous neurogenesis by anti-EFRH immunization in a transgenic mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 1691–1696.

Benzel, I., Barde, Y. A., & Casademunt, E. (2001). Strain-specific complementation between NRIF1 and NRIF2, two zinc finger proteins sharing structural and biochemical properties. *Gene, 281*, 19–30.

Berkemeier, L. R., Winslow, J. W., Kaplan, D. R., Nikolics, K., Goeddel, D. V., & Rosenthal, A. (1991). Neurotrophin-5: a novel neurotrophic factor that activates trk and trkB. *Neuron, 7*, 857–866.

Bernabeu, R. O., & Longo, F. M. (2010). The p75 neurotrophin receptor is expressed by adult mouse dentate progenitor cells and regulates neuronal and non-neuronal cell genesis. *BMC Neuroscience, 11*, 136.

Burns, K. A., Ayoub, A. E., Breunig, J. J., Adhami, F., Weng, W. L., Colbert, M. C., Rakic, P., & Kuan, C. Y. (2007). Nestin-CreER mice reveal DNA synthesis by nonapoptotic neurons following cerebral ischemia hypoxia. *Cerebral Cortex, 17*, 2585–2592.

Byrnes, K. R., Stoica, B. A., Fricke, S., Di Giovanni, S., & Faden, A. I. (2007). Cell cycle activation contributes to post-mitotic cell death and secondary damage after spinal cord injury. *Brain, 130*, 2977–2992.

Calzà, L., Giardino, L., Pozza, M., Bettelli, C., Micera, A., & Aloe, L. (1998). Proliferation and phenotype regulation in the subventricular zone during experimental allergic encephalomyelitis: in vivo evidence of a role for nerve growth factor. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 3209–3214.

Casaccia-Bonnefil, P., Carter, B. D., Dobrowsky, R. T., & Chao, M. V. (1996). Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. *Nature, 383*, 716–719.

Casademunt, E., Carter, B. D., Benzel, I., Frade, J. M., Dechant, G., & Barde, Y. A. (1999). The zinc finger protein NRIF interacts with the neurotrophin receptor $p75^{NTR}$ and participates in programmed cell death. *The EMBO Journal, 18*, 6050–6061.

Catts, V. S., Al-Menhali, N., Burne, T. H., Colditz, M. J., & Coulson, E. J. (2008). The p75 neurotrophin receptor regulates hippocampal neurogenesis and related behaviours. *The European Journal of Neuroscience, 28*, 883–892.

Chao, M. V., Bothwell, M. A., Ross, A. H., Koprowski, H., Lanahan, A. A., Buck, C. R., & Sehgal, A. (1986). Gene transfer and molecular cloning of the human NGF receptor. *Science, 232*, 518–521.

Chen, L. W., Yung, K. K., Chan, Y. S., Shum, D. K., & Bolam, J. P. (2008). The proNGF-$p75^{NTR}$-sortilin signalling complex as new target for the therapeutic treatment of Parkinson's disease. *CNS & Neurological Disorders Drug Targets, 7*, 512–523.

Chittka, A., & Chao, M. V. (1999). Identification of a zinc finger protein whose subcellular distribution is regulated by serum and nerve growth factor. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 10705–10710.

Chittka, A., Arevalo, J. C., Rodriguez-Guzman, M., Pérez, P., Chao, M. V., & Sendtner, M. (2004). The $p75^{NTR}$-interacting protein SC1 inhibits cell cycle progression by transcriptional repression of cyclin E. *The Journal of Cell Biology, 164*, 985–996.

Coggeshall, R. E., Yaksta, B. A., & Swartz, F. J. (1970). A cytophotometric analysis of the DNA in the nucleus of the giant cell, R-2, in Aplysia. *Chromosoma, 32*, 205–212.

Cohen, S., Levi-Montalcini, R., & Hamburger, V. (1954). A nerve growth-stimulating factor isolated from sarcom AS 37 and 180. *Proceedings of the National Academy of Sciences of the United States of America, 40*, 1014–1018.

Colditz, M. J., Catts, V. S., Al-menhali, N., Osborne, G. W., Bartlett, P. F., & Coulson, E. J. (2010). p75 neurotrophin receptor regulates basal and fluoxetine-stimulated hippocampal neurogenesis. *Experimental Brain Research, 200*, 161–167.

Costantini, C. F., Rossi, E., Formaggio, R., Bernardoni, D., Cecconi, V., & Della-Bianca, V. (2005). Characterization of the signaling pathway downstream p75 neurotrophin receptor involved in beta-amyloid peptide-dependent cell death. *Journal of Molecular Neuroscience, 25*, 141–156.

Curtis, M. A., Penney, E. B., Pearson, A. G., van Roon-Mom, W. M., Butterworth, N. J., Dragunow, M., Connor, B., & Faull, R. L. (2003). Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 9023–9027.

Dechant, G., & Barde, Y. A. (2002). The neurotrophin receptor $p75^{NTR}$: novel functions and implications for diseases of the nervous system. *Nature Neuroscience, 5*, 1131–1136.

Di Giovanni, S., Movsesyan, V., Ahmed, F., Cernak, I., Schinelli, S., Stoica, B., & Faden, A. I. (2005). Cell cycle inhibition provides neuroprotection and reduces glial proliferation and scar formation after traumatic brain injury. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 8333–8888.

Edgar, B. A., & Orr-Weaver, T. L. (2001). Endoreplication cell cycles: more for less. *Cell, 105*, 297–306.

Fahnestock, M., Michalski, B., Xu, B., & Coughlin, M. D. (2001). The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Molecular and Cellular Neurosciences, 18*, 210–220.

Fallon, J., Reid, S., Kinyamu, R., Opole, I., Opole, R., Baratta, J., Korc, M., Endo, T. L., Duong, A., Nguyen, G., Karkehabadhi, M., Twardzik, D., Patel, S., & Loughlin, S. (2000). In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 14686–14691.

Fernandez-Fernandez, M. R., Ferrer, I., & Lucas, J. J. (2011). Impaired ATF6α processing, decreased Rheb and neuronal cell cycle re-entry in Huntington's disease. *Neurobiology of Disease, 41*, 23–32.

Ferraiuolo, L., Heath, P. R., Holden, H., Kasher, P., Kirby, J., & Shaw, P. J. (2007). Microarray analysis of the cellular pathways involved in the adaptation to and progression of motor neuron injury in the SOD1 G93A mouse model of familial ALS. *The Journal of Neuroscience, 27*, 9201–9219.

Foltz, G., Ryu, G. Y., Yoon, J. G., Nelson, T., Fahey, J., Frakes, A., Lee, H., Field, L., Zander, K., Sibenaller, Z., Ryken, T. C., Vibhakar, R., Hood, L., & Madan, A. (2006). Genome-wide analysis of epigenetic silencing identifies BEX1 and BEX2 as candidate tumor suppressor genes in malignant glioma. *Cancer Research, 66*, 6665–6674.

Frade, J. M. (2000). Unscheduled re-entry into the cell cycle induced by NGF precedes cell death in nascent retinal neurones. *Journal of Cell Science, 113*, 1139–1148.

Frade, J. M. (2005). Nuclear translocation of the p75 neurotrophin receptor cytoplasmic domain in response to neurotrophin binding. *The Journal of Neuroscience, 25*, 1407–1411.

Frade, J. M., & Barde, Y. A. (1999). Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord. *Development, 126*, 683–690.

Frade, J. M., & López-Sánchez, N. (2010). A novel hypothesis for Alzheimer disease based on neuronal tetraploidy induced by $p75^{NTR}$. *Cell Cycle, 9*, 1934–1941.

Frade, J. M., Bovolenta, P., Martínez-Morales, J. R., Arribas, A., Barbas, J. A., & Rodríguez-Tébar, A. (1997). Control of early cell death by BDNF in the chick retina. *Development, 124*, 3313–3320.

Fritz, M. D., Mirnics, Z. K., Nylanderm, K. D., & Schor, N. F. (2006). $p75^{NTR}$ enhances PC12 cell tumor growth by a non-receptor mechanism involving downregulation of cyclin D2. *Experimental Cell Research, 312*, 3287–3297.

Gentry, J. J., Rutkoski, N. J., Burke, T. L., & Carter, B. D. (2004). A functional interaction between the p75 neurotrophin receptor interacting factors, TRAF6 and NRIF. *The Journal of Biological Chemistry, 279*, 16646–16656.

Giuliani, A., D'Intino, G., Paradisi, M., Giardino, L., & Calzà, L. (2004). $p75^{NTR}$-immunoreactivity in the subventricular zone of adult male rats: expression by cycling cells. *Journal of Molecular Histology, 35*, 749–758.

Greenberg, M. E., Xu, B., Lu, B., & Hempstead, B. L. (2009). New insights in the biology of BDNF synthesis and release: implications in CNS function. *The Journal of Neuroscience, 29*, 12764–12767.

Hallböök, F., Ibáñez, C. F., & Persson, H. (1991). Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. *Neuron, 6*, 845–858.

Hapner, S. J., Boeshore, K. L., Large, T. H., & Lefcort, F. (1998). Neural differentiation promoted by truncated trkC receptors in collaboration with $p75^{NTR}$. *Developmental Biology, 201*, 90–100.

Höglinger, G. U., Breunig, J. J., Depboylu, C., Rouaux, C., Michel, P. P., Alvarez-Fischer, D., Boutillier, A. L., Degregori, J., Oertel, W. H., Rakic, P., Hirsch, E. C., & Hunot, S. (2007). The pRb/E2F cell-cycle pathway mediates cell death in Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 3585–3590.

Hohn, A., Leibrock, J., Bailey, K., & Barde, Y. A. (1990). Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature, 344*, 339–341.

Hosomi, S., Yamashita, T., Aoki, M., & Tohyama, M. (2003). The p75 receptor is required for BDNF-induced differentiation of neural precursor cells. *Biochemical and Biophysical Research Communications, 301*, 1011–1015.

Hu, X. Y., Zhang, H. Y., Qin, S., Xu, H., Swaab, D. F., & Zhou, J. N. (2002). Increased $p75^{NTR}$ expression in hippocampal neurons containing hyperphosphorylated tau in Alzheimer patients. *Experimental Neurology, 178*, 104–111.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. *Annual Review of Neuroscience, 24*, 677–736.

Jiang, Y., Chen, G., Zhang, Y., Lu, L., Liu, S., & Cao, X. (2007). Nerve growth factor promotes TLR4 signaling-induced maturation of human dendritic cells in vitro through inducible $p75^{NTR}$. *Journal of Immunology, 179*, 6297–6304.

Jin, H., Pan, Y., Zhao, L., Zhai, H., Li, X., Sun, L., He, L., Chen, Y., Hong, L., Du, Y., & Fan, D. (2007). p75 neurotrophin receptor suppresses the proliferation of human gastric cancer cells. *Neoplasia, 9*, 471–478.

Johnson, D., Lanahan, A., Buck, C. R., Sehgal, A., Morgan, C., Mercer, E., Bothwell, M., & Chao, M. (1986). Expression and structure of the human NGF receptor. *Cell, 47*, 545–554.

Jones, K. R., & Reichardt, L. F. (1990). Molecular cloning of a human gene that is a member of the nerve growth factor family. *Proceedings of the National Academy of Sciences of the United States of America, 87*, 8060–8064.

Kanning, K. C., Hudson, M., Amieux, P. S., Wiley, J. C., Bothwell, M., & Schecterson, L. C. (2003). Proteolytic processing of the p75 neurotrophin receptor and two homologs generates C-terminal fragments with signaling capability. *The Journal of Neuroscience, 23*, 5425–5436.

Kaplan, D. R., Hempstead, B. L., Martin-Zanca, D., Chao, M. V., & Parada, L. F. (1991). The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. *Science, 252*, 554–558.

Kawaguchi, J., Kano, K., & Naito, K. (2009). Expression profiling of tetraploid mouse embryos in the developmental stages using a cDNA microarray analysis. *The Journal of Reproduction and Development, 55*, 670–675.

Kenchappa, R. S., Zampieri, N., Chao, M. V., Barker, P. A., Teng, H. K., Hempstead, B. L., & Carter, B. D. (2006). Ligand-dependent cleavage of the p75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. *Neuron, 50*, 219–232.

Kenchappa, R. S., Tep, C., Korade, Z., Urra, S., Bronfman, F. C., Yoon, S. O., & Carter, B. D. (2010). p75 neurotrophin receptor-mediated apoptosis in sympathetic neurons involves a biphasic activation of JNK and up-regulation of tumor necrosis factor-alpha-converting enzyme/ADAM17. *The Journal of Biological Chemistry, 285*, 20358–20368.

Khwaja, F., & Djakiew, D. (2003). Inhibition of cell-cycle effectors of proliferation in bladder tumor epithelial cells by the $p75^{NTR}$ tumor suppressor. *Molecular Carcinogenesis, 36*, 153–160.

Khwaja, F., Tabassum, A., Allen, J., & Djakiew, D. (2006). The $p75^{NTR}$ tumor suppressor induces cell cycle arrest facilitating caspase mediated apoptosis in prostate tumor cells. *Biochemical and Biophysical Research Communications, 341*, 1184–1192.

Klein, R., Jing, S. Q., Nanduri, V., O'Rourke, E., & Barbacid, M. (1991a). The trk proto-oncogene encodes a receptor for nerve growth factor. *Cell, 65*, 189–197.

Klein, R., Nanduri, V., Jing, S. A., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F., & Barbacid, M. (1991b). The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell, 66*, 395–403.

Klein, R., Lamballe, F., Bryant, S., & Barbacid, M. (1992). The trkB tyrosine protein kinase is a receptor for neurotrophin-4. *Neuron, 8*, 947–956.

Krygier, S., & Djakiew, D. (2001). The neurotrophin receptor $p75^{NTR}$ is a tumor suppressor in human prostate cancer. *Anticancer Research, 21*, 3749–3755.

Kuan, C. Y., Schloemer, A. J., Lu, A., Burns, K. A., Weng, W. L., Williams, M. T., Strauss, K. I., Vorhees, C. V., Flavell, R. A., Davis, R. J., Sharp, F. R., & Rakic, P. (2004). Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. *The Journal of Neuroscience, 24*, 10763–10772.

Kuwako, K., Taniura, H., & Yoshikawa, K. (2004). Necdin-related MAGE proteins differentially interact with the E2F1 transcription factor and the p75 neurotrophin receptor. *The Journal of Biological Chemistry, 279*, 1703–1712.

Lamballe, F., Klein, R., & Barbacid, M. (1991). trkC a new member of the trk family of tyrosine protein kinases is a receptor for neurotrophin-3. *Cell, 66*, 967–979.

Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*, 1945–1948.

Levi-Montalcini, R., & Hamburger, V. (1951). Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. *The Journal of Experimental Zoology, 116*, 321–361.

Li, H. Y., Say, E. H., & Zhou, X. F. (2007). Isolation and characterization of neural crest progenitors from adult dorsal root ganglia. *Stem Cells, 25*, 2053–2065.

López-Sánchez, N., & Frade, J. M. (2002). Control of the cell cycle by neurotrophins: lessons from the p75 neurotrophin receptor. *Histology and Histopathology, 17*, 1227–1237.

López-Sánchez, N., González-Fernández, Z., Niinobe, M., Yoshikawa, K., & Frade, J. M. (2007). Single mage gene in the chicken genome encodes CMage, a protein with functional similarities to mammalian type II Mage proteins. *Physiological Genomics, 30*, 156–171.

López-Sánchez, N., Ovejero-Benito, M. C., Borreguero, L., & Frade, J. M. (2011). Control of neuronal ploidy during vertebrate development. *Results and Problems in Cell Differentiation, 53*, 547–563.

Lowry, K. S., Murray, S. S., McLean, C. A., Talman, P., Mathers, S., Lopes, E. C., & Cheema, S. S. (2001). A potential role for the p75 low-affinity neurotrophin receptor in spinal motor neuron degeneration in murine and human amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 2*, 127–134.

Lu, B. (2003). Pro-region of neurotrophins: role in synaptic modulation. *Neuron, 39*, 735–738.

Maisonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M., & Yancopoulos, G. D. (1990). Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science, 247*, 1446–1451.

Majdan, M., Lachance, C., Gloster, A., Aloyz, R., Zeindler, C., Bamji, S., Bhakar, A., Belliveau, D., Fawcett, J., Miller, F. D., & Barker, P. A. (1997). Transgenic mice expressing the intracellular domain of the p75 neurotrophin receptor undergo neuronal apoptosis. *The Journal of Neuroscience, 17*, 6988–9698.

Manfredi Romanini, M. G., Fraschini, A., & Bernocchi, G. (1973). DNA content and nuclear area in the neurons of the cerebral ganglion in Helix pomatia. *Annales d'Histochimie, 18*, 49–58.

Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., Levesque, M., Allaire, N., Perrin, S., Sands, B., Crowell, T., Cate, R. L., McCoy, J. M., & Pepinsky, R. B. (2004). LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nature Neuroscience, 7*, 221–228.

Michalski, B., & Fahnestock, M. (2003). Pro-brain-derived neurotrophic factor is decreased in parietal cortex in Alzheimer's disease. *Brain Research. Molecular Brain Research, 111*, 148–154.

Morillo, S. M., & Frade, J. M. (2008). Nerve growth factor signaling in neural cancer and metastasis. In G. K. McIntire (Ed.), *Nerve growth factor: new research* (pp. 203–227). New York: NOVA Science Publishers.

Morillo, S. M., Escoll, P., de la Hera, A., & Frade, J. M. (2010). Somatic tetraploidy in specific chick retinal ganglion cells induced by nerve growth factor. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 109–114.

Mosch, B., Morawski, M., Mittag, A., Lenz, D., Tarnok, A., & Arendt, T. (2007). Aneuploidy and DNA replication in the normal human brain and Alzheimer's disease. *The Journal of Neuroscience, 27*, 6859–6867.

Mukai, J., Hachiya, T., Shoji-Hoshino, S., Kimura, M. T., Nadano, D., Suvanto, P., Hanaoka, T., Li, Y., Irie, S., Greene, L. A., & Sato, T. A. (2000). NADE, a $p75^{NTR}$-associated cell death executor, is involved in signal transduction mediated by the common neurotrophin receptor $p75^{NTR}$. *The Journal of Biological Chemistry, 275*, 17566–17570.

Nakamura, T., Endo, K., & Kinoshita, S. (2007). Identification of human oral keratinocyte stem/progenitor cells by neurotrophin receptor p75 and the role of neurotrophin/p75 signaling. *Stem Cells, 25*, 628–638.

Nykjaer, A., Lee, R., Teng, K. K., Jansen, P., Madsen, P., Nielsen, M. S., Jacobsen, C., Kliemannel, M., Schwarz, E., Willnow, T. E., Hempstead, B. L., & Petersen, C. M. (2004). Sortilin is essential for proNGF-induced neuronal cell death. *Nature, 427*, 843–848.

Okano, H. J., Pfaffm, D. W., & Gibbs, R. B. (1996). Expression of EGFR-, p75NGFR-, and PSTAIR^{cdc2}-like immunoreactivity by proliferating cells in the adult rat hippocampal formation and forebrain. *Developmental Neurosciences, 18*, 199–209.

Okumura, T., Shimada, Y., Imamura, M., & Yasumoto, S. (2003). Neurotrophin receptor p75^{NTR} characterizes human esophageal keratinocyte stem cells in vitro. *Oncogene, 22*, 4017–4026.

Osuga, H., Osuga, S., Wang, F., Fetni, R., Hogan, M. J., Slack, R. S., Hakim, A. M., Ikeda, J.-E., & Park, D. S. (2000). Cyclin-dependent kinases as a therapeutic target for stroke. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 10254–10259.

Parkhurst, C. N., Zampieri, N., & Chao, M. V. (2010). Nuclear localization of the p75 neurotrophin receptor intracellular domain. *The Journal of Biological Chemistry, 285*, 5361–5368.

Pelegrí, C., Duran-Vilaregut, J., del Valle, J., Crespo-Biel, N., Ferrer, I., Pallàs, M., Camins, A., & Vilaplana, J. (2008). Cell cycle activation in striatal neurons from Huntington's disease patients and rats treated with 3-nitropropionic acid. *International Journal of Developmental Neuroscience, 26*, 665–671.

Pincheira, R., Baerwald, M., Dunbar, J. D., & Donner, D. B. (2009). Sall2 is a novel p75^{NTR}-interacting protein that links NGF signalling to cell cycle progression and neurite outgrowth. *The EMBO Journal, 28*, 261–273.

Podlesniy, P., Kichev, A., Pedraza, C., Saurat, J., Encinas, M., Perez, B., Ferrer, I., & Espinet, C. (2006). Pro-NGF from Alzheimer's disease and normal human brain displays distinctive abilities to induce processing and nuclear translocation of intracellular domain of p75^{NTR} and apoptosis. *The American Journal of Pathology, 169*, 119–131.

Radeke, M. J., Misko, T. P., Hsu, C., Herzenberg, L. A., & Shooter, E. M. (1987). Gene transfer and molecular cloning of the rat nerve growth factor receptor. *Nature, 325*, 593–597.

Ranganathan, S., & Bowser, R. (2003). Alterations in G_1 to S phase cell-cycle regulators during amyotrophic lateral sclerosis. *The American Journal of Pathology, 162*, 823–835.

Ranganathan, S., & Bowser, R. (2010). p53 and cell cycle proteins participate in spinal motor neuron cell death in ALS. *The Open Pathology Journal, 4*, 11–22.

Reynolds, B. A., & Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science, 255*, 1707–1710.

Rodríguez-Tébar, A., Dechant, G., & Barde, Y. A. (1990). Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron, 4*, 487–492.

Rodríguez-Tébar, A., Dechant, G., Götz, R., & Barde, Y. A. (1992). Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. *The EMBO Journal, 11*, 917–922.

Rosenthal, A., Goeddel, D. V., Nguyen, T., Lewis, M., Shih, A., Laramee, G. R., Nikolics, K., & Winslow, J. W. (1990). Primary structure and biological activity of a novel human neurotrophic factor. *Neuron, 4*, 767–773.

Salehi, A. H., Roux, P. P., Kubu, C. J., Zeindler, C., Bhakar, A., Tannis, L. L., Verdi, J. M., & Barker, P. A. (2000). NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis. *Neuron, 27*, 279–288.

Skeldal, S., Matusica, D., Nykjaer, A., & Coulson, E. J. (2011). Proteolytic processing of the p75 neurotrophin receptor: a prerequisite for signalling?: Neuronal life, growth and death signalling are crucially regulated by intra-membrane proteolysis and trafficking of p75(NTR). *Bioessays, 33*, 614–625.

Sotthibundhu, A., Li, Q. X., Thangnipon, W., & Coulson, E. J. (2009). $A\beta_{1\text{-}42}$ stimulates adult SVZ neurogenesis through the p75 neurotrophin receptor. *Neurobiology of Aging, 30*, 1975–1985.

Stone, J. G., Siedlak, S. L., Tabaton, M., Hirano, A., Castellani, R. J., Santocanale, C., Perry, G., Smith, M. A., Zhu, X., & Lee, H. G. (2011). The cell cycle regulator phosphorylated retinoblastoma protein is associated with tau pathology in several tauopathies. *Journal of Neuropathology and Experimental Neurology, 70*, 578–587.

Susen, K., Heumann, R., & Blöchl, A. (1999). Nerve growth factor stimulates MAPK via the low affinity receptor p75^{LNTR}. *FEBS Letters, 463*, 231–234.

Swartz, F. J., & Bhatnagar, K. P. (1981). Are CNS neurons polyploid? A critical analysis based upon cytophotometric study of the DNA content of cerebellar and olfactory bulbar neurons of the bat. *Brain Research, 208*, 267–281.

Swift, H. (1953). Quantitative aspects of nuclear nucleoproteins. *International Review of Cytology, 2*, 1–76.

Szaro, B. G., & Tompkins, R. (1987). Effect of tetraploidy on dendritic branching in neurons and glial cells of the frog, Xenopus laevis. *The Journal of Comparative Neurology, 258*, 304–316.

Taniura, H., Taniguchi, N., Hara, M., & Yoshikawa, K. (1998). Necdin, a postmitotic neuron-specific growth suppressor, interacts with viral transforming proteins and cellular transcription factor E2F1. *The Journal of Biological Chemistry, 273*, 720–728.

Tcherpakov, M., Bronfman, F. C., Conticello, S. G., Vaskovsky, A., Levy, Z., Niinobe, M., Yoshikawa, K., Arenas, E., & Fainzilber, M. (2002). The p75 neurotrophin receptor interacts with multiple MAGE proteins. *The Journal of Biological Chemistry, 277*, 49101–49104.

Teng, H. K., Teng, K. K., Lee, R., Wright, S., Tevar, S., Almeida, R. D., Kermani, P., Torkin, R., Chen, Z. Y., Lee, F. S., Kraemer, R. T., Nykjaer, A., & Hempstead, B. L. (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of $p75^{NTR}$ and sortilin. *The Journal of Neuroscience, 25*, 5455–5463.

Ullah, Z., Lee, C. Y., Lilly, M. A., & DePamphilis, M. L. (2009). Developmentally programmed endoreduplication in animals. *Cell Cycle, 8*, 1501–1509.

Urdiales, J. L., Becker, E., Andrieu, M., Thomas, A., Jullien, J., van Grunsven, L. A., Menut, S., Evan, G. I., Martín-Zanca, D., & Rudkin, B. B. (1998). Cell cycle phase-specific surface expression of nerve growth factor receptors TrkA and $p75^{NTR}$. *The Journal of Neuroscience, 18*, 6767–6775.

Veeriah, S., Morris, L., Solit, D., & Chan, T. A. (2010). The familial Parkinson disease gene PARK2 is a multisite tumor suppressor on chromosome 6q25.2-27 that regulates cyclin E. *Cell Cycle, 9*, 1451–1452.

Verbeke, S., Meignan, S., Lagadec, C., Germain, E., Hondermarck, H., Adriaenssens, E., & Le Bourhis, X. (2010). Overexpression of $p75^{NTR}$ increases survival of breast cancer cells through $p21^{waf1}$. *Cellular Signalling, 22*, 1864–1873.

Verdaguer, E., Jiménez, A., Canudas, A. M., Jordà, E. G., Sureda, F. X., Pallàs, M., & Camins, A. (2004). Inhibition of cell cycle pathway by flavopiridol promotes survival of cerebellar granule cells after an excitotoxic treatment. *The Journal of Pharmacology and Experimental Therapeutics, 308*, 609–616.

Vilar, M., Murillo-Carretero, M., Mira, H., Magnusson, K., Besset, V., & Ibáñez, C. F. (2006). Bex1, a novel interactor of the p75 neurotrophin receptor, links neurotrophin signaling to the cell cycle. *The EMBO Journal, 25*, 1219–1230.

Volosin, M., Trotter, C., Cragnolini, A., Kenchappa, R. S., Light, M., Hempstead, B. L., Carter, B. D., & Friedman, W. J. (2008). Induction of proneurotrophins and activation of $p75^{NTR}$-mediated apoptosis via neurotrophin receptor-interacting factor in hippocampal neurons after seizures. *The Journal of Neuroscience, 28*, 9870–9879.

Wang, K. C., Kimm, J. A., Sivasankaran, R., Segal, R., & He, Z. (2002). p75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature, 420*, 74–78.

Wang, W., Bu, B., Xie, M., Zhang, M., Yu, Z., & Tao, D. (2009). Neural cell cycle dysregulation and central nervous system diseases. *Progress in Neurobiology, 89*, 1–17.

Wen, C. J., Xue, B., Qin, W. X., Yu, M., Zhang, M. Y., Zhao, D. H., Gao, X., Gu, J. R., & Li, C. J. (2004). hNRAGE, a human neurotrophin receptor interacting MAGE homologue, regulates p53 transcriptional activity and inhibits cell proliferation. *FEBS Letters, 564*, 171–176.

Woo, N. H., Teng, H. K., Siao, C. J., Chiaruttini, C., Pang, P. T., Milner, T. A., Hempstead, B. L., & Lu, B. (2005). Activation of $p75^{NTR}$ by proBDNF facilitates hippocampal long-term depression. *Nature Neuroscience, 8*, 1069–1077.

Woods, J., Snape, M., & Smith, M. A. (2007). The cell cycle hypothesis of Alzheimer's disease: suggestions for drug development. *Biochimica et Biophysica Acta, 1772*, 503–508.

Yang, Y., Geldmacher, D. S., & Herrup, K. (2001). DNA replication precedes neuronal cell death in Alzheimer's disease. *The Journal of Neuroscience, 21*, 2661–2668.

Yankner, B. A., & Shooter, E. M. (1982). The biology and mechanism of action of nerve growth factor. *Annual Review of Biochemistry, 51*, 845–868.

Yano, H., Torkin, R., Martin, L. A., Chao, M. V., & Teng, K. K. (2009). Proneurotrophin-3 is a neuronal apoptotic ligand: evidence for retrograde-directed cell killing. *The Journal of Neuroscience, 29*, 14790–14802.

Young, K. M., Mersonm, T. D., Sotthibundhum, A., Coulson, E. J., & Bartlett, P. F. (2007). p75 neurotrophin receptor expression defines a population of BDNF-responsive neurogenic precursor cells. *The Journal of Neuroscience, 27*, 5146–5155.

Yuanlong, H., Haifeng, J., Xiaoyin, Z., Jialin, S., Jie, L., Li, Y., Huahong, X., Jiugang, S., Yanglin, P., Kaichun, W., Jie, D., & Daiming, F. (2008). The inhibitory effect of p75 neurotrophin receptor on growth of human hepatocellular carcinoma cells. *Cancer Letters, 268*, 110–119.

Zhang, W., & Liu, H. T. (2002). MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Research, 12*, 9–18.

Zhang, W., Zeng, Y. S., Wang, J. M., Ding, Y., Li, Y., & Wu, W. (2009). Neurotrophin-3 improves retinoic acid-induced neural differentiation of skin-derived precursors through a $p75^{NTR}$-dependent signaling pathway. *Neuroscience Research, 64*, 170–176.

Zhu, W., Cheng, S., Xu, G., Ma, M., Zhou, Z., Liu, D., & Liu, X. (2011). Intranasal nerve growth factor enhances striatal neurogenesis in adult rats with focal cerebral ischemia. *Drug Delivery, 18*, 338–343.

Zuccato, C., Marullo, M., Conforti, P., MacDonald, M. E., Tartari, M., & Cattaneo, E. (2008). Systematic assessment of BDNF and its receptor levels in human cortices affected by Huntington's disease. *Brain Pathology, 18*, 225–238.

p75NTR Processing and Signaling: Functional Role

Ramiro D. Almeida and Carlos B. Duarte

Contents

Abstract

The p75 neurotrophin receptor ($p75^{NTR}$) was the first neurotrophin receptor to be identified and, more than two decades after, significant advances have been made in understanding its biological actions. $p75^{NTR}$ belongs to the tumor necrosis factor receptor superfamily (TNFRS) and is particularly important during developmental neural cell death and under injury conditions in the adulthood. However, recent studies shed new light into the functions of $p75^{NTR}$ in the nervous system. $p75^{NTR}$ either acting autonomously or as part of a complex with other co-receptors regulates neuronal survival and differentiation, cell cycle and proliferation, axonal outgrowth, and synaptic

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plasticity. Here, we will review the main findings of the last two decades as well as new findings that reveal additional and unexpected roles for $p75^{NTR}$.

Keywords
Axonal growth • Cell cycle • Neuronal death • Neurotrophins • p75NTR • Synaptic plasticity

1 Introduction

The p75 neurotrophin receptor ($p75^{NTR}$) belongs to the tumor necrosis factor receptor superfamily (TNFRS) and was first identified by Shooter and colleagues (reviewed in Herrup and Shooter 1973). $p75^{NTR}$ has an important role in the development of the nervous system and accordingly is mainly expressed during development, decreasing in the adulthood, except in a few selected populations of neurons and under pathological conditions, when it is re-expressed (reviewed in Dechant and Barde 2002). It should also be emphasized that $p75^{NTR}$ has critical roles in glial biology and outside the nervous system, namely, in the immune and vascular systems.

All known mammalian neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT-4/5), bind to $p75^{NTR}$ with similar affinities. However, they bind their cognate tropomyosin-related kinase (Trk) receptor with different specificities. Generally, NGF binds TrkA, BDNF and NT-4/5 activate TrkB, and NT-3 recognizes TrkC, and this selectivity is believed to promote neuronal survival and differentiation in distinct populations of neurons (reviewed in Lu et al. 2005). Neurotrophins can also induce cell death by activating $p75^{NTR}$ and this causes an apparent contradiction. The latter effects are mediated by proneurotrophins, the immature uncleaved form, which bind $p75^{NTR}$ with relatively higher affinity than to Trk receptors, thereby promoting cell death. Accordingly, the mature forms of neurotrophins preferentially bind Trk receptors (Lee et al. 2001; Teng et al. 2005). This specificity is achieved through the formation of molecular complexes with different co-receptors. While mature neurotrophins bind to a complex of $p75^{NTR}$ and Trk, the proforms preferentially bind to a complex of $p75^{NTR}$ and Sortilin, another type I transmembrane protein, but structurally unrelated to $p75^{NTR}$ (reviewed in Nykjaer et al. 2005). This feature reveals a distinctive characteristic of $p75^{NTR}$: signaling independently and in conjunction with its co-receptors. The best studied $p75^{NTR}$ partner is the Trk receptor, and the molecular basis of their interaction has been extensively studied. $p75^{NTR}$ is able to form complexes with TrkA, TrkB, and TrkC, altering its affinity and signaling properties (reviewed in Chao 2003). Recent studies revealed new roles for $p75^{NTR}$ in the nervous system and along with it new co-receptors and cytosolic interactors were described. In fact, $p75^{NTR}$ was described to regulate additional aspects of neuronal development like axonal growth, cell cycle, and synaptic plasticity (reviewed in Ibanez and Simi 2012). Although exciting, these novel observations raise the question of how $p75^{NTR}$ signaling specificity is achieved

with a relatively high number of signaling partners. It is believed that such specificity of $p75^{NTR}$ actions is likely to be dependent on the developmental and cellular context in which the receptor is integrated.

2 Expression: Isoforms and Co-receptors

The $p75^{NTR}$ is widely expressed in the nervous system during development. In the developing CNS, the $p75^{NTR}$ is present in spinal motor neurons and brain stem motor nuclei, lateral geniculate nucleus, medial terminal nucleus of the accessory optic tract, ventral and dorsal cochlear nucleus, thalamic nucleus, nucleus of the lateral lemniscus, amygdala, cortical subplate neurons, olivary pretectal nucleus, cuneate nucleus, and gracile nucleus, in addition to Purkinje neurons and the external granule layer and deep nuclei of the cerebellum (Buck et al. 1988; Ernfors et al. 1988; Heuer et al. 1990; Schatteman et al. 1988). In the adult nervous system, the $p75^{NTR}$ is found mainly within the basal forebrain (Hefti et al. 1986; Schatteman et al. 1988; Springer et al. 1987; Yan and Johnson 1988), and lower expression levels are observed in the caudate/putamen neurons (Henry et al. 1994), cerebellar Purkinje neurons (Cohen-Cory et al. 1991; Koh et al. 1989; Mufson et al. 1991; Shelton and Reichardt 1986), motor neurons (Armstrong et al. 1991; Ernfors et al. 1989), and within several central nuclei (Cohen-Cory et al. 1991; Koh et al. 1989; Schatteman et al. 1988; Shelton and Reichardt 1986; Sofroniew et al. 1989). $p75^{NTR}$ is expressed in neurons and glia (Cragnolini and Friedman 2008), and their expression is induced by many types of injury, including transient forebrain or global cerebral ischemia (Junier et al. 1994; Lee et al. 1995; Oderfeld-Nowak et al. 2003), in ischemia induced by cortical devascularization (Angelo et al. 2009), and in the brain of rats subjected to pilocarpine-induced seizures (Roux et al. 1999; Unsain et al. 2008) [for a detailed review see Ibanez and Simi (2012)]. In the peripheral nervous system, the $p75^{NTR}$ is expressed in dorsal root ganglia sensory neurons as well as in a subpopulation of enteric and parasympathetic neurons (Carroll et al. 1992; Schatteman et al. 1993; Verge et al. 1992; Yan and Johnson 1988). Axotomy of peripheral motor neurons, resembling the lesions resulting from severe traumatic injuries of the limbs, was also shown to upregulate $p75^{NTR}$ expression in adult animals (Ferri et al. 2002). Furthermore, $p75^{NTR}$ is present outside the nervous system, where the highest expression of the receptor is found (reviewed in Barker 1998).

The $p75^{NTR}$ belongs to the tumor necrosis factor family of receptors (TNFR), comprising an extracellular region with four cysteine-rich domains, each with six cysteine residues, a single transmembrane domain, and the intracellular region. The latter region contains two domains that are able to mediate intracellular signaling, the Chopper domain and the death domain homologous to that present in the TNFR family (reviewed in Skeldal et al. 2011). The Chopper domain is a juxtamembrane region with the ability to induce cell death when bound to the membrane (Coulson et al. 2000; Underwood et al. 2008) (Fig. 1). The $p75^{NTR}$ can bind all four neurotrophins (NGF, BDNF, NT3, and NT4/NT5) (Rodriguez-Tebar et al. 1992;

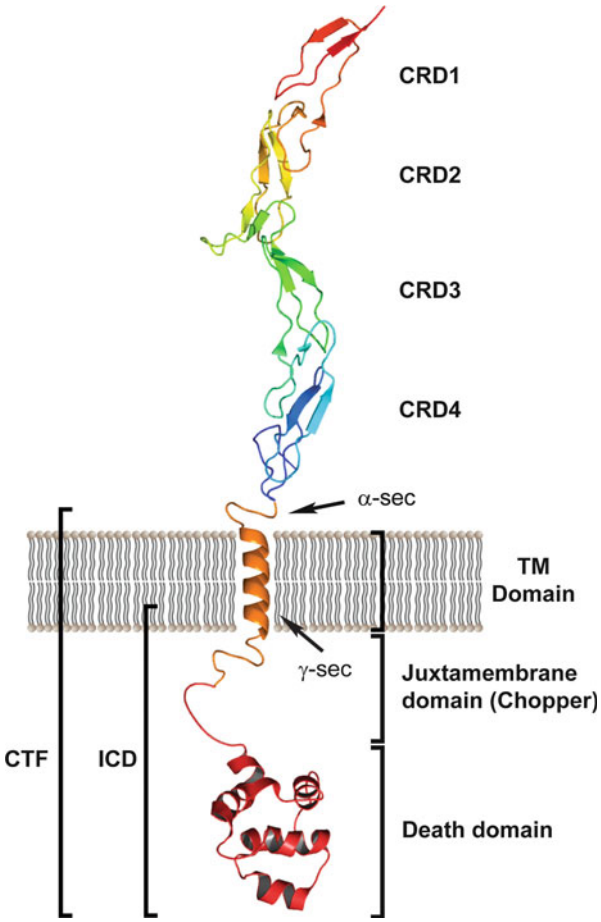

Fig. 1 $p75^{NTR}$ protein structure. $p75^{NTR}$ has an extracellular domain with four cysteine-rich repeats, characteristic of TNFR superfamily members, a transmembrane domain, and an intracellular death domain. Neurotrophins bind to cysteine-rich domains 2–4, which are coded in Exon III, leading to a conformational change that ultimately results in the recruitment and activation of downstream signaling pathways. The *black arrows* indicate the sites of α- and γ-secretase cleavage (regulated intramembrane proteolysis). Abbreviations: *CRD* cysteine-rich domain, *TM domain* transmembrane domain, *CTF* C-terminal fragment, *ICD* intracellular domain

Ryden et al. 1995; Urfer et al. 1994), as well as to proNGF and proBDNF (Lee et al. 2001; Teng et al. 2005). The crystal structure of the extracellular domain of $p75^{NTR}$ complexed with NGF (Aurikko et al. 2005) or NT3 (Gong et al. 2008) showed a symmetrical 2:2 receptor-ligand complex. However, a different study reported that $p75^{NTR}$ binds NGF in a 1:2 stoichiometry (He and Garcia 2004), which may correspond to an intermediate step before a symmetrical 2:2 receptor-ligand complex is formed. The $p75^{NTR}$ forms dimers even in the absence of neurotrophins through cysteinyl residues within the transmembrane domain, and it was proposed that the interaction of the neurotrophins with the receptor complex induces a conformational change in the $p75^{NTR}$ intracellular domain which may allow the recruitment of the effector molecules NRIF and TRAF6 (Vilar et al. 2009a, b). The activation of $p75^{NTR}$ by all neurotrophins contrasts with the more stringent specificity of the Trk family of receptors for neurotrophins.

The biological effects of proNGF and proBDNF mediated by activation of $p75^{NTR}$ require the co-receptor sortilin which interacts with the prodomain of the

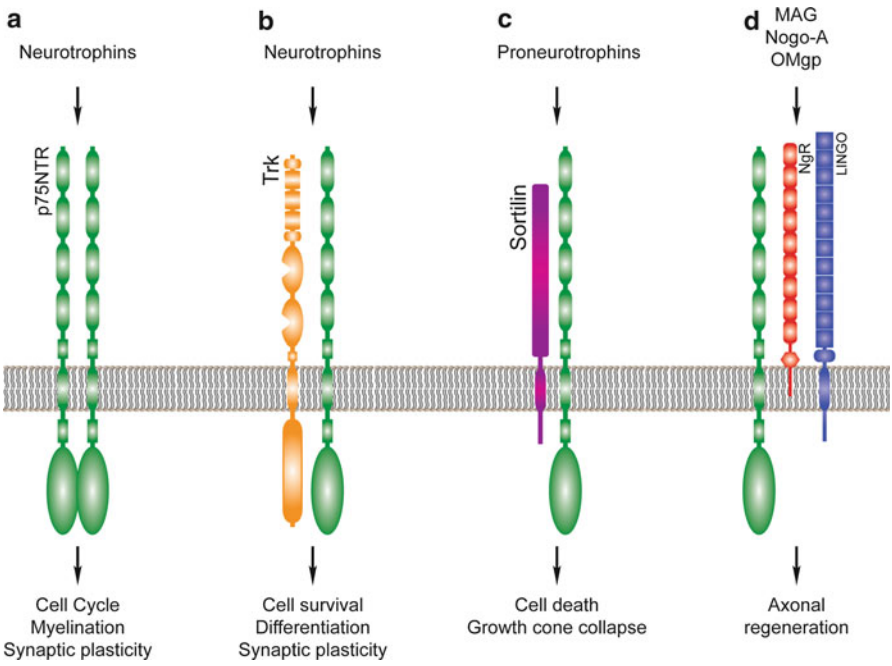

Fig. 2 $p75^{NTR}$ co-receptor complexes and their biological function. To date, $p75^{NTR}$ was shown to interact with a diversity of receptors, namely, Sortilin, LINGO-1, NgR, and Trk receptors. The outcome of these interactions is a variety of cellular responses including cell death, synaptic plasticity, neurite outgrowth and cell cycle regulation. The C-terminal fragment of $p75^{NTR}$ can also bind other cytosolic interactors (not depicted here), increasing the diversity and complexity of signaling mechanisms that are regulated by this receptor. Abbreviations: *MAG* myelin-associated glycoprotein, *OMGP* oligodendrocyte myelin glycoprotein

neurotrophins (Nykjaer et al. 2004; Teng et al. 2005) (Fig. 2c). However, it is still not yet clear whether each prodomain of the dimeric neurotrophin molecules binds to a different sortilin molecule or whether two prodomains bind to the same sortilin protein (Feng et al. 2010). During development, sortilin is expressed in the brain cortex, hippocampus, and neural retina, and this pattern is preserved in the adulthood, when the protein is particularly abundant in pyramidal cells of the hippocampus (Hermans-Borgmeyer et al. 1999; Sarret et al. 2003). Activation of the $p75^{NTR}$/sortilin complex by proNGF or proBFNF was shown to induce apoptotic cell death in cultured sympathetic neurons, but this effect was not observed in cells derived from mice lacking $p75^{NTR}$ or in the presence of the sortilin antagonist neurotensin (Nykjaer et al. 2004; Teng et al. 2005).

A truncated isoform of $p75^{NTR}$ has been described, resulting from alternative splicing and lacking the cysteine-rich repeats 2, 3 and 4 (s-$p75^{NTR}$) (Paul et al. 2004; von Schack et al. 2001), which are required for neurotrophin binding (Aurikko et al. 2005; Feng et al. 2010; Gong et al. 2008; He and Garcia 2004). Although these variants are unable to bind neurotrophins, they may regulate cellular activity by

interacting with some of the intracellular signaling proteins regulated by the full-length $p75^{NTR}$. Furthermore, a neurotrophin receptor homologue-2 (NRH2, also termed PLAIDD or NRADD) was described, which lacks the ligand-binding domain present in the $p75^{NTR}$ but has some similarity with its transmembrane, juxtamembrane and the death domain regions (Kanning et al. 2003; Murray et al. 2004). The expression of NRH2 receptors is developmentally regulated, decreasing in adult tissues (Frankowski et al. 2002; Wang et al. 2003). Although the homology between $p75^{NTR}$ and NRH2 suggests that it may act as a functional analogue of the s-$p75^{NTR}$, the differences between the intracellular domains of the two receptor proteins may provide some specificity in the biological activity of NRH2 receptors (Kim and Hempstead 2009). NRH2 facilitates the formation of a complex between $p75^{NTR}$ and sortilin, and was proposed to act by impairing the degradation of sortilin in the lysosomes and by redistributing sortilin to the surface (Kim and Hempstead 2009). However, additional studies are required to determine the stoichiometry of the interactions between $p75^{NTR}$, sortilin, and NRH2.

In addition to its signaling activity, the $p75^{NTR}$ may also interact with the TrkA (Huber and Chao 1995), TrkB, and TrkC receptors (Bibel et al. 1999), thereby modulating their activity. In particular, the $p75^{NTR}$/TrkA complexes show an increased affinity for NGF when compared with each one of the receptors expressed independently (Hempstead et al. 1991), and the co-expression of the two classes of receptors allows TrkA-mediated responses at lower concentrations of NGF (Horton et al. 1997; Lee et al. 1994). The TrkA and $p75^{NTR}$ were shown to interact through their intracellular domains even before NGF stimulation, and this association may contribute to the observed high-affinity NGF-binding sites (Iacaruso et al. 2011).

3 $p75^{NTR}$ Proteolysis and Signaling in Cell Death and Survival

The absence of catalytic activity in the $p75^{NTR}$ intracellular region suggests that the intracellular signaling activity is mediated by binding partners that may be recruited upon receptor activation and/or constitutively bound. In fact, more than 20 different intracellular binding partners of $p75^{NTR}$ were identified up to now (Barker 2004; Bronfman and Fainzilber 2004; Dechant and Barde 2002; Roux and Barker 2002; Schecterson and Bothwell 2010).

Initial studies showed a role for $p75^{NTR}$ in the facilitation of survival signals induced by Trk receptors, activated by the mature form of neurotrophins, and this effect has been attributed to an increase in the affinity of the Trk receptor for the ligand (Roux and Barker 2002) (Fig. 2b). Furthermore, $p75^{NTR}$ increases the specificity of Trk receptors for particular ligands, which may change the pattern of signaling response to stimulation by each neurotrophin (Clary and Reichardt 1994). The mechanism whereby $p75^{NTR}$ increases Trk receptor activity has not been elucidated, but should involve the intracellular region of the receptor since extracellular domain mutations of $p75^{NTR}$ proteins also allow the formation of

high-affinity Trk binding sites (Esposito et al. 2001). This is supported by results obtained with Förster resonance energy transfer showing that $p75^{NTR}$ and TrkA receptors physically interact through their intracellular domains, even before stimulation with NGF (Iacaruso et al. 2011). A model was proposed according to which Trk receptor activation leads to activation of the transmembrane cysteine protease α-secretase tumor necrosis factor-α converting enzyme/a disintegrin and metallopeptidase domain (TACE/ADAM) 17 by MEK-dependent phosphorylation, which is followed by $p75^{NTR}$ cleavage. TACE/ADAM17 displays α-secretase activity, cleaving $p75^{NTR}$ in the extracellular juxtamembrane domain, releasing the extracellular domain and giving rise to a product that remains anchored at the membrane. According to this model, the $p75^{NTR}$ cleavage product feeds back to enhance Trk-induced neurotrophin signaling, thereby increasing ERK and Akt activity which enhance cell survival (Ceni et al. 2010; Kommaddi et al. 2011). Whether the $p75^{NTR}$ cleavage products directly interact with Trk receptors to increase the affinity for ligands remains to be elucidated.

The $p75^{NTR}$ may also be coupled to cell death when activated by proneurotrophins, and in this case, sortilin has been shown to act as a co-receptor, increasing the affinity of $p75^{NTR}$ for the ligand (Nykjaer et al. 2004). Sortilin interacts with the prodomain of neurotrophins, and the signaling that leads to cell death may occur in the absence or in the presence of Trk receptors. The role of $p75^{NTR}$ and sortilin in proneurotrophin-induced cell death is supported by studies showing that antibodies against sortilin, $p75^{NTR}$, or proNGF protect cells from apoptotic death (Beattie et al. 2002; Nykjaer et al. 2004; Volosin et al. 2006, 2008). Furthermore, an excess of the prodomain of proNGF or neurotensin, which also binds sortilin (Quistgaard et al. 2009), inhibits proNGF-induced cell death (Jansen et al. 2007; Nykjaer et al. 2004; Rogers et al. 2010; Volosin et al. 2006).

An interesting feature of $p75^{NTR}$ function is its ability to undergo proteolysis with the generation of fragments with signaling capability. $p75^{NTR}$ proteolysis was described to be required for cell survival (addressed below), cell migration, neurite growth inhibition, and cell cycle. In fact, several intracellular domain interactors that regulate cell cycle, like Schwann cell factor-1 (Sc-1), brain-expressed X-linked 1 (Bex1), and melanoma antigen gene (MAGE), have been found in the nucleus after $p75^{NTR}$ activation (reviewed in Skeldal et al. 2011), but $p75^{NTR}$ regulated intramembrane proteolysis (RIP) has been best studied in neuronal death. The process of $p75^{NTR}$ proteolytic cleavage occurs through the sequential action of two distinct proteins. First, the extracellular domain (ECD) of $p75^{NTR}$ is cleaved by TACE/ADAM17, generating a membrane-bound C-terminal fragment (CTF). The CTF undergoes a second cleavage by the presenilin-dependent γ-secretase, releasing the intracellular domain (ICD) into the cytoplasm (Jung et al. 2003; Kanning et al. 2003) (Fig. 3). NRH2 can also undergo proteolytic cleavage, releasing its cytoplasmic domain, which unlike the ICD has higher stability (Kanning et al. 2003).

The $p75^{NTR}$ ICD regulates neuronal cell death in vitro and in vivo. Neurotrophin binding to $p75^{NTR}$ was shown to promote γ-secretase-dependent

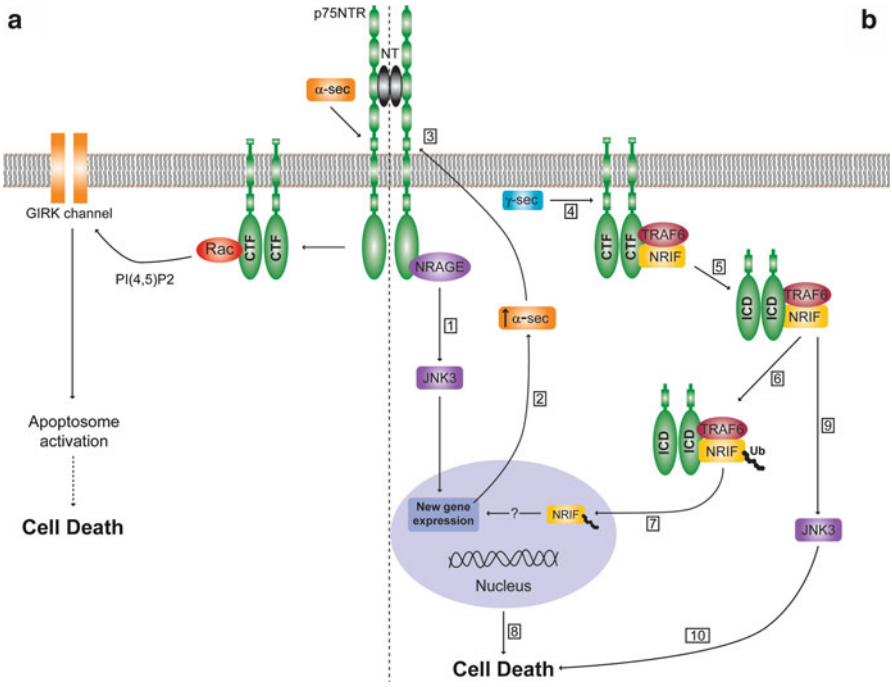

Fig. 3 $p75^{NTR}$ proteolysis and cell death signaling pathways. Regulated intracellular proteolysis of $p75^{NTR}$ leading to neuronal cell death can act through two distinct pathways. (**a**) In one model, the 29 amino acid intracellular juxtamembrane region of $p75^{NTR}$ named "chopper" induces cell death through activation of G-protein-coupled inwardly rectifying potassium (GIRK/Kir3) channels. The corresponding potassium efflux supposedly leads to apoptosome activation and by consequence neuronal cell death. (**b**) In another model $p75^{NTR}$ activates JNK 3 (*1*), which leads to the transcription-dependent upregulation of α-secretase (*2*) that cleaves $p75^{NTR}$ extracellular domain (*3*). γ-secretase releases the ICD into the cytoplasm (*4*) which associates with the DNA-binding protein NRIF and the E3 ubiquitin ligase TRAF6 (*5*). The formation of this ternary complex leads to TRAF6-dependent ubiquitination of NRIF (*6*) and nuclear translocation (*7*), a process required for $p75^{NTR}$-dependent cell death (*8*). In parallel, this ternary complex further activates JNK3 (*9*), amplifying the apoptotic signal (*10*). Abbreviations: *JNK3* c-Jun N-terminal kinase 3, *NRAGE* neurotrophin-receptor-interacting MAGE (melanoma-associated antigen) homologue

cleavage, with the cytosolic release of ICD and subsequent nuclear translocation (Frade 2005; Kenchappa et al. 2006). The complete picture is however considerably more complicated and involves a dual-phase signaling mechanism. Stimulation of the receptor with BDNF induces the activation of JNK3, leading to the upregulation of TACE/ADAM17 by a transcription-dependent mechanism (Kenchappa et al. 2010). The $p75^{NTR}$ is first cleaved by TACE/ADAM17 and then by the γ-secretase complex, releasing the intracellular domain (ICD) of the receptor and allowing the translocation of neurotrophin-receptor-interacting factor (NRIF) to the nucleus. The $p75^{NTR}$ ICD can also translocate to the nucleus

where it regulates gene expression (Parkhurst et al. 2010). The nuclear translocation of NRIF depends on its polyubiquitination mediated by the E3 ubiquitin ligase TRAF6, and is necessary for $p75^{NTR}$-mediated apoptosis (Geetha et al. 2005; Kenchappa et al. 2006). In addition, the ICD/TRAF6/NRIF complex can activate JNK3 directly further amplifying the cell death cascade (Fig. 3b). NRIF nuclear translocation seems to be of particular importance in the period of naturally occurring superior cervical ganglion (SCG) neuron death since this protein is observed in the nucleus of SCG neurons at postnatal day 4 but not at postnatal day 24, a developmental time point when neuron elimination has diminished (Kenchappa et al. 2010). How the nuclear translocation of NRIF induces apoptosis has not yet been elucidated, but considering that NRIF is a zinc finger DNA-binding protein, it may act through altering the expression of proapoptotic genes (Kenchappa et al. 2006). NRIF was also shown to mediate the apoptotic signaling initiated by $p75^{NTR}$ in hippocampal neurons after pilocarpine-induced seizures (Volosin et al. 2008). The protein NRAGE (neurotrophin-receptor-interacting MAGE homolog), a member of the MAGE (melanoma antigen) family, also interacts with the $p75^{NTR}$ intracellular region, facilitating the receptor-induced apoptotic death (Salehi et al. 2000). The effects of NRAGE are mediated by stimulation of JNK and c-Jun, which is followed by the cytochrome C release from the mitochondria, and activation of caspases–9, –3, and –7 to induce cell death (Salehi et al. 2002).

Under certain conditions, the C-terminal fragment of $p75^{NTR}$ resulting from metalloprotease cleavage may induce cell death without additional cleavage by γ-secretase to generate the ICD (Coulson et al. 2000, 2008; Underwood et al. 2008). The $p75^{NTR}$ CTF contains a cytoplasmic juxtamembrane sequence of 29 amino acids that is both necessary and sufficient to trigger cell death (Coulson et al. 2000). According to the model proposed, the cleavage of $p75^{NTR}$ followed by palmitoylation of the CTF causes its translocation to cholesterol-rich domains of the plasma membrane (Underwood et al. 2008). The $p75^{NTR}$ CTF upregulates the production of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) through activation of Rac, and the locally generated PtdIns(4,5)P_2 activate G-protein-coupled inwardly rectifying potassium (GIRK/Kir3) channels (Coulson et al. 2008) (Fig. 3a). The resulting increase in K^+ permeability with consequent reduction in the $[K^+]_i$ may contribute to induce the apoptotic pathway. However, additional studies are required to determine under which conditions this pathway is active in vivo and how the CTF- and the ICD-mediated pathways are differentially regulated.

In addition to the activation of apoptotic signaling pathways, $p75^{NTR}$ may also be coupled to the induction of cellular alterations that prevent the trophic effects of Trk neurotrophin receptors, thereby contributing to cell death. Such a mechanism was described in basal forebrain neurons, one of the few CNS populations that express $p75^{NTR}$ throughout life. Stimulation of $p75^{NTR}$ with proNGF upregulates PTEN (phosphatase and tensin homolog deleted on chromosome 10) in basal forebrain neurons, a protein known to antagonize the activation of Akt by PI3-K. When the cells are stimulated simultaneously with proNGF and BDNF, to activate

$p75^{NTR}$ and TrkB receptors, respectively, the inhibition of the TrkB-induced PI3-K signaling by PTEN is sufficient to abrogate the trophic effects of TrkB signaling (Song et al. 2010). Accordingly, inhibition of PTEN was shown to prevent neuronal loss in the hippocampus following pilocarpine-induced seizures, a model of $p75^{NTR}$-mediated neuronal death (Song et al. 2010).

4 Axonal Growth

The antagonistic functions of neurotrophins and their receptors are well depicted in sympathetic neuron growth and target innervations. NGF acting through TrkA induces axonal growth, while BDNF acting on $p75^{NTR}$ inhibits it (Kohn et al. 1999). These evidences are supported in vivo where the pineal gland, a sympathetic target organ, of BDNF −/− mice is hyperinnervated (Kohn et al. 1999). This axonal pruning depends on activity-dependent release of BDNF by the wining axons that in turn acts on $p75^{NTR}$ present in the losing axons, antagonizing NGF/TrkA (Singh and Miller 2005; Singh et al. 2008). This model provides an elegant explanation of how competing axons can promote growth inhibition and axonal degeneration on less favored neighboring axons.

Currently, it is widely accepted that central nervous system (CNS) neurons do not have the ability to regenerate upon injury. Several factors account for this phenomenon such as the scar generation at the injury site, reduced regrowth capability of mature neurons, and myelin-associated inhibitory factors (MAIFs) (Chen et al. 2009). Myelin-derived molecules such as myelin-associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (oMgp), and Nogo exert their action through a common receptor, the Nogo receptor (NgR) (Fournier et al. 2001), and this effect is mediated by activation of a small G protein, the Ras homologue member A (RhoA) (Niederost et al. 2002; Yamashita et al. 2002). However, NgR is a glycosyl-phosphatidylinositol (GPI)-linked membrane protein, with no cytoplasmic domain and therefore, it is not capable of transducing an appropriate signal upon ligand binding. Subsequently, it was demonstrated that $p75^{NTR}$ is a co-receptor for NgR (Wang et al. 2002; Wong et al. 2002). Neurons from $p75^{NTR}$ knockout mice are no longer responsive to myelin, and blocking NgR-$p75^{NTR}$ interaction has a similar effect (Wang et al. 2002). However, in a reconstituted nonneuronal system, NgR and $p75^{NTR}$ alone are not able to activate RhoA, indicating that an additional molecule or molecules are required. Exogenous expression of a dominant negative LINGO-1 in cerebellar granular neurons reduced myelin inhibition (Mi et al. 2004) (Fig. 2d), indicating LINGO-1 forms a signaling complex with NgR and $p75^{NTR}$.

$p75^{NTR}$ directly regulates RhoA activation. Barde and colleagues demonstrated that neurotrophin binding to $p75^{NTR}$ inhibits RhoA activation, increasing the rate of neurite outgrowth (Yamashita et al. 1999). A similar effect is observed on filopodial dynamics where neurotrophin treatment of retinal or dorsal root ganglion (DRG) neurons leads to a decrease in RhoA activity, which in turns increases filopodial length. On the other hand, when MAIFs bind to $p75^{NTR}$, RhoA is activated and

neurite outgrowth reduced. This effect is mediated by the Rho-GDP dissociation inhibitor (Rho-GDI) which interacts with the cytoplasmic domain of $p75^{NTR}$. Usually, RhoA is bound to Rho-GDI, but the MAG or Nogo proteins facilitate the interaction between $p75^{NTR}$ and Rho-GDI, releasing RhoA (Yamashita and Tohyama 2003), which then leads to actin depolymerization. Additionally, MAG induces α- and then γ-secretase-mediated proteolysis of $p75^{NTR}$ and this cleavage is required for the activation of RhoA and neurite outgrowth inhibition (Domeniconi et al. 2005). However, $p75^{NTR}$ is only expressed in selected populations of mature neurons, raising the question of how myelin inhibitory signals are transduced in $p75^{NTR}$-lacking cells. TROY/TAJ, another TNFR homologue, broadly expressed in postnatal and adult neurons, can also bind NgR and LINGO-1, to form a functional complex and activate RhoA in the presence of myelin inhibitors (Park et al. 2005; Shao et al. 2005). Together, these studies established that $p75^{NTR}$ has a crucial role in axonal regeneration.

It is puzzling why the nervous system would increase the expression of $p75^{NTR}$ when its effects are so deleterious to neurons. Currently, there is no clear answer to this question, but we can speculate that the detrimental effects of $p75^{NTR}$ in neuronal injury could be due to a deregulation of a homeostatic mechanism (reviewed in Ibanez and Simi 2012). In line with this, a recent study in the uninjured adult mice brain showed that myelin acting through $p75^{NTR}$ is responsible for the axonal degeneration of aberrant septal cholinergic axons projecting to the cerebral cortex and hippocampus through the supra-callosal pathway (Park et al. 2010). Interestingly, this effect required BDNF binding to $p75^{NTR}$ since an antibody against BDNF rescued myelin-induced inhibition of axonal growth and axonal degeneration (Park et al. 2010). These results provide an explanation to the intrinsic inability of CNS neurons to regrow on myelin inhibitory substrates.

$p75^{NTR}$ also regulates growth cone collapse. Recently, it was shown that proNGF induces growth cone collapse in cultured hippocampal neurons. Binding of proNGF to a $p75^{NTR}$ and sortilin-related VPS10 domain-containing receptor 2 (SorCS2) complex decreases Rac activity and inactivates fascin, an actin-bundling protein, regulating actin dynamics (Deinhardt et al. 2011). A single nucleotide polymorphism (SNP) is present on the 5′ proBDNF sequence at codon 66, producing an amino acid substitution (Val66Met). In humans, the Met66BDNF was associated with abnormal hippocampal activation and poorer episodic memory (Egan et al. 2003). Interestingly, preliminary studies suggest that recombinant Met66 but not Val66 BDNF prodomain induces growth cone collapse in cultured hippocampal neurons and this effect is dependent on $p75^{NTR}$ and SorCS2 (Anastasia et al. 2013). The authors claim Met66 prodomain is intrinsically disordered with some transient secondary structure. However, it is not clear how proNGF and proBDNF(Val66), which share a high degree of homology in their prodomain, elicit different results.

Recently, $p75^{NTR}$ has been involved in two other systems of axonal guidance, Sema3A and ephrin-A. Eph/ephrins are membrane proteins that regulate neuronal migration, synapse formation, and axon guidance. The eph/ephrin system is divided into class A and B, and they can signal bidirectionally; eph signaling is considered

forward while ephrin signaling is considered reverse (reviewed in Xu and Henkemeyer 2012). Similarly to the Nogo receptor, class A ephrins are GPI-linked proteins and require a co-receptor as a transducing unit. Using retinal ganglion cells (RGCs), a widely used axon guidance model system, O'Leary and colleagues reported that ephrin-As colocalize with $p75^{NTR}$ in retinal axons and this association is required for Fyn phosphorylation upon ephA binding (Lim et al. 2008). Moreover, this requirement is specific to ephrin reverse signaling since eph forward signaling is not affected (Lim et al. 2008). Sema3A is a secreted protein that regulates growth cone collapse and repulsion, and signals through plexins and neuropilins (Pasterkamp 2012). Sema3A was found to increase the co-localization of $p75^{NTR}$ and neuropilin-1 in DRG growth cones, and inhibits the direct association of plexinA4 with neuropilin-1, presumably by sequestering the latter receptor. Less plexinA4/neuropilin-1 complex leads to increased sensitivity of DRG neurons from $p75^{NTR}$ (ExonIII−/−) mice to Sema3A (Ben-Zvi et al. 2007).

5 Regulation of Cell Cycle

The $p75^{NTR}$ is expressed very early during development of the nervous system (Yan and Johnson 1987, 1988), when many precursor cells are still not differentiated and before targeting specific neuronal populations for programmed cell death. The role of $p75^{NTR}$ signaling in the modulation of cell cycle progression has been investigated in vitro and in vivo. Thus, the pheochromocytoma PC12 cells differentiate in response to NGF when stimulated in the G1 phase (but not during the other phases of the cell cycle) (Rudkin et al. 1989), and this effect correlates with the transient surface expression of $p75^{NTR}$ (Ito et al. 2002, 2003; Urdiales et al. 1998). Furthermore, stimulation of $p75^{NTR}$ with NGF was shown to prevent the progression through the G1 phase of the cell cycle in astrocytes (Cragnolini et al. 2009; Cragnolini et al. 2012). Activation of $p75^{NTR}$ was also shown to play a role in BDNF-induced differentiation of neural precursor cells in culture (Hosomi et al. 2003) and may be involved in the development of dendrites by purified subventricular cells cultured in the presence of neurotrophins (Gascon et al. 2005).

In contrast with the differentiating effects described above, $p75^{NTR}$ receptors were shown to mediate the NGF-induced proliferation of mouse embryonic stem cells in culture (Moscatelli et al. 2009). $p75^{NTR}$ is also expressed in adult dentate gyrus progenitor cells, playing a role in the proliferation and/or early maturation of neuronal and glial cells, as well as other cell types (Bernabeu and Longo 2010). Furthermore, in vitro and in vivo studies identified a population of highly proliferative subventricular zone cells in the adult rat, characterized by the expression of $p75^{NTR}$ and showing an increased rate of neuroblast generation in response to BDNF (Giuliani et al. 2004; Young et al. 2007). However, under disease or injury conditions, the potential of these $p75^{NTR}$-expressing cells present in the subventricular zone may be altered, to yield glial progenitors in addition to their neurogenic potential. In patients and in animal models of multiple sclerosis,

the $p75^{NTR}$-positive cells in the subventricular zone may give rise to oligodendrocyte progenitors (Petratos et al. 2004). The amyloid-beta peptide (Aβ), which is accumulated in amyloid plaques and generally recognized as a key feature of Alzheimer's disease, was also shown to stimulate adult SVZ neurogenesis, by a mechanism dependent on the expression of $p75^{NTR}$ by the precursor cells and activation of the receptors through cleavage by metalloproteases (Sotthibundhu et al. 2009). This correlates with the results showing an interaction of Aβ with $p75^{NTR}$ (Yaar et al. 1997, 2002), but was observed mainly in younger animals. Based on these observations, it was suggested that overstimulation of $p75^{NTR}$-positive progenitor cells early in life may lead to the depletion of a pool of stem cells with a consequent downregulation in basal neurogenesis (Sotthibundhu et al. 2009). Excitotoxic stimulation of the adult striatum also induces the expression of the $p75^{NTR}$ protein in astrocytic progenitor cells (Hanbury et al. 2002), and pilocarpine-induced seizures upregulated $p75^{NTR}$ protein levels in neurons and astrocytes (Cragnolini et al. 2009; Cragnolini et al. 2012). Since $p75^{NTR}$ controls the astrocytic proliferation (see above) and its ligand (NGF) is produced by astrocytes under inflammatory conditions (Friedman et al. 1996; Oderfeld-Nowak and Bacia 1994), NGF may have an autocrine or paracrine effect in the regulation of reactive gliosis in vivo after injury.

The diversity of mechanisms contributing to the differential effects of $p75^{NTR}$ and NGF on cell cycle is not fully understood, but these responses are likely to be mediated by the interaction of the receptor with different intracellular proteins under distinct cellular contexts. Several of the $p75^{NTR}$ binding partners are regulators of the cell cycle, and their interaction with the receptors is controlled by ligand binding. The zinc finger protein SC1 interacts with the intracellular region of the $p75^{NTR}$, being translocated to the nucleus following stimulation of COS cells with NGF (Chittka and Chao 1999). SC1 acts as a transcriptional repressor and may interact with histone deacetylases to repress the promitotic genes cyclin E and B, thereby inducing growth arrest (Chittka et al. 2004). The NRAGE (neurotrophin-receptor-interacting MAGE homolog) protein also interacts with the $p75^{NTR}$, and it is thought to compete with TrkA receptors for binding to a juxtamembrane domain of the receptor (Salehi et al. 2000). Interaction of NRAGE with the $p75^{NTR}$ is mediated by a conserved sequence called MAGE homology domain, which also determines the interaction of necdin and MAGE-H1 (two MAGE proteins) with $p75^{NTR}$. The interaction of these two MAGE proteins with the $p75^{NTR}$ is enhanced by ligand stimulation, which may contribute to the recruitment of a signaling complex to induce differentiation (Tcherpakov et al. 2002). Similarly, the $p75^{NTR}$ binding partner NRAGE was shown to facilitate cell cycle exit in HEK293 cells (Salehi et al. 2000). Sall2 also interacts and sequesters $p75^{NTR}$, and activation of TrkA receptors is required to induce the nuclear translocation of the protein with consequent activation of target genes. The induction of $p21^{WAF1/CIP1}$ may mediate the effects of Sall2 on NGF-dependent cell cycle arrest and growth inhibition (Pincheira et al. 2009). In contrast with SC1, MAGE proteins, and Sall2, which induce cell cycle arrest, Bex1 (brain-expressed X-linked 1) competes with the receptor interacting protein 2 (RIP2) for binding to the $p75^{NTR}$, thereby maintaining

proliferation and preventing differentiation. Studies performed in astrocytes, which express $p75^{NTR}$ but not TrkA receptors, showed that NGF attenuates the EGF-induced upregulation of cyclins D1 and E, inhibits the interaction between cyclin D1 and Cdk4, which is required for progression from G1 into S phase, and upregulates cyclin kinase inhibitory proteins (Cragnolini et al. 2012). These evidences indicate that $p75^{NTR}$ receptors may act at different levels to induce cell cycle arrest in a given cell.

6 Synaptic Plasticity

Neurotrophins were found to modulate synaptic transmission and plasticity. Valuable insight came from observing the peripheral nervous system, particularly the heart sympathetic innervation. This system can be reproduced in vitro by co-culturing SGCs neurons with cardiac myocytes where acute application of norepinephrine increases the contraction frequency of myocytes while acetylcholine has the opposite effect (Furshpan et al. 1976). Neurotrophins regulate sympathetic neuron cotransmission by modulating the activity-dependent release of norepinephrine and acetylcholine (Lockhart et al. 1997; Yang et al. 2002). Similarly to other neuronal functions described throughout this chapter, Trk receptors and $p75^{NTR}$ function antagonistically. NGF induces potentiation of excitatory noradrenergic transmission that is mediated by the TrkA receptor (Lockhart et al. 1997), while BDNF induces a shift from excitatory to inhibitory (cholinergic) transmission acting through the $p75^{NTR}$ (Yang et al. 2002). CaMKII acts downstream of $p75^{NTR}$ to control the shift from excitatory to inhibitory transmission since presynaptic inhibition of CaMKII activity or expression of constitutively active CaMKII blocks or mimics BDNF-induced inhibitory transmission respectively (Slonimsky et al. 2006).

Synaptic plasticity in the CNS induced by BDNF has been particularly well studied (Carvalho et al. 2008; Santos et al. 2010). Application of BDNF or NT-3 induced an enhancement of synaptic strength at the Schaffer collateral-CA1 synapses (Kang and Schuman 1995). Accordingly, BDNF regulates long-term potentiation (LTP) in the hippocampus (Figurov et al. 1996; Korte et al. 1996; Patterson et al. 1996) and in the visual cortex (Akaneya et al. 1997; Huber et al. 1998). Application of exogenous BDNF is able to induce LTP in hippocampal slices, and this effect is blocked by BDNF scavengers like BDNF antibodies or TrkB-IgG fusion proteins (Chen et al. 1999; Figurov et al. 1996; Pozzo-Miller et al. 1999). In line with these evidences, BDNF knockout animals show impaired LTP in the CA1 region of the hippocampus, and exogenous BDNF or re-expression of BDNF using a virus-based strategy restored LTP in BDNF-deficient mice (Korte et al. 1996; Patterson et al. 1996). An additional layer of complexity was added when Pang et al. showed tPA is responsible for plasmin-dependent conversion of proBDNF into mature BDNF and this is required for late-phase long-term potentiation (L-LTP) (Pang et al. 2004). However, another study reports proBDNF is rapidly converted intracellularly to mature BDNF and the mature but not the precursor form

is secreted upon stimulation. Moreover, the authors show hippocampal long-term depression (LTD) is unaffected in slices prepared from conditional BDNF KO animals (Matsumoto et al. 2008). The reasons for these conflicting results are still elusive and additional studies are required to clarify these divergent observations.

The opposing roles of $p75^{NTR}$ and Trk receptors in the nervous system led the investigators to ask if the $p75^{NTR}$ could negatively regulate structural and functional plasticity. As hypothesized, $p75^{NTR-/-}$ mice have higher spine density and greater dendritic complexity than wild-type mice. Accordingly, overexpression of $p75^{NTR}$ decreased dendritic complexity and the number of spines (Zagrebelsky et al. 2005). Interestingly the phenotype of p75 (ExonIV−/−) animals, which lack both the FL-p75 and s-p75 isoforms, is more dramatic than the one observed in p75 (EXONIII −/−) animals. The authors claim this could be due to a compensatory effect by the s-p75 isoform (Zagrebelsky et al. 2005). Additionally, Korte and colleagues showed that $p75^{NTR}$-null animals have impaired hippocampal LTD while LTP is unchanged, with the expression levels of GluA2 and GluA3 subunits of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor in the hippocampus being altered in the $p75^{NTR}$ null animals (Rosch et al. 2005). In line with these studies, proBNDF was shown to induce NMDA-dependent LTD in the hippocampus which is mediated by $p75^{NTR}$ and the GluN2B subunit. Blocking the function of $p75^{NTR}$ or GluN2B abolishes proBDNF enhancement of LTD (Woo et al. 2005). Furthermore, GluN2B-mediated synaptic currents and expression are reduced in the hippocampus of $p75^{NTR-/-}$ mice (Woo et al. 2005). However, at the moment, there is no direct link between $p75^{NTR}$ and NMDA receptors and it remains to be determined if $p75^{NTR}$ regulates the trafficking or endocytosis of GluN2B and/or of GluA2/3. In Xenopus neuromuscular synapses, exogenous proBDNF induces synaptic depression. Moreover, stimulation-induced release of proBDNF from myocytes leads to synaptic depression and retraction and this effect is mediated by presynaptic $p75^{NTR}$ (Yang et al. 2009).

Paralleling the effects attributed to $p75^{NTR}$ in synaptic plasticity has proven more difficult in behavioral animal models. Some studies claim $p75^{NTR}$ null animals have behavioral impairments, particularly deficits in memory task acquisition (Peterson et al. 1999; Wright et al. 2004), while others presented evidences for better spatial learning performance in $p75^{NTR}$ knockout mice when compared to control animals (Barrett et al. 2010; Greferath et al. 2000). The different genetic background of the animals used in these studies could account for the conflicting results, but further investigation is required to reconcile this discrepancies. Taken together, these results show that $p75^{NTR}$ has a role in activity-dependent synaptic plasticity.

7 Conclusion

Although $p75^{NTR}$ was the first neurotrophin receptor to be identified, for many years, it stood in the shadow of the Trk receptor with a mere supporting role. We now know that $p75^{NTR}$ plays a major role in the nervous system, especially during development and in injury scenarios, but with the latest developments, we face the

risk of this vision also becoming outdated. Recent studies provided a fresh new look into $p75^{NTR}$ function, for example, the diversity of mechanisms induced to promote cell cycle arrest in a given cell, its role in axonal pruning, and the specificity of neural connectivity in the adult brain (Park et al. 2010; Singh et al. 2008). Also, unforeseen $p75^{NTR}$ partners and consequently new functions were identified, adding new layers of complexity to $p75^{NTR}$ function. It will be of the outmost importance to design experiments to understand how these different ligands, co-receptors, and cytosolic interactors, acting through the same receptor, activate the specific signaling pathways that are responsible for the variety of physiological functions attributed to the $p75^{NTR}$. It is tempting to speculate that the different microdomains within a neuronal cell could provide the most needed functional specificity but this is unlikely to be the sole answer to this question. A major challenge will be to understand the spatial and temporal expression patterns of $p75^{NTR}$, its co-receptors, homologues, and intracellular partners, to have an integrative view of $p75^{NTR}$ function in the nervous system.

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References

Akaneya, Y., Tsumoto, T., Kinoshita, S., & Hatanaka, H. (1997). Brain-derived neurotrophic factor enhances long-term potentiation in rat visual cortex. *The Journal of Neuroscience, 17*, 6707–6716.

Anastasia, A., Deinhardt, K., Chao, M. V., Will, N. E., Irmady, K., Lee, F. S., Hempstead, B. L., & Bracken, C. (2013). Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. *Nat Commun, 4*, 2490.

Angelo, M. F., Aviles-Reyes, R. X., Villarreal, A., Barker, P., Reines, A. G., & Ramos, A. J. (2009). $p75^{NTR}$ expression is induced in isolated neurons of the penumbra after ischemia by cortical devascularization. *Journal of Neuroscience Research, 87*, 1892–1903.

Armstrong, D. M., Brady, R., Hersh, L. B., Hayes, R. C., & Wiley, R. G. (1991). Expression of choline acetyltransferase and nerve growth factor receptor within hypoglossal motoneurons following nerve injury. *The Journal of Comparative Neurology, 304*, 596–607.

Aurikko, J. P., Ruotolo, B. T., Grossmann, J. G., Moncrieffe, M. C., Stephens, E., Leppanen, V. M., Robinson, C. V., Saarma, M., Bradshaw, R. A., & Blundell, T. L. (2005). Characterization of symmetric complexes of nerve growth factor and the ectodomain of the pan-neurotrophin receptor, $p75^{NTR}$. *The Journal of Biological Chemistry, 280*, 33453–33460.

Barker, P. A. (1998). $p75^{NTR}$: A study in contrasts. *Cell Death and Differentiation, 5*, 346–356.

Barker, P. A. (2004). $p75^{NTR}$ is positively promiscuous: Novel partners and new insights. *Neuron, 42*, 529–533.

Barrett, G. L., Reid, C. A., Tsafoulis, C., Zhu, W., Williams, D. A., Paolini, A. G., Trieu, J., & Murphy, M. (2010). Enhanced spatial memory and hippocampal long-term potentiation in p75 neurotrophin receptor knockout mice. *Hippocampus, 20*, 145–152.

Beattie, M. S., Harrington, A. W., Lee, R., Kim, J. Y., Boyce, S. L., Longo, F. M., Bresnahan, J. C., Hempstead, B. L., & Yoon, S. O. (2002). ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron, 36*, 375–386.

Ben-Zvi, A., Ben-Gigi, L., Klein, H., & Behar, O. (2007). Modulation of semaphorin3A activity by p75 neurotrophin receptor influences peripheral axon patterning. *The Journal of Neuroscience, 27*, 13000–13011.

Bernabeu, R. O., & Longo, F. M. (2010). The p75 neurotrophin receptor is expressed by adult mouse dentate progenitor cells and regulates neuronal and non-neuronal cell genesis. *BMC Neuroscience, 11*, 136.

Bibel, M., Hoppe, E., & Barde, Y. A. (1999). Biochemical and functional interactions between the neurotrophin receptors trk and $p75^{NTR}$. *The EMBO Journal, 18*, 616–622.

Bronfman, F. C., & Fainzilber, M. (2004). Multi-tasking by the p75 neurotrophin receptor: Sortilin things out? *EMBO Reports, 5*, 867–871.

Buck, C. R., Martinez, H. J., Chao, M. V., & Black, I. B. (1988). Differential expression of the nerve growth factor receptor gene in multiple brain areas. *Brain Research. Developmental Brain Research, 44*, 259–268.

Carroll, S. L., Silos-Santiago, I., Frese, S. E., Ruit, K. G., Milbrandt, J., & Snider, W. D. (1992). Dorsal root ganglion neurons expressing trk are selectively sensitive to NGF deprivation in utero. *Neuron, 9*, 779–788.

Carvalho, A. L., Caldeira, M. V., Santos, S. D., & Duarte, C. B. (2008). Role of the brain-derived neurotrophic factor at glutamatergic synapses. *British Journal of Pharmacology, 153*(Suppl 1), S310–S324.

Ceni, C., Kommaddi, R. P., Thomas, R., Vereker, E., Liu, X., McPherson, P. S., Ritter, B., & Barker, P. A. (2010). The $p75^{NTR}$ intracellular domain generated by neurotrophin-induced receptor cleavage potentiates Trk signaling. *Journal of Cell Science, 123*, 2299–2307.

Chao, M. V. (2003). Neurotrophins and their receptors: A convergence point for many signalling pathways. *Nature Reviews. Neuroscience, 4*, 299–309.

Chen, G., Kolbeck, R., Barde, Y. A., Bonhoeffer, T., & Kossel, A. (1999). Relative contribution of endogenous neurotrophins in hippocampal long-term potentiation. *The Journal of Neuroscience, 19*, 7983–7990.

Chen, Y., Zeng, J., Cen, L., Wang, X., Yao, G., Wang, W., Qi, W., & Kong, K. (2009). Multiple roles of the p75 neurotrophin receptor in the nervous system. *The Journal of International Medical Research, 37*, 281–288.

Chittka, A., & Chao, M. V. (1999). Identification of a zinc finger protein whose subcellular distribution is regulated by serum and nerve growth factor. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 10705–10710.

Chittka, A., Arevalo, J. C., Rodriguez-Guzman, M., Perez, P., Chao, M. V., & Sendtner, M. (2004). The $p75^{NTR}$-interacting protein SC1 inhibits cell cycle progression by transcriptional repression of cyclin E. *The Journal of Cell Biology, 164*, 985–996.

Clary, D. O., & Reichardt, L. F. (1994). An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 11133–11137.

Cohen-Cory, S., Dreyfus, C. F., & Black, I. B. (1991). NGF and excitatory neurotransmitters regulate survival and morphogenesis of cultured cerebellar Purkinje cells. *The Journal of Neuroscience, 11*, 462–471.

Coulson, E. J., Reid, K., Baca, M., Shipham, K. A., Hulett, S. M., Kilpatrick, T. J., & Bartlett, P. F. (2000). Chopper, a new death domain of the p75 neurotrophin receptor that mediates rapid neuronal cell death. *The Journal of Biological Chemistry, 275*, 30537–30545.

Coulson, E. J., May, L. M., Osborne, S. L., Reid, K., Underwood, C. K., Meunier, F. A., Bartlett, P. F., & Sah, P. (2008). p75 neurotrophin receptor mediates neuronal cell death by activating GIRK channels through phosphatidylinositol 4,5-bisphosphate. *The Journal of Neuroscience, 28*, 315–324.

Cragnolini, A. B., & Friedman, W. J. (2008). The function of $p75^{NTR}$ in glia. *Trends in Neurosciences, 31*, 99–104.

Cragnolini, A. B., Huang, Y., Gokina, P., & Friedman, W. J. (2009). Nerve growth factor attenuates proliferation of astrocytes via the p75 neurotrophin receptor. *Glia, 57*, 1386–1392.

Cragnolini, A. B., Volosin, M., Huang, Y., & Friedman, W. J. (2012). Nerve growth factor induces cell cycle arrest of astrocytes. *Developmental Neurobiology, 72*, 766–776.

Dechant, G., & Barde, Y. A. (2002). The neurotrophin receptor p75(NTR): Novel functions and implications for diseases of the nervous system. *Nature Neuroscience, 5*, 1131–1136.

Deinhardt, K., Kim, T., Spellman, D. S., Mains, R. E., Eipper, B. A., Neubert, T. A., Chao, M. V., & Hempstead, B. L. (2011). Neuronal growth cone retraction relies on proneurotrophin receptor signaling through Rac. *Science Signaling, 4*, ra82.

Domeniconi, M., Zampieri, N., Spencer, T., Hilaire, M., Mellado, W., Chao, M. V., & Filbin, M. T. (2005). MAG induces regulated intramembrane proteolysis of the p75 neurotrophin receptor to inhibit neurite outgrowth. *Neuron, 46*, 849–855.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., et al. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257–269.

Ernfors, P., Hallbook, F., Ebendal, T., Shooter, E. M., Radeke, M. J., Misko, T. P., & Persson, H. (1988). Developmental and regional expression of beta-nerve growth factor receptor mRNA in the chick and rat. *Neuron, 1*, 983–996.

Ernfors, P., Henschen, A., Olson, L., & Persson, H. (1989). Expression of nerve growth factor receptor mRNA is developmentally regulated and increased after axotomy in rat spinal cord motoneurons. *Neuron, 2*, 1605–1613.

Esposito, D., Patel, P., Stephens, R. M., Perez, P., Chao, M. V., Kaplan, D. R., & Hempstead, B. L. (2001). The cytoplasmic and transmembrane domains of the p75 and Trk A receptors regulate high affinity binding to nerve growth factor. *The Journal of Biological Chemistry, 276*, 32687–32695.

Feng, D., Kim, T., Ozkan, E., Light, M., Torkin, R., Teng, K. K., Hempstead, B. L., & Garcia, K. C. (2010). Molecular and structural insight into proNGF engagement of $p75^{NTR}$ and sortilin. *Journal of Molecular Biology, 396*, 967–984.

Ferri, C. C., Ghasemlou, N., Bisby, M. A., & Kawaja, M. D. (2002). Nerve growth factor alters p75 neurotrophin receptor-induced effects in mouse facial motoneurons following axotomy. *Brain Research, 950*, 180–185.

Figurov, A., Pozzo-Miller, L. D., Olafsson, P., Wang, T., & Lu, B. (1996). Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature, 381*, 706–709.

Fournier, A. E., GrandPre, T., & Strittmatter, S. M. (2001). Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. *Nature, 409*, 341–346.

Frade, J. M. (2005). Nuclear translocation of the p75 neurotrophin receptor cytoplasmic domain in response to neurotrophin binding. *The Journal of Neuroscience, 25*, 1407–1411.

Frankowski, H., Castro-Obregon, S., del Rio, G., Rao, R. V., & Bredesen, D. E. (2002). PLAIDD, a type II death domain protein that interacts with p75 neurotrophin receptor. *Neuromolecular Medicine, 1*, 153–170.

Friedman, W. J., Thakur, S., Seidman, L., & Rabson, A. B. (1996). Regulation of nerve growth factor mRNA by interleukin-1 in rat hippocampal astrocytes is mediated by NFkappaB. *The Journal of Biological Chemistry, 271*, 31115–31120.

Furshpan, E. J., MacLeish, P. R., O'Lague, P. H., & Potter, D. D. (1976). Chemical transmission between rat sympathetic neurons and cardiac myocytes developing in microcultures: Evidence for cholinergic, adrenergic, and dual-function neurons. *Proceedings of the National Academy of Sciences of the United States of America, 73*, 4225–4229.

Gascon, E., Vutskits, L., Zhang, H., Barral-Moran, M. J., Kiss, P. J., Mas, C., & Kiss, J. Z. (2005). Sequential activation of p75 and TrkB is involved in dendritic development of subventricular zone-derived neuronal progenitors in vitro. *The European Journal of Neuroscience, 21*, 69–80.

Geetha, T., Kenchappa, R. S., Wooten, M. W., & Carter, B. D. (2005). TRAF6-mediated ubiquitination regulates nuclear translocation of NRIF, the p75 receptor interactor. *The EMBO Journal, 24*, 3859–3868.

Giuliani, A., D'Intino, G., Paradisi, M., Giardino, L., & Calza, L. (2004). p75(NTR)-immunoreactivity in the subventricular zone of adult male rats: Expression by cycling cells. *Journal of Molecular Histology, 35*, 749–758.

Gong, Y., Cao, P., Yu, H. J., & Jiang, T. (2008). Crystal structure of the neurotrophin-3 and $p75^{NTR}$ symmetrical complex. *Nature, 454*, 789–793.

Greferath, U., Bennie, A., Kourakis, A., Bartlett, P. F., Murphy, M., & Barrett, G. L. (2000). Enlarged cholinergic forebrain neurons and improved spatial learning in p75 knockout mice. *The European Journal of Neuroscience, 12*, 885–893.

Hanbury, R., Charles, V., Chen, E. Y., Leventhal, L., Rosenstein, J. M., Mufson, E. J., & Kordower, J. H. (2002). Excitotoxic and metabolic damage to the rodent striatum: Role of the P75 neurotrophin receptor and glial progenitors. *The Journal of Comparative Neurology, 444*, 291–305.

He, X. L., & Garcia, K. C. (2004). Structure of nerve growth factor complexed with the shared neurotrophin receptor p75. *Science, 304*, 870–875.

Hefti, F., Hartikka, J., Salvatierra, A., Weiner, W. J., & Mash, D. C. (1986). Localization of nerve growth factor receptors in cholinergic neurons of the human basal forebrain. *Neuroscience Letters, 69*, 37–41.

Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R., Parada, L. F., & Chao, M. V. (1991). High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. *Nature, 350*, 678–683.

Henry, M. A., Westrum, L. E., Bothwell, M., & Press, S. (1994). Electron microscopic localization of nerve growth factor receptor (p75)-immunoreactivity in pars caudalis/medullary dorsal horn of the cat. *Brain Research, 642*, 137–145.

Hermans-Borgmeyer, I., Hermey, G., Nykjaer, A., & Schaller, C. (1999). Expression of the 100-kDa neurotensin receptor sortilin during mouse embryonal development. *Brain Research. Molecular Brain Research, 65*, 216–219.

Herrup, K., & Shooter, E. M. (1973). Properties of the beta nerve growth factor receptor of avian dorsal root ganglia. *Proceedings of the National Academy of Sciences of the United States of America, 70*, 3884–3888.

Heuer, J. G., Fatemie-Nainie, S., Wheeler, E. F., & Bothwell, M. (1990). Structure and developmental expression of the chicken NGF receptor. *Developmental Biology, 137*, 287–304.

Horton, A., Laramee, G., Wyatt, S., Shih, A., Winslow, J., & Davies, A. M. (1997). NGF binding to p75 enhances the sensitivity of sensory and sympathetic neurons to NGF at different stages of development. *Molecular and Cellular Neurosciences, 10*, 162–172.

Hosomi, S., Yamashita, T., Aoki, M., & Tohyama, M. (2003). The p75 receptor is required for BDNF-induced differentiation of neural precursor cells. *Biochemical and Biophysical Research Communications, 301*, 1011–1015.

Huber, L. J., & Chao, M. V. (1995). A potential interaction of p75 and trkA NGF receptors revealed by affinity crosslinking and immunoprecipitation. *Journal of Neuroscience Research, 40*, 557–563.

Huber, K. M., Sawtell, N. B., & Bear, M. F. (1998). Brain-derived neurotrophic factor alters the synaptic modification threshold in visual cortex. *Neuropharmacology, 37*, 571–579.

Iacaruso, M. F., Galli, S., Marti, M., Villalta, J. I., Estrin, D. A., Jares-Erijman, E. A., & Pietrasanta, L. I. (2011). Structural model for p75(NTR)-TrkA intracellular domain interaction: A combined FRET and bioinformatics study. *Journal of Molecular Biology, 414*, 681–698.

Ibanez, C. F., & Simi, A. (2012). p75 neurotrophin receptor signaling in nervous system injury and degeneration: Paradox and opportunity. *Trends in Neurosciences, 37*, 431–440.

Ito, H., Nomoto, H., & Furukawa, S. (2002). Role of low-affinity p75 receptor in nerve growth factor-inducible growth arrest of PC12 cells. *Journal of Neuroscience Research, 69*, 653–661.

Ito, H., Nomoto, H., & Furukawa, S. (2003). Growth arrest of PC12 cells by nerve growth factor is dependent on the phosphatidylinositol 3-kinase/Akt pathway via p75 neurotrophin receptor. *Journal of Neuroscience Research, 72*, 211–217.

Jansen, P., Giehl, K., Nyengaard, J. R., Teng, K., Lioubinski, O., Sjoegaard, S. S., Breiderhoff, T., Gotthardt, M., Lin, F., Eilers, A., et al. (2007). Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. *Nature Neuroscience, 10*, 1449–1457.

Jung, K. M., Tan, S., Landman, N., Petrova, K., Murray, S., Lewis, R., Kim, P. K., Kim, D. S., Ryu, S. H., Chao, M. V., & Kim, T. W. (2003). Regulated intramembrane proteolysis of the p75 neurotrophin receptor modulates its association with the TrkA receptor. *The Journal of Biological Chemistry, 278*, 42161–42169.

Junier, M. P., Suzuki, F., Onteniente, B., & Peschanski, M. (1994). Target-deprived CNS neurons express the NGF gene while reactive glia around their axonal terminals contain low and high affinity NGF receptors. *Brain Research. Molecular Brain Research, 24*, 247–260.

Kang, H., & Schuman, E. M. (1995). Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science, 267*, 1658–1662.

Kanning, K. C., Hudson, M., Amieux, P. S., Wiley, J. C., Bothwell, M., & Schecterson, L. C. (2003). Proteolytic processing of the p75 neurotrophin receptor and two homologs generates C-terminal fragments with signaling capability. *The Journal of Neuroscience, 23*, 5425–5436.

Kenchappa, R. S., Zampieri, N., Chao, M. V., Barker, P. A., Teng, H. K., Hempstead, B. L., & Carter, B. D. (2006). Ligand-dependent cleavage of the p75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. *Neuron, 50*, 219–232.

Kenchappa, R. S., Tep, C., Korade, Z., Urra, S., Bronfman, F. C., Yoon, S. O., & Carter, B. D. (2010). p75 neurotrophin receptor-mediated apoptosis in sympathetic neurons involves a biphasic activation of JNK and up-regulation of tumor necrosis factor-alpha-converting enzyme/ADAM17. *The Journal of Biological Chemistry, 285*, 20358–20368.

Kim, T., & Hempstead, B. L. (2009). NRH2 is a trafficking switch to regulate sortilin localization and permit proneurotrophin-induced cell death. *The EMBO Journal, 28*, 1612–1623.

Koh, S., Oyler, G. A., & Higgins, G. A. (1989). Localization of nerve growth factor receptor messenger RNA and protein in the adult rat brain. *Experimental Neurology, 106*, 209–221.

Kohn, J., Aloyz, R. S., Toma, J. G., Haak-Frendscho, M., & Miller, F. D. (1999). Functionally antagonistic interactions between the TrkA and p75 neurotrophin receptors regulate sympathetic neuron growth and target innervation. *The Journal of Neuroscience, 19*, 5393–5408.

Kommaddi, R. P., Thomas, R., Ceni, C., Daigneault, K., & Barker, P. A. (2011). Trk-dependent ADAM17 activation facilitates neurotrophin survival signaling. *The FASEB Journal, 25*, 2061–2070.

Korte, M., Griesbeck, O., Gravel, C., Carroll, P., Staiger, V., Thoenen, H., & Bonhoeffer, T. (1996). Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 12547–12552.

Lee, K. F., Davies, A. M., & Jaenisch, R. (1994). p75-deficient embryonic dorsal root sensory and neonatal sympathetic neurons display a decreased sensitivity to NGF. *Development, 120*, 1027–1033.

Lee, T. H., Abe, K., Kogure, K., & Itoyama, Y. (1995). Expressions of nerve growth factor and p75 low affinity receptor after transient forebrain ischemia in gerbil hippocampal CA1 neurons. *Journal of Neuroscience Research, 41*, 684–695.

Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*, 1945–1948.

Lim, Y. S., McLaughlin, T., Sung, T. C., Santiago, A., Lee, K. F., & O'Leary, D. D. (2008). p75(NTR) mediates ephrin-A reverse signaling required for axon repulsion and mapping. *Neuron, 59*, 746–758.

Lockhart, S. T., Turrigiano, G. G., & Birren, S. J. (1997). Nerve growth factor modulates synaptic transmission between sympathetic neurons and cardiac myocytes. *The Journal of Neuroscience, 17*, 9573–9582.

Lu, B., Pang, P. T., & Woo, N. H. (2005). The yin and yang of neurotrophin action. *Nature Reviews. Neuroscience, 6*, 603–614.

Matsumoto, T., Rauskolb, S., Polack, M., Klose, J., Kolbeck, R., Korte, M., & Barde, Y. A. (2008). Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. *Nature Neuroscience, 11*, 131–133.

Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., Levesque, M., Allaire, N., Perrin, S., Sands, B., et al. (2004). LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nature Neuroscience, 7*, 221–228.

Moscatelli, I., Pierantozzi, E., Camaioni, A., Siracusa, G., & Campagnolo, L. (2009). p75 neurotrophin receptor is involved in proliferation of undifferentiated mouse embryonic stem cells. *Experimental Cell Research, 315*, 3220–3232.

Mufson, E. J., Higgins, G. A., & Kordower, J. H. (1991). Nerve growth factor receptor immunoreactivity in the new world monkey (*Cebus apella*) and human cerebellum. *The Journal of Comparative Neurology, 308*, 555–575.

Murray, S. S., Perez, P., Lee, R., Hempstead, B. L., & Chao, M. V. (2004). A novel p75 neurotrophin receptor-related protein, NRH2, regulates nerve growth factor binding to the TrkA receptor. *The Journal of Neuroscience, 24*, 2742–2749.

Niederost, B., Oertle, T., Fritsche, J., McKinney, R. A., & Bandtlow, C. E. (2002). Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1. *The Journal of Neuroscience, 22*, 10368–10376.

Nykjaer, A., Lee, R., Teng, K. K., Jansen, P., Madsen, P., Nielsen, M. S., Jacobsen, C., Kliemannel, M., Schwarz, E., Willnow, T. E., et al. (2004). Sortilin is essential for proNGF-induced neuronal cell death. *Nature, 427*, 843–848.

Nykjaer, A., Willnow, T. E., & Petersen, C. M. (2005). $p75^{NTR}$ – live or let die. *Current Opinion in Neurobiology, 15*, 49–57.

Oderfeld-Nowak, B., & Bacia, A. (1994). Expression of astroglial nerve growth factor in damaged brain. *Acta Neurobiologiae Experimentalis (Wars), 54*, 73–80.

Oderfeld-Nowak, B., Orzylowska-Sliwinska, O., Soltys, Z., Zaremba, M., Januszewski, S., Janeczko, K., & Mossakowski, M. (2003). Concomitant up-regulation of astroglial high and low affinity nerve growth factor receptors in the CA1 hippocampal area following global transient cerebral ischemia in rat. *Neuroscience, 120*, 31–40.

Pang, P. T., Teng, H. K., Zaitsev, E., Woo, N. T., Sakata, K., Zhen, S., Teng, K. K., Yung, W. H., Hempstead, B. L., & Lu, B. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science, 306*, 487–491.

Park, J. B., Yiu, G., Kaneko, S., Wang, J., Chang, J., He, X. L., Garcia, K. C., & He, Z. (2005). A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. *Neuron, 45*, 345–351.

Park, K. J., Grosso, C. A., Aubert, I., Kaplan, D. R., & Miller, F. D. (2010). $p75^{NTR}$-dependent, myelin-mediated axonal degeneration regulates neural connectivity in the adult brain. *Nature Neuroscience, 13*, 559–566.

Parkhurst, C. N., Zampieri, N., & Chao, M. V. (2010). Nuclear localization of the p75 neurotrophin receptor intracellular domain. *The Journal of Biological Chemistry, 285*, 5361–5368.

Pasterkamp, R. J. (2012). Getting neural circuits into shape with semaphorins. *Nature Reviews. Neuroscience, 13*, 605–618.

Patterson, S. L., Abel, T., Deuel, T. A., Martin, K. C., Rose, J. C., & Kandel, E. R. (1996). Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron, 16*, 1137–1145.

Paul, C. E., Vereker, E., Dickson, K. M., & Barker, P. A. (2004). A pro-apoptotic fragment of the p75 neurotrophin receptor is expressed in $p75^{NTR}$ExonIV null mice. *The Journal of Neuroscience, 24*, 1917–1923.

Peterson, D. A., Dickinson-Anson, H. A., Leppert, J. T., Lee, K. F., & Gage, F. H. (1999). Central neuronal loss and behavioral impairment in mice lacking neurotrophin receptor p75. *The Journal of Comparative Neurology, 404*, 1–20.

Petratos, S., Gonzales, M. F., Azari, M. F., Marriott, M., Minichiello, R. A., Shipham, K. A., Profyris, C., Nicolaou, A., Boyle, K., Cheema, S. S., & Kilpatrick, T. J. (2004). Expression of the low-affinity neurotrophin receptor, p75(NTR), is upregulated by oligodendroglial progenitors adjacent to the subventricular zone in response to demyelination. *Glia, 48*, 64–75.

Pincheira, R., Baerwald, M., Dunbar, J. D., & Donner, D. B. (2009). Sall2 is a novel $p75^{NTR}$-interacting protein that links NGF signalling to cell cycle progression and neurite outgrowth. *The EMBO Journal, 28*, 261–273.

Pozzo-Miller, L. D., Gottschalk, W., Zhang, L., McDermott, K., Du, J., Gopalakrishnan, R., Oho, C., Sheng, Z. H., & Lu, B. (1999). Impairments in high-frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice. *The Journal of Neuroscience, 19*, 4972–4983.

Quistgaard, E. M., Madsen, P., Groftehauge, M. K., Nissen, P., Petersen, C. M., & Thirup, S. S. (2009). Ligands bind to sortilin in the tunnel of a ten-bladed beta-propeller domain. *Nature Structural & Molecular Biology, 16*, 96–98.

Rodriguez-Tebar, A., Dechant, G., Gotz, R., & Barde, Y. A. (1992). Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. *The EMBO Journal, 11*, 917–922.

Rogers, M. L., Bailey, S., Matusica, D., Nicholson, I., Muyderman, H., Pagadala, P. C., Neet, K. E., Zola, H., Macardle, P., & Rush, R. A. (2010). ProNGF mediates death of natural killer cells through activation of the $p75^{NTR}$-sortilin complex. *Journal of Neuroimmunology, 226*, 93–103.

Rosch, H., Schweigreiter, R., Bonhoeffer, T., Barde, Y. A., & Korte, M. (2005). The neurotrophin receptor p75NTR modulates long-term depression and regulates the expression of AMPA receptor subunits in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 7362–7367.

Roux, P. P., & Barker, P. A. (2002). Neurotrophin signaling through the p75 neurotrophin receptor. *Progress in Neurobiology, 67*, 203–233.

Roux, P. P., Colicos, M. A., Barker, P. A., & Kennedy, T. E. (1999). p75 neurotrophin receptor expression is induced in apoptotic neurons after seizure. *The Journal of Neuroscience, 19*, 6887–6896.

Rudkin, B. B., Lazarovici, P., Levi, B. Z., Abe, Y., Fujita, K., & Guroff, G. (1989). Cell cycle-specific action of nerve growth factor in PC12 cells: Differentiation without proliferation. *The EMBO Journal, 8*, 3319–3325.

Ryden, M., Murray-Rust, J., Glass, D., Ilag, L. L., Trupp, M., Yancopoulos, G. D., McDonald, N. Q., & Ibanez, C. F. (1995). Functional analysis of mutant neurotrophins deficient in low-affinity binding reveals a role for p75LNGFR in NT-4 signalling. *The EMBO Journal, 14*, 1979–1990.

Salehi, A. H., Roux, P. P., Kubu, C. J., Zeindler, C., Bhakar, A., Tannis, L. L., Verdi, J. M., & Barker, P. A. (2000). NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis. *Neuron, 27*, 279–288.

Salehi, A. H., Xanthoudakis, S., & Barker, P. A. (2002). NRAGE, a p75 neurotrophin receptor-interacting protein, induces caspase activation and cell death through a JNK-dependent mitochondrial pathway. *The Journal of Biological Chemistry, 277*, 48043–48050.

Santos, A. R., Comprido, D., & Duarte, C. B. (2010). Regulation of local translation at the synapse by BDNF. *Progress in Neurobiology, 92*, 505–516.

Sarret, P., Krzywkowski, P., Segal, L., Nielsen, M. S., Petersen, C. M., Mazella, J., Stroh, T., & Beaudet, A. (2003). Distribution of NTS3 receptor/sortilin mRNA and protein in the rat central nervous system. *The Journal of Comparative Neurology, 461*, 483–505.

Schatteman, G. C., Gibbs, L., Lanahan, A. A., Claude, P., & Bothwell, M. (1988). Expression of NGF receptor in the developing and adult primate central nervous system. *The Journal of Neuroscience, 8*, 860–873.

Schatteman, G. C., Langer, T., Lanahan, A. A., & Bothwell, M. A. (1993). Distribution of the 75-kD low-affinity nerve growth factor receptor in the primate peripheral nervous system. *Somatosensory & Motor Research, 10*, 415–432.

Schecterson, L. C., & Bothwell, M. (2010). Neurotrophin receptors: Old friends with new partners. *Developmental Neurobiology, 70*, 332–338.

Shao, Z., Browning, J. L., Lee, X., Scott, M. L., Shulga-Morskaya, S., Allaire, N., Thill, G., Levesque, M., Sah, D., McCoy, J. M., et al. (2005). TAJ/TROY, an orphan TNF receptor family member, binds Nogo-66 receptor 1 and regulates axonal regeneration. *Neuron, 45*, 353–359.

Shelton, D. L., & Reichardt, L. F. (1986). Studies on the expression of the beta nerve growth factor (NGF) gene in the central nervous system: Level and regional distribution of NGF mRNA suggest that NGF functions as a trophic factor for several distinct populations of neurons. *Proceedings of the National Academy of Sciences of the United States of America, 83*, 2714–2718.

Singh, K. K., & Miller, F. D. (2005). Activity regulates positive and negative neurotrophin-derived signals to determine axon competition. *Neuron, 45*, 837–845.

Singh, K. K., Park, K. J., Hong, E. J., Kramer, B. M., Greenberg, M. E., Kaplan, D. R., & Miller, F. D. (2008). Developmental axon pruning mediated by BDNF-p75^{NTR}-dependent axon degeneration. *Nature Neuroscience, 11*, 649–658.

Skeldal, S., Matusica, D., Nykjaer, A., & Coulson, E. J. (2011). Proteolytic processing of the p75 neurotrophin receptor: A prerequisite for signalling?: Neuronal life, growth and death signalling are crucially regulated by intra-membrane proteolysis and trafficking of p75(NTR). *Bioessays, 33*, 614–625.

Slonimsky, J. D., Mattaliano, M. D., Moon, J. I., Griffith, L. C., & Birren, S. J. (2006). Role for calcium/calmodulin-dependent protein kinase II in the p75-mediated regulation of sympathetic cholinergic transmission. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 2915–2919.

Sofroniew, M. V., Isacson, O., & O'Brien, T. S. (1989). Nerve growth factor receptor immunoreactivity in the rat suprachiasmatic nucleus. *Brain Research, 476*, 358–362.

Song, W., Volosin, M., Cragnolini, A. B., Hempstead, B. L., & Friedman, W. J. (2010). ProNGF induces PTEN via p75^{NTR} to suppress Trk-mediated survival signaling in brain neurons. *The Journal of Neuroscience, 30*, 15608–15615.

Sotthibundhu, A., Li, Q. X., Thangnipon, W., & Coulson, E. J. (2009). Abeta(1–42) stimulates adult SVZ neurogenesis through the p75 neurotrophin receptor. *Neurobiology of Aging, 30*, 1975–1985.

Springer, J. E., Koh, S., Tayrien, M. W., & Loy, R. (1987). Basal forebrain magnocellular neurons stain for nerve growth factor receptor: Correlation with cholinergic cell bodies and effects of axotomy. *Journal of Neuroscience Research, 17*, 111–118.

Tcherpakov, M., Bronfman, F. C., Conticello, S. G., Vaskovsky, A., Levy, Z., Niinobe, M., Yoshikawa, K., Arenas, E., & Fainzilber, M. (2002). The p75 neurotrophin receptor interacts with multiple MAGE proteins. *The Journal of Biological Chemistry, 277*, 49101–49104.

Teng, H. K., Teng, K. K., Lee, R., Wright, S., Tevar, S., Almeida, R. D., Kermani, P., Torkin, R., Chen, Z. Y., Lee, F. S., et al. (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75^{NTR} and sortilin. *The Journal of Neuroscience, 25*, 5455–5463.

Underwood, C. K., Reid, K., May, L. M., Bartlett, P. F., & Coulson, E. J. (2008). Palmitoylation of the C-terminal fragment of p75(NTR) regulates death signaling and is required for subsequent cleavage by gamma-secretase. *Molecular and Cellular Neurosciences, 37*, 346–358.

Unsain, N., Nunez, N., Anastasia, A., & Masco, D. H. (2008). Status epilepticus induces a TrkB to p75 neurotrophin receptor switch and increases brain-derived neurotrophic factor interaction with p75 neurotrophin receptor: An initial event in neuronal injury induction. *Neuroscience, 154*, 978–993.

Urdiales, J. L., Becker, E., Andrieu, M., Thomas, A., Jullien, J., van Grunsven, L. A., Menut, S., Evan, G. I., Martin-Zanca, D., & Rudkin, B. B. (1998). Cell cycle phase-specific surface expression of nerve growth factor receptors TrkA and p75(NTR). *The Journal of Neuroscience, 18*, 6767–6775.

Urfer, R., Tsoulfas, P., Soppet, D., Escandon, E., Parada, L. F., & Presta, L. G. (1994). The binding epitopes of neurotrophin-3 to its receptors trkC and gp75 and the design of a multifunctional human neurotrophin. *The EMBO Journal, 13*, 5896–5909.

Verge, V. M., Merlio, J. P., Grondin, J., Ernfors, P., Persson, H., Riopelle, R. J., Hokfelt, T., & Richardson, P. M. (1992). Colocalization of NGF binding sites, trk mRNA, and low-affinity NGF receptor mRNA in primary sensory neurons: Responses to injury and infusion of NGF. *The Journal of Neuroscience, 12*, 4011–4022.

Vilar, M., Charalampopoulos, I., Kenchappa, R. S., Reversi, A., Klos-Applequist, J. M., Karaca, E., Simi, A., Spuch, C., Choi, S., Friedman, W. J., et al. (2009a). Ligand-independent signaling by disulfide-crosslinked dimers of the p75 neurotrophin receptor. *Journal of Cell Science, 122*, 3351–3357.

Vilar, M., Charalampopoulos, I., Kenchappa, R. S., Simi, A., Karaca, E., Reversi, A., Choi, S., Bothwell, M., Mingarro, I., Friedman, W. J., et al. (2009b). Activation of the p75 neurotrophin receptor through conformational rearrangement of disulphide-linked receptor dimers. *Neuron, 62*, 72–83.

Volosin, M., Song, W., Almeida, R. D., Kaplan, D. R., Hempstead, B. L., & Friedman, W. J. (2006). Interaction of survival and death signaling in basal forebrain neurons: Roles of neurotrophins and proneurotrophins. *The Journal of Neuroscience, 26*, 7756–7766.

Volosin, M., Trotter, C., Cragnolini, A., Kenchappa, R. S., Light, M., Hempstead, B. L., Carter, B. D., & Friedman, W. J. (2008). Induction of proneurotrophins and activation of $p75^{NTR}$-mediated apoptosis via neurotrophin receptor-interacting factor in hippocampal neurons after seizures. *The Journal of Neuroscience, 28*, 9870–9879.

von Schack, D., Casademunt, E., Schweigreiter, R., Meyer, M., Bibel, M., & Dechant, G. (2001). Complete ablation of the neurotrophin receptor $p75^{NTR}$ causes defects both in the nervous and the vascular system. *Nature Neuroscience, 4*, 977–978.

Wang, K. C., Kim, J. A., Sivasankaran, R., Segal, R., & He, Z. (2002). P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature, 420*, 74–78.

Wang, X., Shao, Z., Zetoune, F. S., Zeidler, M. G., Gowrishankar, K., & Vincenz, C. (2003). NRADD, a novel membrane protein with a death domain involved in mediating apoptosis in response to ER stress. *Cell Death and Differentiation, 10*, 580–591.

Wong, S. T., Henley, J. R., Kanning, K. C., Huang, K. H., Bothwell, M., & Poo, M. M. (2002). A p75(NTR) and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. *Nature Neuroscience, 5*, 1302–1308.

Woo, N. H., Teng, H. K., Siao, C. J., Chiaruttini, C., Pang, P. T., Milner, T. A., Hempstead, B. L., & Lu, B. (2005). Activation of $p75^{NTR}$ by proBDNF facilitates hippocampal long-term depression. *Nature Neuroscience, 8*, 1069–1077.

Wright, J. W., Alt, J. A., Turner, G. D., & Krueger, J. M. (2004). Differences in spatial learning comparing transgenic p75 knockout, New Zealand Black, C57BL/6, and Swiss Webster mice. *Behavioural Brain Research, 153*, 453–458.

Xu, N. J., & Henkemeyer, M. (2012). Ephrin reverse signaling in axon guidance and synaptogenesis. *Seminars in Cell & Developmental Biology, 23*, 58–64.

Yaar, M., Zhai, S., Pilch, P. F., Doyle, S. M., Eisenhauer, P. B., Fine, R. E., & Gilchrest, B. A. (1997). Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis. A possible mechanism for Alzheimer's disease. *Journal of Clinical Investigation, 100*, 2333–2340.

Yaar, M., Zhai, S., Fine, R. E., Eisenhauer, P. B., Arble, B. L., Stewart, K. B., & Gilchrest, B. A. (2002). Amyloid beta binds trimers as well as monomers of the 75-kDa neurotrophin receptor and activates receptor signaling. *The Journal of Biological Chemistry, 277*, 7720–7725.

Yamashita, T., & Tohyama, M. (2003). The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. *Nature Neuroscience, 6*, 461–467.

Yamashita, T., Tucker, K. L., & Barde, Y. A. (1999). Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. *Neuron, 24*, 585–593.

Yamashita, T., Higuchi, H., & Tohyama, M. (2002). The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. *The Journal of Cell Biology, 157*, 565–570.

Yan, Q., & Johnson, E. M., Jr. (1987). A quantitative study of the developmental expression of nerve growth factor (NGF) receptor in rats. *Developmental Biology, 121*, 139–148.

Yan, Q., & Johnson, E. M., Jr. (1988). An immunohistochemical study of the nerve growth factor receptor in developing rats. *The Journal of Neuroscience, 8*, 3481–3498.

Yang, B., Slonimsky, J. D., & Birren, S. J. (2002). A rapid switch in sympathetic neurotransmitter release properties mediated by the p75 receptor. *Nature Neuroscience, 5*, 539–545.

Yang, F., Je, H. S., Ji, Y., Nagappan, G., Hempstead, B., & Lu, B. (2009). Pro-BDNF-induced synaptic depression and retraction at developing neuromuscular synapses. *The Journal of Cell Biology, 185*, 727–741.

Young, K. M., Merson, T. D., Sotthibundhu, A., Coulson, E. J., & Bartlett, P. F. (2007). p75 neurotrophin receptor expression defines a population of BDNF-responsive neurogenic precursor cells. *The Journal of Neuroscience, 27*, 5146–5155.

Zagrebelsky, M., Holz, A., Dechant, G., Barde, Y. A., Bonhoeffer, T., & Korte, M. (2005). The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons. *The Journal of Neuroscience, 25*, 9989–9999.

p75NTR: A Molecule with Multiple Functions in Amyloid-Beta Metabolism and Neurotoxicity

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Abstract

The p75 neurotrophin receptor (p75NTR) is a pan-receptor for neurotrophins including nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin 4/5. P75NTR plays a diverse role from regulating cell survival to modulating neurite outgrowth. Under some pathological conditions, p75NTR expression is activated and plays detrimental roles in disease progression. Alzheimer's disease (AD), the most common form of dementia, is characterized by the deposition of amyloid plaques, accumulation of fibrillary tangles in neurons, neurite degeneration, loss of neurons, and a progressive loss of cognitive function. Recent studies suggest that p75NTR, also a receptor for amyloid-beta (Aβ), is a critical factor involved in the pathogenesis of AD. This chapter is to discuss the roles of p75NTR in the production of amyloid-beta (Aβ), neuronal death, neurite degeneration, tau hyperphosphorylation, cell cycle re-entry, and cognition decline, to propose that p75NTR is a potential target for the development of therapeutic drugs for AD, and to provide perspectives in developing various therapeutic strategies targeting different aspects of AD hallmarks which relate to p75NTR functions, and breaking the p75NTR-mediated positive-feedback loop which promotes the cascades in the pathogenesis of AD.

Keywords

Alzheimer's disease • Amyloid-beta • Cell cycle • Cognition • Extracellular domain • Neurodegeneration • Neuronal death • p75NTR • tau

List of Abbreviations

AD	Alzheimer's disease
APP	Amyloid precursor protein
Aβ	Amyloid-beta
BACE	Beta-site APP-cleaving enzyme
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system
DRG	Dorsal root ganglion
ECD	Extracellular domain
MAG	Myelin-associated glycoprotein
NBM	Nucleus basalis of Meynert
NGF	Nerve growth factor
NT	Neurotrophin
p75NTR	p75NTR neurotrophin receptor
PNS	Peripheral nervous system
TACE	TNF-alpha converting enzyme
TNF	Tumor necrosis factor
Trk	Tropomyosin receptor kinase

1 Introduction

The p75 neurotrophin receptor (p75NTR) was first identified about 20 years ago as the receptor for nerve growth factor (NGF) and was subsequently found to be the receptor for all other neurotrophins including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/neurotrophin-5 (NT-4/NT-5) (Chao and Bothwell 2002; Barker 2004). p75NTR belongs to the tumor necrosis factor receptor superfamily which consists of ten different transmembrane glycoproteins (type I) (Gruss 1996). The role as the pan-receptor for neurotrophins enables p75NTR to play a diverse role in regulating cell survival. It can activate at least three signaling pathways including neuronal growth, apoptosis, and synapse plasticity, and most recently this receptor is also found to be involved in the neuronal cell cycle re-entry, which is considered a key pathological mechanism underlying the development of Alzheimer's disease and other neuronal degenerative diseases. This chapter focuses on the recent progress in understanding the physiological and pathological roles of p75NTR with particular interest in the role of p75NTR in development and intervention of Alzheimer's disease.

2 Biology of p75NTR

2.1 Molecular Biology, Structure, Distribution, and Expression Pattern

The structure of the human p75NTR gene was first described by Santee et al. in 1996 (Santee and Owen-Schaub 1996). It was identified as 19728 base pairs (bp) in size containing six exons and five introns. Sharing an analogous structure and function with the tumor necrosis factor (TNF) receptor, p75NTR is a transmembrane protein that consists of an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain consists of a stalk domain which links the transmembrane domain and four cysteine-rich repeat domains that facilitate neurotrophin binding. There is clear evidence that the third and fourth cysteine repeats are the binding sites for neurotrophins, as the mutant form of p75NTR that lacks these two domains cannot bind neurotrophins. The intracellular part of p75NTR contains an 80-amino acid "death domain" module. It connects the transmembrane domain through a flexible linker region N-terminal domain. The flexibility of the linker domain possibly plays an important role in multiple signal transduction. In addition, the intracellular, cytoplasmic region of p75NTR does not exhibit an intrinsic ligand-inducible enzymatic function, and this is distinctly different from tropomyosin receptor kinase (Trk) receptors. Although p75NTR is a member of the TNF receptor family, there are some differences between p75NTR and other TNF receptors. The death domain of p75NTR is a type-2 molecule, which is different from the type-1 molecules of other TNF receptors. Furthermore, unlike other TNF receptors, p75NTR does not self-associate in solution.

2.2 Expression Location of p75NTR

The p75NTR has a very wide tissue and cellular distribution in a wide range of species and its distribution varies during the different stages of the life. Its expression has been shown at the early developmental stages of the central nervous system (CNS) and peripheral nervous system (PNS). In the developing rat dorsal root ganglion (DRG), p75NTR expression can be found as early as E7 (Ernfors et al. 1988). The receptor was also found to be selectively expressed in the sympathetic ganglia (Buck et al. 1987). In the basal forebrain of the rat, the p75NTR immunoreactivity can be observed as early as E15 (Koh and Loy 1989) and throughout the whole of the adult life (Mufson and Kordower 1992; Hartig et al. 1998). In contrast to the other tissue organs, the expression of p75NTR in these basal forebrain neurons seems to be upregulated during aging while the Trk expression is downregulated (Ginsberg et al. 2006). Interestingly, p75NTR levels tend to be high and widely expressed during development but decrease dramatically in adulthood. In addition, p75NTR can be reexpressed or upregulated to a significant degree in pathological states related to neural cell death or after injury (Barrett and Bartlett 1994). In the adult mouse, p75NTR expression has also been shown to be present in a small subset of spinal cord ascending sensory axons, and in some retinal cells and subsets of dorsal root ganglia, but not the cortex and cerebellum. In addition to neurons, p75NTR is also expressed in a variety of glial tissues such as Schwann cells and oligodendrocytes during development and after injury (Cragnolini and Friedman 2008).

2.3 Physiological Roles of p75NTR and Its Metabolism

In contrast to the Trks receptors, the physiological roles of p75NTR are complicated and difficult to discern. Analysis of the p75NTR function has been complicated by the fact that the major physiological role of p75NTR may change dramatically depending on the cell context. p75NTR is a low-affinity pan-receptor which can functionally collaborate with Trk receptors to either enhance or reduce neurotrophin-mediated Trk receptor activation. The binding of p75NTR and Trks to neurotrophins can trigger several signal transduction pathways, which subsequently promote the survival, differentiation, and development of neuronal cells. In addition to promoting survival of neuronal tissues, another well-established and important functional role of p75NTR is to mediate the apoptosis in normal and pathological conditions. p75NTR has been shown to induce apoptosis in different cell types, including cultured neonatal sympathetic neurons (Bamji et al. 1998), motor neurons (Sedel et al. 1999), sensory neurons (Barrett and Bartlett 1994), oligodendrocytes (Casaccia-Bonnefil et al. 1996), and embryonic proprioceptive neurons when Trk activation is reduced or absent (Davey and Davies 1998). There is evidence indicating that p75NTR is involved in cholinergic neuronal death independent of TrkA expression during development and after injury (Van der Zee et al. 1996; Naumann et al. 2002).

p75NTR has more recently been shown to regulate neurite outgrowth through regulation of RhoA, in some contexts following activation by myelin proteins in conjunction with the Nogo66 receptor (Barker 2004). It can also promote neuronal differentiation and cell cycle. Adult dorsal root ganglion neurons or postnatal cerebellar neurons from mice carrying a mutation in the p75NTR gene are insensitive to MAG with regard to neurite collapse (Yamashita et al. 2002).

Some novel functional roles of p75NTR have been identified recently. NGF stimulates neurite outgrowth from embryonic rat hippocampal neurons and chick ciliary neurons which express p75NTR but not TrkA (Brann et al. 1999). The p75NTR receptor may also be involved in the inhibition of axonal elongation by myelin. In mice lacking p75NTR, aberrant axonal elongation is observed in the myelin-rich area where these axons would not grow (Walsh et al. 1999). These findings suggested that p75NTR may have bidirectional roles in regulating neurite outgrowth (Hasegawa et al. 2004).

In a recent study, a novel p75NTR-interacting protein named Sall2 has been found to link NGF signaling to cell cycle progression and neurite outgrowth (Pincheira et al. 2009).

p75NTR is physiologically cleaved by the enzyme known as TNF-alpha converting enzyme (TACE) in the juxtamembrane domain, to shed the extracellular domain (Weskamp et al. 2004). The exact significance of this process remains unknown. It is possible that the shedded extracellular domain can play an opposing role to negatively regulate the functions of the membrane counterpart, by competitively inhibiting the binding of ligands to membrane p75NTR.

2.4 Signal Pathway of p75NTR Action

p75NTR has a close collaboration with tyrosine kinases receptors. It regulates ligand specificity of Trk receptors and modulates binding affinity of TrkA to NGF (Huang and Reichardt 2003). Although other neurotrophins can interact with TrkB receptors, only BDNF provides a functional response upon co-expression of TrkB with p75NTR. Similarly, both NGF and NT-3 bind to TrkA, but p75NTR restricts TrkA-NGF signaling. In contrast, co-expression of p75NTR and TrkC decreases ligand specificity of TrkC to NT-3.

p75NTR interacts with a dozen of signaling molecules and mediates apoptosis, Schwann cell migration, myelination, axonal growth, and regeneration. The intracellular domain of p75NTR contains several potential motifs which modulates interactions of p75NTR with other proteins, subsequently activating different signaling pathways (Barker 2004; Bronfman and Fainzilber 2004; Dechant and Barde 2002; Roux and Barker 2002) such as NF-κB (Carter et al. 1996), c-jun kinase (JNK) (Friedman 2000; Yoon et al. 1998), and caspases (Troy et al. 2002). In addition, p75NTR activates a small GTPase Rho (Yamashita et al. 1999) involving different ligands derived from myelin, such as myelin-associated glycoprotein (MAG) and Nogo (Wang et al. 2002a; Wong et al. 2002; Yamashita et al. 2002) and two different co-receptors: a lipid-anchored ligand-binding subunit known as Nogo receptor (NgR) (Fournier et al. 2001) and Lingo-1 (Mi et al. 2004). For instance, BDNF binds to

p75NTR and induces apoptosis through activation of JNK pathway (Bhakar et al. 2003; Palmada et al. 2002). p75NTR and sortilin form a very-high-affinity receptor complex for the binding of proNGF and other proneurotrophins which promote apoptosis (Nykjaer et al. 2004). p75NTR also mediates biological activity of MAG and Nogo which inhibit axonal elongation and neurite growth via interacting with the Nogo receptor (Wang et al. 2002a, b).

3 Pathological Roles of p75NTR in the Nervous System

Although the expression of p75NTR is developmentally regulated in the nervous system, marked increase is observed under certain pathological conditions; however, the significance of the expression in these conditions is still not very clear.

In the dorsal root ganglia, the reduction of p75NTR levels by antisense oligonucleotides prevents the loss of axotomized neurons (Cheema et al. 1996). Similarly, the loss of motor neuron occurring after transection of the neonatal facial nerve is attenuated in mice carrying a mutation in the p75NTR gene (Ferri et al. 1998; Ferri and Bisby 1999). In contrast, the administration of NGF into the transected neonatal facial nerve of animals increases cell death (Sendtner et al. 1992). p75NTR is reexpressed in the transected neurons at levels comparable to those seen during the developmental stages. The upregulation of p75NTR is also observed in the cerebral cortex in Alzheimer's disease (Mufson and Kordower 1992). In a mouse model of amyotrophic lateral sclerosis, in which a mutant form of superoxide dismutase is overexpressed, p75NTR is reexpressed in lumbar motor neurons (Lowry et al. 2001) as the disease progresses. Consistently, the expression of p75NTR is also observed in motor neurons in the cervical spinal cords of patients with amyotrophic lateral sclerosis.

After axotomy, cortical spinal neurons strongly reexpress p75NTR three days after lesion, when neuronal death occurs. The activation of p75NTR by NT-3 causes the death of these neurons, as shown by experiments using blocking antibodies against p75NTR or NT-3 (Giehl et al. 2001). In spinal cord injury, p75NTR is essential for neuronal cell survival and functional recovery after injury (Chu et al. 2007). Activation of p75NTR contributes to NMDAR-mediated apoptosis in the neonatal brain (Griesmaier et al. 2010). These findings further suggest that p75NTR may be involved in neuronal death in neurological disorders. In a study using experimental autoimmune encephalomyelitis (EAE) animal models, in contrast to the clinical manifestation of EAE observed in wild-type C57/BL6 mice, mice deficient of p75NTR developed severe or lethal diseases and concomitant increased levels of inflammation in the CNS (Copray et al. 2004). This may suggest p75NTR is involved in the inflammation process but the detailed molecular mechanism is not clear. In retina degeneration, p75NTR is upregulated and exerts neurotoxic effect through paracrine mechanism (Bai et al. 2010). It appears that in pathologies involved in neuronal degeneration and death, p75NTR expression has a tendency to be upregulated in most experimental models used so far. However, the mechanism underlying the upregulation is not completely understood.

4 Roles of p75NTR in Pathogenesis of Alzheimer's Disease

4.1 p75NTR Expression in the Brain of Alzheimer's Disease

It is well known that cholinergic neurons in the basal forebrain especially in the nucleus basalis of Meynert (NBM) are most vulnerable and severely affected in Alzheimer's disease and they express p75NTR-positive, TrkA, TrkB, and TrkC (Salehi et al. 2000). Strikingly, very little or no p75NTR-positive neurons are normally observed in other brain regions. How the level of p75NTR expression changes in Alzheimer's disease patients and during the aging process remains controversial. Some researchers find that the expression of the nerve growth factor (NGF) receptor (including p75NTR) is increased in both cortical neurons and CA1 and CA2 subfields of the hippocampus in Alzheimer's disease patients compared with controls (Mufson and Kordower 1992; Hu et al. 2002). Some other studies show a significant reduction of p75NTR in Alzheimer's disease brains (Arendt et al. 1997; Salehi et al. 2000). Meanwhile, no significant differences in expression levels for p75NTR were observed across control, mild cognitive impairment (MCI, a pre-stage of Alzheimer's disease), nor Alzheimer's disease in other reports (Goedert et al. 1989; Treanor et al. 1991; Mufson et al. 2002; Ginsberg et al. 2006). The discrepancy on p75NTR expression in Alzheimer's disease may be due to the differences in investigated brain regions and measurements. The severity of Alzheimer's disease also influences the level of p75NTR, because it is certain that the greater the loss of cholinergic neurons occurring in Alzheimer's disease, the less that p75NTR is present in the basal forebrain. More work is required before a definite conclusion about this problem can be drawn regarding this issue. Studies on p75NTR expression in the Alzheimer's disease animal models and cell lines provide a clearer answer. In two different strains of transgenic Alzheimer's disease mice, the triple transgenic mice (3xTg-AD, harboring PS1M146V, APPswe, and tauP301L) and APPswe/PS1dE9 mice, the level of p75NTR is significantly increased and associated with the age-dependent rise of Aβ. Meanwhile, Aβ has promoted p75NTR expression in the membrane of SH-SY5Y human neuroblastoma cells (Chakravarthy et al. 2010). They further demonstrated that the microinjected Aβ oligomer can also increase p75NTR expression, which according to the results of in vitro study may be mediated through an insulin-like growth factor 1 receptor (IGF-1R) phosphorylation (Ito et al. 2012). Consistently, it was found that p75NTR expression increases in an age-dependent manner in wild-type mice and is higher in APPswe/PS1dE9 mice relative to their wild-type littermates at the same age (Wang et al. 2011). Although many of the human studies show a reduction of the p75NTR level in the brain, reflecting the loss of p75NTR-positive cholinergic neurons, these animal studies suggest that p75NTR expression increases with aging and is further activated by Aβ. The neurons of the transgenic animal models are more resistant to Aβ toxicity as reflected by the loss of very few neurons in the brain of the animal models, relative to humans (Gotz and Ittner 2008). Moreover, it was recently discovered that p75NTR would express in the cultured epithelial cells of choroid plexus, mainly in the cytoplasm and CSF side (Spuch and Carro 2011), which is an indication of its potential role to transport Aβ through BBB (Zhou and Wang 2011).

4.2 p75NTR Pathway Promotes Aβ Production

The level of p75NTR is significantly elevated in Alzheimer's disease brains, but little is known about the reason and meaning of this change. In our studies, the expression of p75NTR is much higher in the neurons of APPswe transgenic mice than that of the wild-type, suggesting Aβ is very likely to cause the high level of p75NTR in Alzheimer's disease. Our further studies showed that after the p75NTR gene knockout in APPswe transgenic mice, the production of Aβ was reduced in the cortex neurons, and the level of soluble Aβ also decreased (Wang, Wang et al. 2011). This indicates that p75NTR is able to promote Aβ production. Based on these results, it is speculated that Aβ upregulates the expression of p75NTR in Alzheimer's disease brains, and the activated p75NTR in turn stimulates Aβ production. Consequently they form a vicious cycle and finally result in Aβ overproduction. This hypothesis can explain the reason why Aβ production accelerates in Alzheimer's disease brains (Kawarabayashi et al. 2001) and why sporadic Alzheimer's disease patients have overproduction of Aβ when they do not have any known gene mutations with Aβ production (Selkoe 2001). It is demonstrated that beta-site APP-cleaving enzyme 1 (BACE1) protein increases in an annulus around amyloid plaques cores, and it occurs without any change in BACE1 mRNA level. Therefore, it can be concluded that amyloid plaques lead to the increased level of BACE1 surrounding neurons at an early stage before neuronal death occurs, and this may drive a positive-feedback loop in Aβ production (Zhao et al. 2007) (Fig. 1). Consistent with this conclusion, further research has described the same interactions between BACE1 and Aβ in the pathogenesis of Alzheimer's disease, no matter whether it is familial or sporadic (Tabaton et al. 2010). The positive-feedback loop in Aβ production is an important issue worthy of further investigations (Fig. 1).

Aging is the most important risk factor for Alzheimer's disease. Recently some researchers have identified the p75NTR system as a molecular link between normal aging and Alzheimer's disease. When p75NTR, which activates β cleavage of APP, is upregulated and TrkA, which reduces the cleavage, is downregulated during aging, the p75NTR-ceramide pathway is significantly activated, causing the increase of steady-state levels and activity of BACE1 downstream and eventually resulting in the acceleration of Aβ production (Costantini et al. 2005b). The caloric restriction and nSMase inhibitors could reduce Aβ production and the risks associated with aging, by interrupting this signaling pathway. Both the upregulation of p75NTR expression and the acceleration of Aβ production follow an age-dependent fashion. Further study of this research demonstrated that signaling through IGF1-R controls the level of TrkA and p75NTR in both human neuroblastoma cell lines and primary neurons from the mouse brain, and IGF1-R regulates Aβ production in the upstream of p75NTR-ceramide pathway (Costantini et al. 2006).

4.3 p75NTR Regulates Aβ Deposition and Clearance

Aβ mainly deposits in the cerebral neocortex, hippocampus, and vessel walls, which are coincident with the projection area of cholinergic neurons and

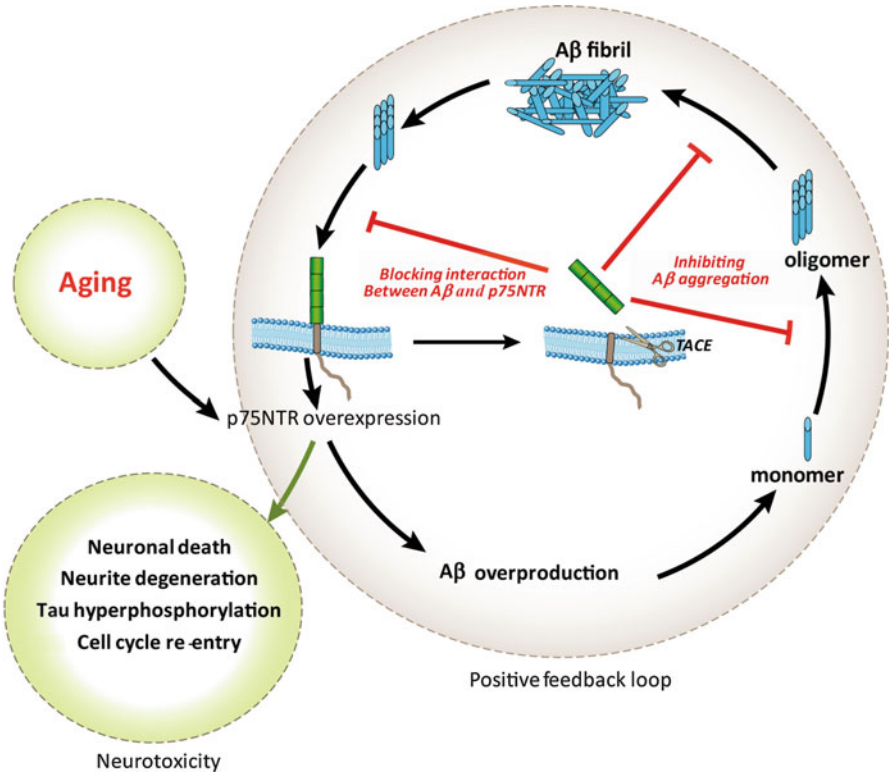

Fig. 1 Schematic diagram of p75NTR functions in Alzheimer's disease. In the brain of Alzheimer's disease, p75NTR expression is upregulated by aging and further activated by Aβ. p75NTR signaling increases Aβ production and thus enhances steady-state levels of Aβ in the interstitial space which may increase AD pathology. Aβ again promotes p75NTR expression. P75NTR/Aβ forms a positive-feedback loop. Besides Aβ production, p75NTR also mediates neurotoxicity of Aβ, such as neuron death, neurite degeneration, tau hyperphosphorylation, and cell cycle re-entry. p75NTR is to be cleaved by an enzyme named TACE to shed its extracellular domain. The extracellular domain of p75NTR after shedding from the membrane may bind to the 25–35 amino acid of Aβ, which is mainly responsible to Aβ aggregation and neurotoxicity, thus suppressing Aβ aggregation and reducing Aβ deposition in the brain. Meanwhile, the extracellular domain of p75NTR may also block the interaction between Aβ and membrane p75NTR by competitive binding Aβ, thus attenuating the p75NTR signaling which leads to both Aβ production and neurotoxicity. The shedded extracellular domain of p75NTR might be an endogenous protective molecule against Aβ production, deposition, and toxicity and would be an important and promising therapeutic target of Alzheimer's disease

sympathetic neurons expressing p75NTR. The underlying mechanism remains unclear. It was found that in the cerebral cortex and hippocampus of APPswe transgenic Alzheimer's disease mice, more than 90 % of the Aβ plaques contained p75NTR-positive fibers. More importantly, the p75NTR-positive fibers are mostly located in the cores of Aβ plaques, while the p75NTR-negative degenerated ones are located around the plaques. The appearance of p75NTR-positive neurites is

prior to Aβ plaques in the brain of Alzheimer's disease mice. These findings all indicate that p75NTR expressed on the fibers is probably an initiating or promoting factor for Aβ deposition. However, deletion of p75NTR can increase total Aβ level in the brain, with increased fibrillar Aβ levels but decreased soluble Aβ level. Therefore, how p75NTR influences Aβ deposition may be complicated.

As a member of the tumor necrosis factor receptor superfamily, p75NTR is cleaved by metalloproteases mainly TACE or ADAM17 in the normal metabolic process, and then the extracellular domain of p75NTR (p75NTR-ECD) is released (Weskamp et al. 2004). The physiological function of p75NTR-ECD has not been well studied. In our studies, after deleting the p75NTR gene of APPswe transgenic mice, insoluble Aβ deposition is significantly increased in the brain (Wang, Wang et al. 2011). Further study demonstrates that recombinant p75NTR-ECD inhibits Aβ oligomerization in a dose-dependent manner in vitro, and injection of p75NTR-ECD in the hippocampus of Alzheimer's disease mice can reduce local Aβ plaques. Based on these results, it can be concluded that p75NTR-ECD, which is released during the metabolic process of p75NTR, is a key regulatory factor of inhibiting Aβ aggregation and facilitating disaggregation of Aβ fibrils. It may act as an Aβ-sequestering molecule to clear Aβ in vivo. Seeking the Aβ binding sites on p75NTR-ECD is very valuable for revealing the mechanism about how p75NTR regulates Aβ deposition. After that, the effective control and interference with the deposition and clearance of Aβ can be achieved. It is worthy to note that since p75NTR is also expressed in endothelial cells of BBB, it may help transport Aβ from CSF to blood, thus facilitating Aβ clearance to some extent (Zhou and Wang 2011).

In the progression of Alzheimer's disease, the level of p75NTR is related to Aβ load and other factors in the brain. How is the extracellular shedding of p75NTR regulated in vivo? Is there any change of p75NTR-ECD level in the Alzheimer's disease brain, cerebral spinal fluid, blood, or urine? Are the function and metabolism of p75NTR-ECD impaired in Alzheimer's disease patients? Can Aβ influence p75NTR-ECD production? By what means does p75NTR transport Aβ through BBB? Are there any single nucleotide polymorphisms of the p75NTR gene associated with the risk of Alzheimer's disease? All these important questions need to be answered in future studies.

4.4 p75NTR and Neurite Degeneration

Neurite degeneration is a pivotal event of Alzheimer's disease and a main contributor of the memory loss. Aβ is an important factor which leads to neurite degeneration in Alzheimer's disease patients, but its receptor pathway and mechanism are still unknown (Petratos et al. 2008). It is demonstrated that intact p75NTR mediates or enables Aβ-induced neurite degeneration in vitro and in vivo via the c-Jun pathways, a known mediator of Aβ-induced deleterious signaling. After the removal of p75NTR extracellular domain, the Aβ-induced neuritic dystrophy in cultured hippocampus neurons and in the basal forebrain of chronic Alzheimer's

disease mice could be completely reversed, and the neuritic dystrophy in the hippocampus was also significantly diminished (Knowles et al. 2009). Besides, non-peptide small molecule p75NTR ligands which block the binding of Aβ to p75NTR are found to be capable of inhibiting Aβ-induced neurite degeneration, synapse impairment, and the activation of some molecules involved in Alzheimer's disease pathology including calpain/cdk5, GSK3β and c-Jun, and tau phosphorylation. They can also prevent Aβ-induced inactivation of AKT and CREB (Yang et al. 2008). In accordance with results above, it was observed that abundant degenerative fibers and neurites in the neocortex and hippocampus express p75NTR in APPswe/PS1dE9 mice (Wang et al. 2010b), suggesting that p75NTR is a key receptor modulating Aβ-induced neurite degeneration. At the same time, a recent study found that the sympathetic nervous system is severely impaired in p75NTR-deficient Alzheimer's disease model, which leads to critical dysfunction of sympathetic innervation to multiple organs and finally results in the death of the animal. The protective role of p75NTR against Aβ-induced neurite degeneration showed in this study could be the result of the combined effect of p75NTR and its co-receptors under in vivo condition, such as the enhanced NGF and TrkA signaling, and possibly the promoted glial cell line-derived neurotrophic factor (GDNF) signaling, especially in the sympathetic nervous system (Bengoechea et al. 2009).

The reduction of NGF has also been suggested to be involved in the pathogenesis of Alzheimer's disease, but the exact role of its precursor (proNGF) in Alzheimer's disease remains elusive. It is reported that proNGF is the predominant form of this neurotrophin in the human brain, and it binds to p75NTR more strongly than mature NGF (Fahnestock et al. 2001). ProNGF has been shown to induce apoptosis in neuronal cell cultures through its interaction with p75NTR and its co-receptor, sortilin (Niewiadomska et al. 2011). Besides, the binding of proNGF to p75NTR-SorCS2 (sortilin-related VPS10 domain-containing receptor 2) complex can disassociate the guanine nucleotide exchange factor Trio from the complex, which brings down the activity of the small GTPase Rac and finally leads to destabilization and collapse of actin filaments, as well as growth cone retraction and collapse in cultured hippocampus neurons (Deinhardt et al. 2011). Since the level of proNGF has been found to increase in the cerebral cortex in Alzheimer's disease, it may mediate neurite degeneration through this mechanism. Besides, blocking proNGF can inhibit neuronal loss (Friedman 2000; Podlesniy et al. 2006; Volosin et al. 2008). The effects of proNGF in vitro and in vivo were investigated, and the results showed that proNGF is localized to the Aβ plaques in the brain of Alzheimer's disease mice, which also induced the neuronal death and neurite degeneration of different neuronal cell lines and primary neurons in culture, likely dependent on the expression of p75NTR (Wang et al. 2010a) (Fig. 1).

4.5 p75NTR and Neuronal Death

Neuronal death is another important pathological character of Alzheimer's disease. As one ligand of p75NTR, Aβ is best known for mediating neuronal death and has

been consistently linked to the pathology of Alzheimer's disease. It is believed that the interactions of Aβ with p75NTR and other neuronal cell surface receptors can trigger the production of reactive oxygen species, expression of caspases and pro-apoptotic genes (such as p53, p35), and enhance mitochondrial permeability (Dias Bda et al. 2011). Evidence shows that p75NTR plays a critical role in the early and characteristic loss of cholinergic neurons in response to Aβ in the septohippocampal pathway in Alzheimer's disease (Sotthibundhu et al. 2008). A recent study demonstrated that overexpression of tumor necrosis factor receptor-associated factor 6 (TRAF6) and p62, which are essential in p75NTR polyubiquitination and NF-κB activation-mediated cell survival signaling downstream, can reduce Aβ-induced neuronal death (Geetha et al. 2012). It was found that in the presence of Aβ, more neurons survived in culture after the p75NTR gene was deleted. Besides, the application of the recombinant extracellular domain of p75NTR can decrease the Aβ-induced neuronal death. Thus, it is probable that Aβ toxicity can be antagonized by blocking the p75NTR-mediated pathway (Wang et al. 2010b). However, research shows that the role of p75NTR in neurotoxicity depends on the different forms of Aβ interacting with it. For fibrillar Aβ, p75NTR mediates neuronal death, whereas for soluble oligomeric Aβ, it exerts a protective role against the neurotoxicity (Costantini et al. 2005a). The protective effect may be mediated by phosphatidylinositide 3-kinase (PI3K) activity due to the function of the juxtamembrane sequence of the cytoplasmic region of p75NTR. Considering that the activation of p75NTR-mediated pathway may need the role of its specific co-receptors, and that most studies were confined to in vitro experiments, further investigation about how p75NTR mediates Aβ-induced neurotoxicity needs to be carried out in in vivo models (Coulson 2006).

proNGF participates in neuronal death in Alzheimer's disease as well. Some researchers demonstrate that proNGF from the injured spinal cord induces apoptosis in culture among p75NTR-positive, but not p75NTR-negative, oligodendrocytes, and its action can be blocked by proNGF-specific antibodies. ProNGF can eliminate damaged cells by activating the apoptotic machinery of p75NTR (Beattie et al. 2002). Other researchers also found that proNGF secreted by astrocytes under the stimulation of peroxynitrite can specifically cause motor neuron death via the engagement of p75NTR (Domeniconi et al. 2007). As for Alzheimer's disease, some studies showed that proNGF levels increased in glial cells and neurons of the cortex and hippocampus, which is closely related to the progression of Alzheimer's disease. The upregulation of proNGF may represent the dysfunction of maturation of NGF that enhances the vulnerability of cholinergic neurons in Alzheimer's disease (Cuello et al. 2007). Moreover, researchers found proNGF extracted from Alzheimer's disease brains can obviously induce apoptotic neuronal death through p75NTR (Pedraza et al. 2005). And p75NTR intracellular domain (p75NTR-ICD), the level of which was significantly increased and paralleled the increase of proNGF in the entorhinal cortex, should account for proNGF-induced apoptosis in Alzheimer's disease (Podlesniy et al. 2006). Nevertheless, some researchers speculated the possibility that the loss of proNGF-mediated signaling may reduce the TrkA receptors within the cholinergic basal forebrain (CBF) neurons, affecting

cell survival. Whether proNGF binds p75NTR in vivo leading to apoptosis remains unclear (Peng et al. 2004). A recent study indicated that cleavage-resistant mutated forms of proNGF can promote neurite outgrowth, increase cell survival, as well as cause apoptotic cell death through phosphorylation of TrkA and ERK1/2 or initiation of apoptotic pathway by cleavage of caspase 3. So the final result of cell viability or apoptotic effects will depend on the balance of proNGF:NGF and p75NTR:TrkA ratios in Alzheimer's disease brains (Clewes et al. 2008). This is further proved as deletion of p75NTR-ECD which consequently leads to the abrogating of proNGF signaling in AD12 mice, obtained by crossing AD10 mice which express anti-NGF antibodies with $p75NTR^{exonIII(-/-)}$ mice, can still exert a protective role to anti-NGF-induced amyloidogenesis as a result of dominant TrkA survival signaling (Capsoni et al. 2010).

4.6 p75NTR and Neuronal Cell Cycle Re-entry

Adult neurons in the brain are classically thought to be in terminal differentiation permanently, effectively precluding them from cell division. However, around a decade ago, it was shown that susceptible cortical and hippocampal neurons in Alzheimer's disease display an activated cell cycle, suggesting cell cycle re-entry of neurons. Accumulating evidence indicates that neuronal cell cycle re-entry represents an early and critical event in Alzheimer's disease, recapitulating known hallmarks of the disease including NFTs and deposition of Aβ plaques. The cell cycle re-entry hypothesis may be extensively applicable in many neurological diseases because the re-entry of cell cycle also occurs in other neurodegenerative diseases.

The mechanism and the role of p75NTR in cell cycle re-entry in Alzheimer's disease remain obscure. However, there is evidence that illustrates an important position of p75NTR in cell cycle re-entry. Firstly, it was demonstrated that during development of the nervous system, the activation of p75NTR by an endogenous source of NGF (possibly proNGF) can trigger cell cycle re-entry and tetraploidization of retinal ganglion cells (Jansen et al. 2007; Nakamura et al. 2007; Morillo et al. 2010). Secondly, many newly identified p75NTR intracellular interactors have been known to regulate cell cycle. Finally, as a known stress-response receptor, expression of p75NTR in affected neurons in Alzheimer's disease becomes upregulated in response to stress, which has a close relationship with cell cycle re-entry. The exact role of p75NTR in the cell cycle re-entry of neurons in Alzheimer's disease needs further investigation.

p75NTR can be activated by Aβ (Yaar et al. 1997) and promote neuritic dystrophy and apoptosis in vitro and in vivo (Sotthibundhu et al. 2008; Knowles et al. 2009). Binding of aggregated Aβ to p75NTR can also lead to Ras/ERK activation (Susen and Blochl 2005), suggesting that the previously described increase of Ras/ MAPK activity in the Alzheimer's disease brain could be induced by this particular p75NTR-dependent signaling pathway (Arendt et al. 1995; Gartner et al. 1995). Furthermore, p75NTR has also been shown to physically

and functionally interact with APP (Fombonne et al. 2009). p75NTR can interact with different neurotrophin precursors, including proNGF (Fombonne et al. 2009) which represents the predominant form of NGF in the brain. This interaction requires the presence of sortilin, known to bind to the "pro" domain. Interestingly, the expression of sortilin becomes increased in aging basal forebrain neurons (Al-Shawi et al. 2007; Al-Shawi et al. 2008), and this molecule is required for neuronal degeneration associated with aging (Jansen et al. 2007). ProNGF has been shown to trigger pro-apoptotic signals through the p75NTR/sortilin complex (Nykjaer et al. 2004) although proNGF-dependent trophic effects are also observed in vivo (Fahnestock et al. 2004; Al-Shawi et al. 2008). Importantly, proNGF becomes accumulated in the cortex and hippocampus (Fahnestock et al. 2001) of the Alzheimer's disease brain, as well as in the aged brain.

4.7 p75NTR and Cognition, Tau Phosphorylation

In Alzheimer's disease patients degenerated cholinergic neurons in the basal forebrain are closely related to the impaired learning and memory processes. Some researchers found that after deletion of exon III or IV of the p75NTR gene which leads to a partial or complete p75NTR knockout, the cholinergic neurons in the medial septal nucleus increase 13 % and 28 %, respectively, in Alzheimer's disease mice, which is sure to improve cognition to some degree (Naumann et al. 2002). The results also suggest that the cytoplasmic domain of p75NTR is responsible for the cell death effect, and complete deletion of the full-length p75NTR can decrease cholinergic neuronal death.

There is also some evidence to suggest that p75NTR may participate in the formation of NFTs, another pathological character of Alzheimer's disease. When studying the brain of postmortem Alzheimer's disease patients, the ratio of p75NTR-positive neurons to the total number of neurons was found to be significantly higher in the CA1 and CA2 subfields of the hippocampus. Besides, a large proportion of these p75NTR expressing neurons are co-localized with Alz-50, whose antibody can be regarded as an indirect indication of hyperphosphorylated tau in the same subfields of Alzheimer's disease patients, suggesting that p75NTR may be involved in the formation of NFTs (Hu et al. 2002).

Our recent research demonstrated that deletion of the p75NTR gene can also exacerbate other Alzheimer's disease-type pathologies in the brain of Alzheimer's disease mice, such as microgliosis and microhemorrhage but does not reduce the memory deficits of Alzheimer's disease mice until 9 months of age (Wang et al. 2011).

5 Potential Use of p75NTR as a Therapeutic Target for Alzheimer's Disease

The roles of p75NTR in the pathogenesis of Alzheimer's disease described above strongly suggest that p75NTR represents a valid therapeutic target for the

treatment of Alzheimer's disease (Dechant and Barde 2002; Coulson et al. 2009; Knowles et al. 2009). However, as p75NTR has double-faced roles in Aβ metabolism and neurotoxicity, it is cautioned that simply reducing expression of p75NTR may exacerbate Alzheimer's disease pathology, as knockout of the p75NTR gene results in increased Aβ deposition and Alzheimer's pathologies in an APPswe/PS1dE9 transgenic mouse model. However, the use of the extracellular domain not only may attenuate neurotoxic signals by blocking Aβ-p75NTR and proneurotrophin-p75NTR interactions on cell membranes but also may suppress Aβ aggregation and deposition and enhance Aβ clearance from the brain. In addition, it was found that the recombinant extracellular domain of p75NTR is also able to block the Aβ- and proNGF-induced neurotoxicity in vitro (Wang et al. 2010a). This data suggests that recombinant extracellular domain of p75NTR may be a desirable anti-Alzheimer's disease agent with multiple functions to facilitate Aβ clearance by inhibiting Aβ aggregation, and to protect cholinergic neurons from Aβ- and proneurotrophin-induced neurotoxicity by blocking their interaction with p75NTR, and to reduce Aβ production by interrupt feedback loop between Aβ and p75NTR (Fig. 1).

6 Conclusion

p75NTR is an important molecule which has diverse functions linked with neuron development, aging, stress, and neuropathological conditions. Recent studies suggest that p75NTR plays a critical role in the pathogenesis, almost all hallmarks of Alzheimer's disease, starting from the generation of Aβ and removal of Aβ deposition, to mediate Aβ-induced neuronal death, neurite degeneration, tau hyperphosphorylation, and cell cycle re-entry. The shedded p75NTR extracellular domain is regarded as an endogenous protective molecule to interrupt Aβ/p75NTR feedback loop, block Aβ neurotoxicity, and remove Aβ deposits. Thus, the extracellular domain of p75NTR is a promising therapeutic target for the development of therapeutics for Alzheimer's disease.

References

Al-Shawi, R., Hafner, A., et al. (2007). ProNGF, sortilin, and age-related neurodegeneration. *Annals of the New York Academy of Sciences, 1119*, 208–215.

Al-Shawi, R., Hafner, A., et al. (2008). Neurotoxic and neurotrophic roles of proNGF and the receptor sortilin in the adult and ageing nervous system. *European Journal of Neuroscience, 27*(8), 2103–2114.

Arendt, T., Holzer, M., et al. (1995). Increased expression and subcellular translocation of the mitogen activated protein kinase kinase and mitogen-activated protein kinase in Alzheimer's disease. *Neuroscience, 68*(1), 5–18.

Arendt, T., Schindler, C., et al. (1997). Plastic neuronal remodeling is impaired in patients with Alzheimer's disease carrying apolipoprotein epsilon 4 allele. *Journal of Neuroscience, 17*(2), 516–529.

Bai, Y., Dergham, P., et al. (2010). Chronic and acute models of retinal neurodegeneration TrkA activity are neuroprotective whereas p75NTR activity is neurotoxic through a paracrine mechanism. *Journal of Biological Chemistry, 285*(50), 39392–39400.

Bamji, S. X., Majdan, M., et al. (1998). The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *The Journal of Cell Biology, 140*(4), 911–923.

Barker, P. A. (2004). p75NTR is positively promiscuous: Novel partners and new insights. *Neuron, 42*(4), 529–533.

Barrett, G. L., & Bartlett, P. F. (1994). The p75 nerve growth factor receptor mediates survival or death depending on the stage of sensory neuron development. *Proceedings of the National Academy of Sciences of the United States of America, 91*(14), 6501–6505.

Beattie, M. S., Harrington, A. W., et al. (2002). ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron, 36*(3), 375–386.

Bengoechea, T. G., Chen, Z., et al. (2009). p75 reduces beta-amyloid-induced sympathetic innervation deficits in an Alzheimer's disease mouse model. *Proceedings of the National Academy of Sciences of the United States of America, 106*(19), 7870–7875.

Bhakar, A. L., Howell, J. L., et al. (2003). Apoptosis induced by p75NTR overexpression requires Jun kinase-dependent phosphorylation of Bad. *Journal of Neuroscience, 23*(36), 11373–11381.

Brann, A. B., Scott, R., et al. (1999). Ceramide signaling downstream of the p75 neurotrophin receptor mediates the effects of nerve growth factor on outgrowth of cultured hippocampal neurons. *Journal of Neuroscience, 19*(19), 8199–8206.

Bronfman, F. C., & Fainzilber, M. (2004). Multi-tasking by the p75 neurotrophin receptor: Sortilin things out? *EMBO Reports, 5*(9), 867–871.

Buck, C. R., Martinez, H. J., et al. (1987). Developmentally regulated expression of the nerve growth factor receptor gene in the periphery and brain. *Proceedings of the National Academy of Sciences of the United States of America, 84*(9), 3060–3063.

Capsoni, S., Tiveron, C., et al. (2010). Dissecting the involvement of tropomyosin-related kinase A and p75 neurotrophin receptor signaling in NGF deficit-induced neurodegeneration. *Proceedings of the National Academy of Sciences of the United States of America, 107*(27), 12299–12304.

Carter, B. D., Kaltschmidt, C., et al. (1996). Selective activation of NF-kappa B by nerve growth factor through the neurotrophin receptor p75. *Science, 272*(5261), 542–545.

Casaccia-Bonnefil, P., Aibel, L., et al. (1996). Central glial and neuronal populations display differential sensitivity to ceramide-dependent cell death. *Journal of Neuroscience Research, 43*(3), 382–389.

Chakravarthy, B., Gaudet, C., et al. (2010). Amyloid-beta peptides stimulate the expression of the p75NTR neurotrophin receptor in SHSY5Y human neuroblastoma cells and AD transgenic mice. *Journal of Alzheimer's Disease, 19*(3), 915–925.

Chao, M. V., & Bothwell, M. (2002). Neurotrophins: To cleave or not to cleave. *Neuron, 33*(1), 9–12.

Cheema, S. S., Barrett, G. L., et al. (1996). Reducing p75 nerve growth factor receptor levels using antisense oligonucleotides prevents the loss of axotomized sensory neurons in the dorsal root ganglia of newborn rats. *Journal of Neuroscience Research, 46*(2), 239–245.

Chu, G. K., Yu, W., et al. (2007). The p75 neurotrophin receptor is essential for neuronal cell survival and improvement of functional recovery after spinal cord injury. *Neuroscience, 148*(3), 668–682.

Clewes, O., Fahey, M. S., et al. (2008). Human ProNGF: Biological effects and binding profiles at TrkA, P75NTR and sortilin. *Journal of Neurochemistry, 107*(4), 1124–1135.

Copray, S., Kust, B., et al. (2004). Deficient p75 low-affinity neurotrophin receptor expression exacerbates experimental allergic encephalomyelitis in C57/BL6 mice. *Journal of Neuroimmunology, 148*(1–2), 41–53.

Costantini, C., Della-Bianca, V., et al. (2005a). The expression of p75 neurotrophin receptor protects against the neurotoxicity of soluble oligomers of beta-amyloid. *Experimental Cell Research, 311*(1), 126–134.

Costantini, C., Weindruch, R., et al. (2005b). A TrkA to p75 NTR molecular switch activates amyloid beta-peptide generation during aging. *Biochemical Journal, 59*(Pt1), 59–67.
Costantini, C., Scrable, H., et al. (2006). An aging pathway controls the TrkA to p75NTR receptor switch and amyloid beta-peptide generation. *The EMBO Journal, 25*(9), 1997–2006.
Coulson, E. J. (2006). Does the p75 neurotrophin receptor mediate Abeta-induced toxicity in Alzheimer's disease? *Journal of Neurochemistry, 98*(3), 654–660.
Coulson, E. J., May, L. M., et al. (2009). The role of the p75 neurotrophin receptor in cholinergic dysfunction in Alzheimer's disease. *The Neuroscientist, 15*(4), 317–323.
Cragnolini, A. B., & Friedman, W. J. (2008). The function of p75NTR in glia. *Trends in Neurosciences, 31*(2), 99–104.
Cuello, A. C., Bruno, M. A., et al. (2007). NGF-cholinergic dependency in brain aging, MCI and Alzheimer's disease. *Current Alzheimer Research, 4*(4), 351–358.
Davey, F., & Davies, A. M. (1998). TrkB signalling inhibits p75-mediated apoptosis induced by nerve growth factor in embryonic proprioceptive neurons. *Current Biology, 8*(16), 915–918.
Dechant, G., & Barde, Y. A. (2002). The neurotrophin receptor p75(NTR): Novel functions and implications for diseases of the nervous system. *Nature Neuroscience, 5*(11), 1131–1136.
Deinhardt, K., Kim, T., et al. (2011). Neuronal growth cone retraction relies on proneurotrophin receptor signaling through Rac. *Science Signaling, 4*(202), ra82.
Dias Bda, C., Jovanovic, K., et al. (2011). Structural and mechanistic commonalities of amyloid-beta and the prion protein. *Prion, 5*(3), 126–137.
Domeniconi, M., Hempstead, B. L., et al. (2007). Pro-NGF secreted by astrocytes promotes motor neuron cell death. *Molecular and Cellular Neuroscience, 34*(2), 271–279.
Ernfors, P., Hallbook, F., et al. (1988). Developmental and regional expression of beta-nerve growth factor receptor mRNA in the chick and rat. *Neuron, 1*(10), 983–996.
Fahnestock, M., Michalski, B., et al. (2001). The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Molecular and Cellular Neuroscience, 18*(2), 210–220.
Fahnestock, M., Yu, G., et al. (2004). ProNGF: A neurotrophic or an apoptotic molecule? *Progress in Brain Research, 146*, 101–110.
Ferri, C. C., & Bisby, M. A. (1999). Improved survival of injured sciatic nerve Schwann cells in mice lacking the p75 receptor. *Neuroscience Letters, 272*(3), 191–194.
Ferri, C. C., Moore, F. A., et al. (1998). Effects of facial nerve injury on mouse motoneurons lacking the p75 low-affinity neurotrophin receptor. *Journal of Neurobiology, 34*(1), 1–9.
Fombonne, J., Rabizadeh, S., et al. (2009). Selective vulnerability in Alzheimer's disease: Amyloid precursor protein and p75(NTR) interaction. *Annals of Neurology, 65*(3), 294–303.
Fournier, A. E., GrandPre, T., et al. (2001). Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. *Nature, 409*(6818), 341–346.
Friedman, W. J. (2000). Neurotrophins induce death of hippocampal neurons via the p75 receptor. *Journal of Neuroscience, 20*(17), 6340–6346.
Gartner, U., Holzer, M., et al. (1995). Induction of p21ras in Alzheimer pathology. *NeuroReport, 6*(10), 1441–1444.
Geetha, T., Zheng, C., et al. (2012). TRAF6 and p62 inhibit amyloid beta-induced neuronal death through p75 neurotrophin receptor. *Neurochemistry International, 61*, 1289–1293.
Giehl, K. M., Rohrig, S., et al. (2001). Endogenous brain-derived neurotrophic factor and neurotrophin-3 antagonistically regulate survival of axotomized corticospinal neurons in vivo. *Journal of Neuroscience, 21*(10), 3492–3502.
Ginsberg, S. D., Che, S., et al. (2006). Down regulation of trk but not p75NTR gene expression in single cholinergic basal forebrain neurons mark the progression of Alzheimer's disease. *Journal of Neurochemistry, 97*(2), 475–487.
Goedert, M., Fine, A., et al. (1989). Nerve growth factor receptor mRNA distribution in human brain: Normal levels in basal forebrain in Alzheimer's disease. *Brain Research. Molecular Brain Research, 5*(1), 1–7.

Gotz, J., & Ittner, L. M. (2008). Animal models of Alzheimer's disease and frontotemporal dementia. *Nature Review Neuroscience, 9*(7), 532–544.

Griesmaier, E., Schlager, G., et al. (2010). Role of p75(NTR) in NMDAR-mediated excitotoxic brain injury in neonatal mice. *Brain Research, 1355*, 31–40.

Gruss, H. J. (1996). Molecular, structural, and biological characteristics of the tumor necrosis factor ligand superfamily. *International Journal of Clinical & Laboratory Research, 26*(3), 143–159.

Hartig, W., Seeger, J., et al. (1998). Selective in vivo fluorescence labelling of cholinergic neurons containing p75(NTR) in the rat basal forebrain. *Brain Research, 808*(2), 155–165.

Hasegawa, Y., Yamagishi, S., et al. (2004). p75 neurotrophin receptor signaling in the nervous system. *Biotechnology Annual Review, 10*, 123–149.

Hu, X. Y., Zhang, H. Y., et al. (2002). Increased p75(NTR) expression in hippocampal neurons containing hyperphosphorylated tau in Alzheimer patients. *Experimental Neurology, 178*(1), 104–111.

Huang, E. J., & Reichardt, L. F. (2003). Trk receptors: Roles in neuronal signal transduction. *Annual Review of Biochemistry, 72*, 609–642.

Ito, S., Menard, M., et al. (2012). Involvement of insulin-like growth factor 1 receptor signaling in the amyloid-beta peptide oligomers-induced p75 neurotrophin receptor protein expression in mouse hippocampus. *Journal of Alzheimer's Disease, 31*(3), 493–506.

Jansen, P., Giehl, K., et al. (2007). Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. *Nature Neuroscience, 10*(11), 1449–1457.

Kawarabayashi, T., Younkin, L. H., et al. (2001). Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *Journal of Neuroscience, 21*(2), 372–381.

Knowles, J. K., Rajadas, J., et al. (2009). The p75 neurotrophin receptor promotes amyloid-beta (1–42)-induced neuritic dystrophy in vitro and in vivo. *Journal of Neuroscience, 29*(34), 10627–10637.

Koh, S., & Loy, R. (1989). Localization and development of nerve growth factor-sensitive rat basal forebrain neurons and their afferent projections to hippocampus and neocortex. *Journal of Neuroscience, 9*(9), 2999–3018.

Lowry, K. S., Murray, S. S., et al. (2001). A potential role for the p75 low-affinity neurotrophin receptor in spinal motor neuron degeneration in murine and human amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 2*(3), 127–134.

Mi, S., Lee, X., et al. (2004). LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nature Neuroscience, 7*(3), 221–228.

Morillo, S. M., Escoll, P., et al. (2010). Somatic tetraploidy in specific chick retinal ganglion cells induced by nerve growth factor. *Proceedings of the National Academy of Sciences of the United States of America, 107*(1), 109–114.

Mufson, E. J., & Kordower, J. H. (1992). Cortical neurons express nerve growth factor receptors in advanced age and Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America, 89*(2), 569–573.

Mufson, E. J., Counts, S. E., et al. (2002). Gene expression profiles of cholinergic nucleus basalis neurons in Alzheimer's disease. *Neurochemical Research, 27*(10), 1035–1048.

Nakamura, K., Namekata, K., et al. (2007). Intracellular sortilin expression pattern regulates proNGF-induced naturally occurring cell death during development. *Cell Death and Differentiation, 14*(8), 1552–1554.

Naumann, T., Casademunt, E., et al. (2002). Complete deletion of the neurotrophin receptor p75NTR leads to long-lasting increases in the number of basal forebrain cholinergic neurons. *Journal of Neuroscience, 22*(7), 2409–2418.

Niewiadomska, G., Mietelska-Porowska, A., et al. (2011). The cholinergic system, nerve growth factor and the cytoskeleton. *Behavioural Brain Research, 221*(2), 515–526.

Nykjaer, A., Lee, R., et al. (2004). Sortilin is essential for proNGF-induced neuronal cell death. *Nature, 427*(6977), 843–848.

Palmada, M., Kanwal, S., et al. (2002). c-jun is essential for sympathetic neuronal death induced by NGF withdrawal but not by p75 activation. *Journal of Cell Biology, 158*(3), 453–461.

Pedraza, C. E., Podlesniy, P., et al. (2005). Pro-NGF isolated from the human brain affected by Alzheimer's disease induces neuronal apoptosis mediated by p75NTR. *American Journal of Pathology, 166*(2), 533–543.

Peng, S., Wuu, J., et al. (2004). Increased proNGF levels in subjects with mild cognitive impairment and mild Alzheimer disease. *Journal of Neuropathology and Experimental Neurology, 63*(6), 641–649.

Petratos, S., Li, Q. X., et al. (2008). The beta-amyloid protein of Alzheimer's disease increases neuronal CRMP-2 phosphorylation by a Rho-GTP mechanism. *Brain, 131*(Pt 1), 90–108.

Pincheira, R., Baerwald, M., et al. (2009). Sall2 is a novel p75NTR-interacting protein that links NGF signalling to cell cycle progression and neurite outgrowth. *The EMBO Journal, 28*(3), 261–273.

Podlesniy, P., Kichev, A., et al. (2006). Pro-NGF from Alzheimer's disease and normal human brain displays distinctive abilities to induce processing and nuclear translocation of intracellular domain of p75NTR and apoptosis. *American Journal of Pathology, 169*(1), 119–131.

Roux, P. P., & Barker, P. A. (2002). Neurotrophin signaling through the p75 neurotrophin receptor. *Progress in Neurobiology, 67*(3), 203–233.

Salehi, A., Ocampo, M., et al. (2000). P75 neurotrophin receptor in the nucleus basalis of meynert in relation to age, sex, and Alzheimer's disease. *Experimental Neurology, 161*(1), 245–258.

Santee, S. M., & Owen-Schaub, L. B. (1996). Human tumor necrosis factor receptor p75/80 (CD120b) gene structure and promoter characterization. *Journal of Biological Chemistry, 271*(35), 21151–21159.

Sedel, F., Bechade, C., et al. (1999). Nerve growth factor (NGF) induces motoneuron apoptosis in rat embryonic spinal cord in vitro. *European Journal of Neuroscience, 11*(11), 3904–3912.

Selkoe, D. J. (2001). Alzheimer's disease: Genes, proteins, and therapy. *Physiological Reviews, 81*(2), 741–766.

Sendtner, M., Holtmann, B., et al. (1992). Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature, 360*(6406), 757–759.

Sotthibundhu, A., Sykes, A. M., et al. (2008). Beta-amyloid(1–42) induces neuronal death through the p75 neurotrophin receptor. *Journal of Neuroscience, 28*(15), 3941–3946.

Spuch, C., & Carro, E. (2011). The p75 neurotrophin receptor localization in blood-CSF barrier: Expression in choroid plexus epithelium. *BMC Neuroscience, 12*, 39.

Susen, K., & Blochl, A. (2005). Low concentrations of aggregated beta-amyloid induce neurite formation via the neurotrophin receptor p75. *Journal of Molecular Medicine, 83*(9), 720–735.

Tabaton, M., Zhu, X., et al. (2010). Signaling effect of amyloid-beta(42) on the processing of AbetaPP. *Experimental Neurology, 221*(1), 18–25.

Treanor, J. J., Dawbarn, D., et al. (1991). Low affinity nerve growth factor receptor binding in normal and Alzheimer's disease basal forebrain. *Neuroscience Letters, 121*(1–2), 73–76.

Troy, C. M., Friedman, J. E., et al. (2002). Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. *Journal of Biological Chemistry, 277*(37), 34295–34302.

Van der Zee, C. E., Ross, G. M., et al. (1996). Survival of cholinergic forebrain neurons in developing p75NGFR-deficient mice. *Science, 274*(5293), 1729–1732.

Volosin, M., Trotter, C., et al. (2008). Induction of proneurotrophins and activation of p75NTR-mediated apoptosis via neurotrophin receptor-interacting factor in hippocampal neurons after seizures. *Journal of Neuroscience, 28*(39), 9870–9879.

Walsh, G. S., Krol, K. M., et al. (1999). Enhanced neurotrophin-induced axon growth in myelinated portions of the CNS in mice lacking the p75 neurotrophin receptor. *Journal of Neuroscience, 19*(10), 4155–4168.

Wang, K. C., Kim, J. A., et al. (2002a). P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature, 420*(6911), 74–78.

Wang, K. C., Koprivica, V., et al. (2002b). Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature, 417*(6892), 941–944.

Wang, Y. J., Valadares, D., et al. (2010a). Effects of proNGF on neuronal viability, neurite growth and amyloid-beta metabolism. *Neurotoxicity Research, 17*(3), 257–267.

Wang, Y. J., Wang, X., et al. (2010b). p75NTR regulates Abeta deposition by increasing Abeta production but inhibiting Abeta aggregation with its extracellular domain. *Journal of Neuroscience, 31*, 2292–2304.

Wang, Y. J., Wang, X., et al. (2011). p75NTR regulates Abeta deposition by increasing Abeta production but inhibiting Abeta aggregation with its extracellular domain. *Journal of Neuroscience, 31*(6), 2292–2304.

Weskamp, G., Schlondorff, J., et al. (2004). Evidence for a critical role of the tumor necrosis factor alpha convertase (TACE) in ectodomain shedding of the p75 neurotrophin receptor (p75NTR). *Journal of Biological Chemistry, 279*(6), 4241–4249.

Wong, S. T., Henley, J. R., et al. (2002). A p75(NTR) and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. *Nature Neuroscience, 5*(12), 1302–1308.

Yaar, M., Zhai, S., et al. (1997). Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis. A possible mechanism for Alzheimer's disease. *The Journal of Clinical Investigation, 100*(9), 2333–2340.

Yamashita, T., Tucker, K. L., et al. (1999). Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. *Neuron, 24*(3), 585–593.

Yamashita, T., Higuchi, H., et al. (2002). The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. *Journal of Cell Biology, 157*(4), 565–570.

Yang, T., Knowles, J. K., et al. (2008). Small molecule, non-peptide p75 ligands inhibit Abeta-induced neurodegeneration and synaptic impairment. *PLoS One, 3*(11), e3604.

Yoon, S. O., Casaccia-Bonnefil, P., et al. (1998). Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival. *Journal of Neuroscience, 18*(9), 3273–3281.

Zhao, J., Fu, Y., et al. (2007). Beta-site amyloid precursor protein cleaving enzyme 1 levels become elevated in neurons around amyloid plaques: Implications for Alzheimer's disease pathogenesis. *Journal of Neuroscience, 27*(14), 3639–3649.

Zhou, X. F., & Wang, Y. J. (2011). The p75NTR extracellular domain: A potential molecule regulating the solubility and removal of amyloid-beta. *Prion, 5*(3), 161–163.

Seizures Tip the Balance of Neurotrophin Signaling Toward Neuronal Death

Wilma J. Friedman, Audrey P. Le, Soyoung Choi, and Marta Volosin

Contents

Abstract

Neurotrophins and proneurotrophins have opposing actions on neuronal survival, mediated by their respective receptors. Trk receptors are necessary for mature neurotrophins to support neuronal survival, while the p75 neurotrophin receptor ($p75^{NTR}$) mediates proneurotrophin-induced apoptosis. Therefore, the consequences of neurotrophin actions depend on expression of the different neurotrophin receptors; regulation of proneurotrophin cleavage, which can determine which receptor is bound; and activation of downstream signaling events supporting neuronal survival or apoptosis. Several types of brain injury, including seizures, lead to altered levels of neurotrophin receptors, with increased neuronal expression of $p75^{NTR}$ in the brain. Seizure-induced

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injury also leads to increased secreted proNGF due to altered regulation of the extracellular proNGF-cleaving enzymes. Downstream signaling pathways triggered by proNGF activation of $p75^{NTR}$ include induction of the phosphatase PTEN, which antagonizes TrkB signaling triggered by BDNF and prevents the activation of Akt. Thus, the increase in extracellular proNGF and elevated levels of $p75^{NTR}$ that occur after injury create a milieu that leads to neuronal death.

Keywords
Brain-derived neurotrophic factor • Hippocampus • Nerve growth factor • p75 Neurotrophin receptor • Trk

1 Introduction

Neurotrophins have been well established as trophic factors in the peripheral and central nervous systems, supporting neuronal survival and promoting differentiation (Barde 1994; Huang and Reichardt 2001). The neurotrophin family, consisting of NGF, BDNF, NT3, and NT4, binds to two distinct types of receptors: a member of the Trk receptor tyrosine kinase family and the p75 neurotrophin receptor ($p75^{NTR}$). The role of Trk receptors in mediating neuronal survival, differentiation, and synaptic function has been well defined, and the major signaling pathways activated include the PI_3 kinase-Akt, ras-MAP kinase, and PLCγ signaling pathways, which have been extensively studied (Friedman and Greene 1999; Kaplan and Miller 2000; Patapoutian and Reichardt 2001). In contrast, the $p75^{NTR}$ has been more of an enigma, since it has been implicated in mediating many diverse cellular functions such as survival (Hamanoue et al. 1999; Chu et al. 2007), apoptosis (Casaccia-Bonnefil et al. 1996; Frade et al. 1996; Coulson et al. 1999), and axonal growth (Yamashita et al. 1999; Sun et al. 2012), depending upon the cellular context. Following numerous different types of injury, including seizures, traumatic brain injury, and axotomy, $p75^{NTR}$ has been shown to signal apoptosis (Friedman 2000; Beattie et al. 2002; Troy et al. 2002; Harrington et al. 2004). Although $p75^{NTR}$ can bind all the neurotrophins with low affinity, uncleaved proneurotrophins bind $p75^{NTR}$ with high affinity (Lee et al. 2001) due to the association with the co-receptor sortilin (Nykjaer et al. 2004) and potently induce neuronal death (Harrington et al. 2004; Volosin et al. 2008). Thus, neurotrophins can signal survival or apoptosis, depending on which form of the factor is secreted, which can determine which receptor and signaling pathways are activated. Therefore, determination of neuronal survival or death following injury depends on several events, such as altered expression of the different neurotrophin receptors, $p75^{NTR}$ and the Trk family. Moreover, regulation of proneurotrophin processing to determine whether the cleaved or uncleaved form is secreted and stabilized in the extracellular milieu can be a critical determinant of which receptor is engaged, leading to neuronal survival or death. Thus, a delicate balance of receptor expression and downstream signaling, as well as proneurotrophin processing, can critically determine the outcome for signaling neuronal survival or death.

2 $p75^{NTR}$ and Apoptotic Signaling

Many injury conditions, including seizures, lesions, and traumatic injuries, have been shown to induce the $p75^{NTR}$, suggesting an important role in mediating neuronal responses to injury (Ernfors et al. 1989; Roux et al. 1999). Moreover, in mice lacking this receptor, neuronal death is significantly reduced following injury (Beattie et al. 2002; Troy et al. 2002). Although $p75^{NTR}$ is a member of the TNF receptor family of death receptors, it does not signal through the same pathways as other members of this family, which generally recruit adapter proteins to the trimerized receptor to activate caspase-8 and the extrinsic apoptotic pathway (Green 2003). In contrast, activation of $p75^{NTR}$-mediated apoptosis requires phosphorylation of c-Jun N-terminal kinase (JNK) (Yoon et al. 1998; Friedman 2000) and phosphorylation of the pro-apoptotic proteins Bad (Bhakar et al. 2003) and BimEL (Becker et al. 2004), eliciting the release of cytochrome c from the mitochondria and the subsequent activation of caspase-9, caspase-6, and caspase-3 (Wang et al. 2001; Troy et al. 2002). Thus, apoptotic signaling via $p75^{NTR}$ is different from the classic members of the TNFR family of death receptors.

The $p75^{NTR}$ has no kinase activity and signals by recruiting intracellular binding proteins to activate downstream events. The receptor undergoes regulated intramembrane proteolysis (RIP), generating a C-terminal fragment (CTF) and ultimately the intracellular domain (ICD) that interacts with intracellular proteins such as NRIF, NRAGE, and NADE to promote cell death (Barker 2004), ultimately activating the intrinsic caspase cascade. Genetic mutation analysis of different domains of the $p75^{NTR}$ has demonstrated that specific amino acids distributed throughout the death domain are required for the activation of the caspase pathway, leading to cell death, while other domains are necessary for activation of RIP2 binding, leading to NF-κB activation and cell survival (Charalampopoulos et al. 2012). Activation of NF-κB by $p75^{NTR}$ has been observed in Schwann cells (Carter et al. 1996), but not in hippocampal neurons or astrocytes (Volosin et al. 2008). The activation of distinct cell-specific signaling pathways is likely regulated by the expression or recruitment of the specific adapter proteins that link the receptor to the different downstream pathways. The scattered amino acid residues that are required for caspase-3 activation suggest that multiple binding proteins are necessary for activation of this pathway, possibly forming a multi-molecular complex binding to the receptor.

Following damage in the brain, numerous cytokines and trophic factors are released that can impact upon the injured neurons. Mature neurotrophins, such as BDNF, are present in the hippocampus and can activate its TrkB receptor to promote signaling via the PI3K-Akt and ras-ERK pathways to support survival of these neurons. The ability of neurotrophins to support neuronal survival can be affected by changes in the neurotrophin receptor levels and signaling that occur following seizures. Seizures, as well as other types of injury, lead to an induction of $p75^{NTR}$ expression in the hippocampus (Roux et al. 1999; Troy et al. 2002). A previous study has shown that along with the increase in $p75^{NTR}$, there is a concomitant decrease in TrkB (Unsain et al. 2008), tilting the balance of signaling towards neuronal death. The induced $p75^{NTR}$ on hippocampal neurons can be activated by proNGF, which is

released following injury, leading to neuronal death. Neurons that may express both $p75^{NTR}$ and TrkB and may be exposed to BDNF and proNGF must integrate these signals to a decisive outcome, survival, or death. Interestingly, in contrast to sympathetic neurons where activation of Trk prevents $p75^{NTR}$-mediated apoptosis (Miller and Kaplan 2001), CNS neurons expressing both $p75^{NTR}$ and TrkB, and exposed to both proNGF and BDNF, were not protected from cell death by the BDNF. This was due to the induction of the PTEN phosphatase by $p75^{NTR}$ activation, which prevented the ability of TrkB to activate Akt, thereby inhibiting the ability of BDNF to promote survival and facilitating the apoptotic signaling of $p75^{NTR}$ (Song et al. 2010). When the induction of PTEN by proNGF was prevented, BDNF was able to rescue hippocampal neurons from death following seizures, indicating that the induction of PTEN is a critical checkpoint in the balance between survival and apoptotic signaling (Song et al. 2010). Thus, induction of neuronal apoptosis by proNGF in the presence of mature neurotrophins requires the suppression of Trk signaling as well as the activation of JNK and subsequent caspase cascade.

3 Seizure-Induced Changes in Connectivity

A major consequence of epileptic seizures is the dendritic sprouting and synaptic reorganization that leads to the formation of aberrant connections and the development of spontaneous recurrent seizures (Williams et al. 2009). Many studies have shown that BDNF is elevated in the hippocampus following different types of seizures (Ballarin et al. 1991). Levels of the TrkB receptor are particularly high in the mossy fibers, projections that undergo sprouting and circuit reorganization, which are thought to participate in the development of recurrent seizures, leading to increased seizure susceptibility (Koyama et al. 2004; McNamara and Scharfman 2012). In contrast to the stimulation of axonal and dendritic sprouting induced by BDNF, recent studies have demonstrated that $p75^{NTR}$ can induce axonal degeneration. In fact, the activation of caspase-6 by $p75^{NTR}$ has been implicated not only in cell death but also in axonal degeneration (Park et al. 2010), suggesting that activation of $p75^{NTR}$ may play a broader role following injury, not only inducing neuronal loss but also potentially triggering more subtle but widespread responses involving axonal loss and circuit remodeling. Thus, in addition to having opposing actions with respect to neuronal survival, TrkB and $p75^{NTR}$ have opposing actions in regulating axonal growth, with TrkB eliciting sprouting and $p75^{NTR}$ evoking degeneration, both of which may come into play following seizures with the consequent formation of aberrant circuits.

4 Regulation of $p75^{NTR}$ Following Injury

The $p75^{NTR}$ is not widely expressed in the adult brain but is induced following numerous different types of insults, including seizures, axotomy, and trauma. This receptor is also upregulated in neurodegenerative diseases such as Alzheimer's

disease. The induction of this receptor in all these pathological situations raises the question of whether there are common signals present in all these conditions that regulate expression of $p75^{NTR}$. One common feature of all these injury and disease conditions is hypo-osmolarity, which has been shown to regulate $p75^{NTR}$ expression via activation of Sp1 transcription factors (Ramos et al. 2007). Another common characteristic of these conditions is the presence of inflammation in the brain and high levels of inflammatory cytokines such as IL-1β and TNFα. These cytokines can induce expression of $p75^{NTR}$ in hippocampal neurons in vitro (Choi and Friedman 2009) and in vivo and may contribute to the mechanism by which $p75^{NTR}$ is induced on vulnerable neurons to promote neuron loss. IL-1β is known to enhance neuronal vulnerability to many types of damage, including traumatic brain injury and stroke. In culture, preexposure of hippocampal neurons to IL-1β enhances the apoptotic response to proNGF, suggesting that proNGF is part of the mechanisms by which IL-1β increases neuronal vulnerability.

5 Regulation of Extracellular Proneurotrophin Stability

Neurotrophins are synthesized as precursor proteins that undergo cleavage in the trans-Golgi network by enzymes such as furin and other proconvertases with the cleaved product being secreted as the mature neurotrophin (Seidah et al. 1996). As the precursors of neurotrophins, proneurotrophins are transiently present in neurons and glial cells in the brain; therefore, detection of intracellular proneurotrophins does not indicate which form of the proteins is secreted. The mechanisms by which proneurotrophins may be secreted without being processed are undefined; however, since neurotrophins and proneurotrophins can have opposing actions in mediating neuronal survival or death, and axonal sprouting and degeneration, it is clearly critical to maintain tight regulation of proneurotrophin cleavage. Therefore, in addition to the intracellular processing of this protein by furin, mechanisms also exist for promoting proneurotrophin processing in the extracellular environment. Extracellular enzymes that can cleave proneurotrophins include matrix metalloproteinases (MMPs) and the tissue plasminogen activator (tPA)-plasminogen cascade (Lee et al. 2001). In the pentylenetetrazole (PTZ) model, seizures increased levels of MMP-9, promoting the conversion of proBDNF to BDNF (Mizoguchi et al. 2011). In addition, electroconvulsive seizures (ECS) increased levels of proconvertase 1 and tPA (tissue plasminogen activator), which also promote processing of proBDNF to BDNF (Segawa et al. 2012). Thus, there is evidence that seizures promote cleavage of proBDNF to BDNF, which then increases the likelihood of recurrent seizures (McNamara and Scharfman 2012). However, in contrast to proBDNF, the unprocessed proNGF has been detected in cerebrospinal fluid under conditions of injury and neurodegenerative disease (Harrington et al. 2004; Le and Friedman 2012), indicating that the NGF precursor protein was secreted without being cleaved and is stable in the extracellular environment and not cleaved by extracellular proteases. This indicates that alterations in the levels and/or activity of these cleaving enzymes occur as

a consequence of the injury leading to stabilization of the proneurotrophin. Unlike proBDNF, proNGF is not cleaved by MMP9 but can undergo extracellular cleavage by MMP7. Seizure-induced injury leads to decreased levels of MMP7 protein and activity and increases in tissue inhibitor of metalloproteinase (TIMP)-1 (Rivera et al. 1997), resulting in inhibition of proNGF cleavage (Le and Friedman 2012). Moreover, infusion of exogenous MMP7 into the hippocampus following seizures resulted in cleavage of extracellular proNGF and provided neuroprotection (Le and Friedman 2012). Thus, the altered balance of these extracellular enzymes and their endogenous inhibitors regulates the stability of proNGF in the extracellular milieu, providing a specific ligand for binding the injury-induced $p75^{NTR}$ and promoting neuronal loss following seizures.

6 Conclusion

A tightly regulated balance exists between the ability of neurotrophins to promote neuronal survival and apoptosis. In the healthy brain, proneurotrophins are

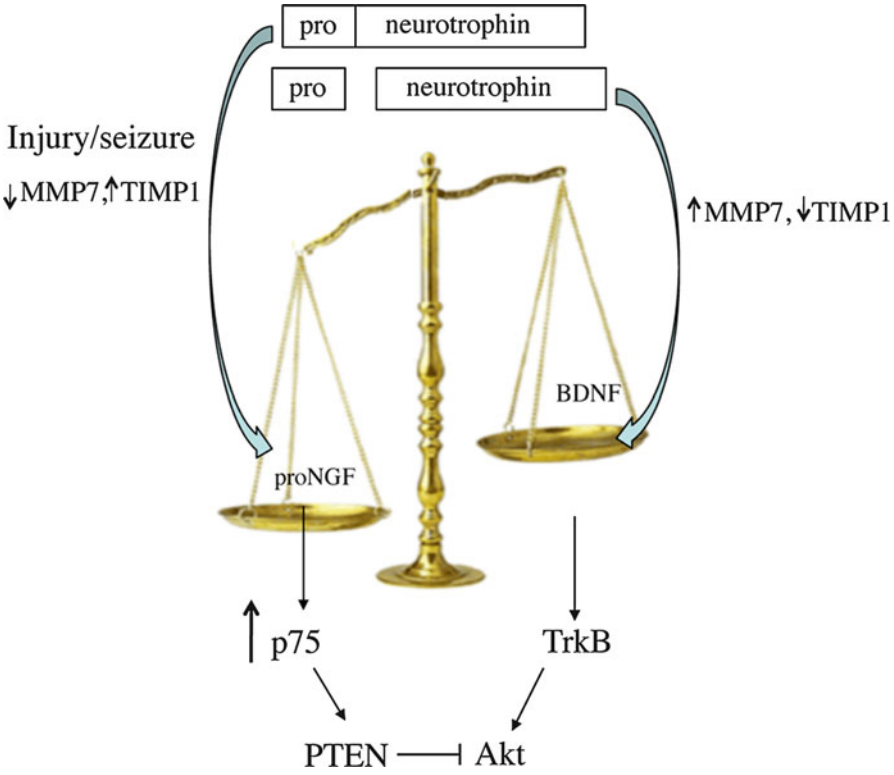

Fig. 1 Tilting the balance: seizure-induced alterations in the balance of proneurotrophin/neurotrophin signaling that lead to neuronal death

processed to generate the mature trophic factors, which signal via their Trk receptors to influence neuronal, survival differentiation and function. Following seizures and other types of injury, the induction of $p75^{NTR}$ expression on injured neurons and extracellular presence of proNGF shift the balance of neurotrophin actions towards inducing apoptosis of the injured cells (Fig. 1).

References

Ballarin, M., Ernfors, P., Lindefors, N., & Persson, P. (1991). Hippocampal damage and kainic acid injection induce a rapid increase in mRNA for BDNF and NGF in the rat brain. *Experimental Neurology, 114*, 35–43.

Barde, Y. A. (1994). Neurotrophins: A family of proteins supporting the survival of neurons. *Progress in Clinical and Biological Research, 390*, 45–56.

Barker, P. A. (2004). $p75^{NTR}$ Is positively promiscuous: Novel partners and new insights. *Neuron, 42*, 529–533.

Beattie, M. S., Harrington, A. W., Lee, R., Kim, J. Y., Boyce, S. L., Longo, F. M., Bresnahan, J. C., Hempstead, B. L., & Yoon, S. O. (2002). ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron, 36*, 375–386.

Becker, E. B., Howell, J., Kodama, Y., Barker, P. A., & Bonni, A. (2004). Characterization of the c-Jun N-terminal kinase-BimEL signaling pathway in neuronal apoptosis. *Journal of Neuroscience, 24*, 8762–8770.

Bhakar, A. L., Howell, J. L., Paul, C. E., Salehi, A. H., Becker, E. B., Said, F., Bonni, A., & Barker, P. A. (2003). Apoptosis induced by $p75^{NTR}$ overexpression requires Jun kinase-dependent phosphorylation of Bad. *Journal of Neuroscience, 23*, 11373–11381.

Carter, B. D., Kaltschmidt, C., Kaltschmidt, B., Offenhäuser, N., Böhm-Matthaei, R., Baeuerle, P. A., & Barde, Y.-A. (1996). Selective activation of NF-kB by nerve growth factor through the neurotrophin receptor p75. *Science, 272*, 542.

Casaccia-Bonnefil, P., Carter, B. D., Dobrowsky, R. T., & Chao, M. V. (1996). Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. *Nature, 383*, 716–719.

Charalampopoulos, I., Vicario, A., Pediaditakis, I., Gravanis, A., Simi, A., & Ib Òez, C. F. (2012). Genetic dissection of neurotrophin signaling through the p75 neurotrophin receptor. *Cell Reports, 2*, 1563–1570.

Choi, S., & Friedman, W. (2009). Inflammatory cytokines IL-1β and TNF-α regulate $p75^{NTR}$ expression in CNS neurons and astrocytes by distinct cell-type-specific signalling mechanisms. *Asn Neuro, 1*(2), art:e00010. doi:10.1042/AN20090009.

Chu, G. K. T., Yu, W., & Fehlings, M. G. (2007). The p75 neurotrophin receptor is essential for neuronal cell survival and improvement of functional recovery after spinal cord injury. *Neuroscience, 148*, 668–682.

Coulson, E. J., Reid, K., & Bartlett, P. F. (1999). Signaling of neuronal cell death by the $p75^{NTR}$ neurotrophin receptor. *Molecular Neurobiology, 20*, 29–44.

Ernfors, P., Henschen, A., Olson, L., & Persson, H. (1989). Expression of nerve growth factor receptor mRNA is developmentally regulated and increased after axotomy in rat spinal cord motoneurons. *Neuron, 2*, 1605–1613.

Frade, J. M., Rodriguez-Tebar, A., & Barde, Y. A. (1996). Induction of cell death by endogenous nerve growth factor through its p75 receptor. *Nature, 383*, 166–168.

Friedman, W. J. (2000). Neurotrophins induce death of Hippocampal neurons via the p75 receptor. *Journal of Neuroscience, 20*, 6340–6346.

Friedman, W. J., & Greene, L. A. (1999). Neurotrophin signaling via Trks and p75. *Experimental Cell Research, 253*, 131–142.

Green, D. R. (2003). Overview: Apoptotic signaling pathways in the immune system. *Immunological Reviews, 193*, 5–9.

Hamanoue, M., Middleton, G., Wyatt, S., Jaffray, E., Hay, R. T., & Davies, A. M. (1999). p75-mediated NF-kappaB activation enhances the survival response of developing sensory neurons to nerve growth factor [in process citation]. *Molecular and Cellular Neurosciences, 14*, 28–40.

Harrington, A., Leiner, B., Blechschmitt, C., Arevalo, J., Lee, R., Morl, K., Meyer, M., Hempstead, B., Yoon, S., & Giehl, K. (2004). Secreted proNGF is a pathophysiological death-inducing ligand after adult CNS injury. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 6226–6230.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: Roles in neuronal development and function. *Annual Review of Neuroscience, 24*, 677–736.

Kaplan, D. R., & Miller, F. D. (2000). Neurotrophin signal transduction in the nervous system. *Current Opinion in Neurobiology, 10*, 381–391.

Koyama, R., Yamada, M. K., Fujisawa, S., Katoh-Semba, R., Matsuki, N., & Ikegaya, Y. (2004). Brain-derived neurotrophic factor induces hyperexcitable reentrant circuits in the dentate gyrus. *Journal of Neuroscience, 24*, 7215–7224.

Le, A. P., & Friedman, W. J. (2012). Matrix Metalloproteinase-7 regulates cleavage of Pro-nerve growth factor and is neuroprotective following kainic acid-induced seizures. *The Journal of Neuroscience, 32*, 703–712.

Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*, 1945–1948.

McNamara, J. O., & Scharfman, H. E. (2012). Temporal lobe epilepsy and the BDNF receptor, TrkB. In J. L. Noebels, M. Avoli, M. A. Rogawski, et al. (Eds.), *Jasper's basic mechanisms of the epilepsies* (4th ed.). Bethesda: National Center for Biotechnology Information.

Miller, F. D., & Kaplan, D. R. (2001). Neurotrophin signalling pathways regulating neuronal apoptosis. *Cellular and Molecular Life Sciences, 58*, 1045–1053.

Mizoguchi, H., Nakade, J., Tachibana, M., Ibi, D., Someya, E., Koike, H., Kamei, H., Nabeshima, T., Itohara, S., Takuma, K., Sawada, M., Sato, J., & Yamada, K. (2011). Matrix Metalloproteinase-9 contributes to kindled seizure development in Pentylenetetrazole-treated mice by converting Pro-BDNF to mature BDNF in the hippocampus. *The Journal of Neuroscience, 31*, 12963–12971.

Nykjaer, A., Lee, R., Teng, K., Jansen, P., Madsen, P., Nielsen, M., Jacobsen, C., Kliemannel, M., Schwarz, E., Willnow, T., Hempstead, B., & Petersen, C. (2004). Sortilin is essential for proNGF-induced neuronal cell death. *Nature, 427*, 843–848.

Park, K. J., Grosso, C. A., Aubert, I., Kaplan, D. R., & Miller, F. D. (2010). $p75^{NTR}$-dependent, myelin-mediated axonal degeneration regulates neural connectivity in the adult brain. *Nature Neuroscience, 13*, 559–566.

Patapoutian, A., & Reichardt, L. F. (2001). Trk receptors: Mediators of neurotrophin action. *Current Opinion in Neurobiology, 11*, 272–280.

Ramos, A., Ho, W. C., Forte, S., Dickson, K., Boutilier, J., Favell, K., & Barker, P. A. (2007). Hypo-osmolar stress induces p75NTR expression by activating Sp1-dependent transcription. *Journal of Neuroscience, 27*, 1498–1506.

Rivera, S., Tremblay, E., Timsit, S., Canals, O., Ben-Ari, Y., & Khrestchatisky, M. (1997). Tissue inhibitor of metalloproteinases-1 (TIMP-1) is differentially induced in neurons and astrocytes after seizures: Evidence for developmental, immediate early gene, and lesion response. *Journal of Neuroscience, 17*, 4223–4235.

Roux, P. P., Colicos, M. A., Barker, P. A., & Kennedy, T. E. (1999). p75 Neurotrophin receptor expression is induced in apoptotic neurons after seizure. *Journal of Neuroscience, 19*, 6887–6896.

Segawa, M., Morinobu, S., Matsumoto, T., Fuchikami, M., & Yamawaki, S. (2012). Electroconvulsive seizure, but not imipramine, rapidly up-regulates pro-BDNF and t-PA, leading to mature BDNF production, in the rat hippocampus. *The International Journal of Neuropsychopharmacology, 16*(2), 339–350.

Seidah, N. G., Benjannet, S., Pareek, S., Savaria, D., Hamelin, J., Goulet, B., Laliberte, J., Lazure, C., Chretien, M., & Murphy, R. A. (1996). Cellular processing of the nerve growth

factor precursor by the mammalian pro-protein convertases. *The Biochemical Journal, 314* (Pt 3), 951–960.

Song, W., Volosin, M., Cragnolini, A. B., Hempstead, B. L., & Friedman, W. J. (2010). ProNGF induces PTEN via $p75^{NTR}$ to suppress Trk-mediated survival signaling in brain neurons. *Journal of Neuroscience, 30*, 15608–15615.

Sun, Y., Lim, Y., Li, F., Liu, S., Lu, J.-J., Haberberger, R., Zhong, J.-H., & Zhou, X.-F. (2012). ProBDNF collapses neurite outgrowth of primary neurons by activating RhoA. *PLoS One, 7*, e35883.

Troy, C. M., Friedman, J. E., & Friedman, W. J. (2002). Mechanisms of p75-mediated death of Hippocampal neurons: Role of Caspases. *Journal of Biological Chemistry, 277*, 34295–34302.

Unsain, N., Nunez, N., Anastasia, A., & Masco, D. H. (2008). Status epilepticus induces a TrkB to p75 neurotrophin receptor switch and increases brain-derived Neurotrophic factor interaction with p75 neurotrophin receptor: An initial event in neuronal injury induction. *Neuroscience, 154*, 978–993.

Volosin, M., Trotter, C., Cragnolini, A., Kenchappa, R. S., Light, M., Hempstead, B. L., Carter, B. D., & Friedman, W. J. (2008). Induction of proneurotrophins and activation of p75NTR-mediated apoptosis via neurotrophin receptor-interacting factor in Hippocampal neurons after seizures. *Journal of Neuroscience, 28*, 9870–9879.

Wang, X., Bauer, J. H., Li, Y., Shao, Z., Zetoune, F. S., Cattaneo, E., & Vincenz, C. (2001). Characterization of a p75NTR apoptotic signaling pathway using a novel cellular model. *The Journal of Biological Chemistry, 12*, 12.

Williams, P. A., White, A. M., Clark, S., Ferraro, D. J., Swiercz, W., Staley, K. J., & Dudek, F. E. (2009). Development of spontaneous recurrent seizures after kainate-induced status epilepticus. *Journal of Neuroscience, 29*, 2103–2112.

Yamashita, T., Tucker, K. L., & Barde, Y. A. (1999). Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. *Neuron, 24*, 585–593.

Yoon, S. O., Casaccia-Bonnefil, P., Carter, B., & Chao, M. V. (1998). Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival. *Journal of Neuroscience, 18*, 3273–3281.

Synthesis, Trafficking and Release of BDNF

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Contents

Abstract

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays an important role in neuronal survival, differentiation, neurite outgrowth, spine formation, synaptic plasticity, and memory. The regulation of its biological activity is well controlled by its gene expression, axonal transport, and release. It is well known that BDNF is synthesized and released in an activity-dependent manner. However, the molecular mechanism for its processing, axonal transport, and release is still not completely understood. In our recent studies we found that Huntingtin-associated protein (HAP1) and sortilin play important roles in BDNF trafficking and processing. HAP1 plays critical roles in BDNF intracellular trafficking, dendritic targeting, and metabolism by binding to the prodomain of

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BDNF and forming a complex with sortilin. In this chapter, we focus on the recent progress made in understanding the molecular mechanism underlying the biosynthesis, transport, and release of BDNF, emphasizing the role of HAP1 and sortilin in the intracellular trafficking of BDNF.

List of Abbreviations

ARF	ADP-ribosylation factor
BDNF	Brain-derived neurotrophic factor
BDNF-GFP	Green fluorescent protein-tagged BDNF
CaRF	Calcium-responsive transcription factor
CREB	cAMP-responsive element-binding protein
HAP1	Huntingtin-associated protein 1
HFS	High-frequency stimulation
LFS	Low-frequency stimulation
LTD	Long-term depression
LTP	Long-term potentiation
MMPs	Matrix-metalloproteinases
MPR	Mannose 6-phosphate receptors
proBDNF	Precursor of brain-derived neurotrophic factor
RGCs	Retinal ganglion cells
SNP	Single nucleotide polymorphism
TGN	Trans-Golgi network
tPA	The tissue-plasminogen activator plasmin
trkB	Tyrosine kinase receptor B

1 Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays important roles in neuronal differentiation and survival, synaptic plasticity, and long-term potentiation during the entire life. BDNF mainly expresses in neural tissues and it is transported both anterogradely and retrogradely in neurons. It has an autocrine and paracrine effect on neuronal cells. BDNF mediates its biological action through binding to its high affinity receptor, tyrosine kinase receptor B (trkB), and p75, the pan receptor for other members of the neurotrophin family. However, the precursor of BDNF (proBDNF) has different binding characteristics and distinct biological activity in comparison with mature BDNF. It is well known that the biological action of BDNF is activity dependent and has been extensively explored. However, the molecular mechanism of its axonal transport and processing is not completely understood. Here in this chapter, we focus on BDNF axonal transport and describe the recent progress in BDNF trafficking, processing, and release.

2 The Expression of BDNF

The BDNF expression starts at an early embryonic stage. Its mRNA is expressed throughout development reaching the highest levels by postnatal day. In adult animals and humans, BDNF is expressed in the brain, with the highest levels in the hippocampus and cortex, suggesting an important role of this factor in the maintenance of the normal central nervous system. In humans, the BDNF gene is located in chromosome 11.

Like all neurotrophins, BDNF is expressed as a precursor encompassing two domains, the prodomain and the mature domain. The BDNF coding sequence has been determined in mice, pigs, rats, and humans. In all of these species, the mature BDNF shows complete identity in amino acid sequence. The expression of BDNF is well controlled in neurons by transcriptional regulation and differential targeting of BDNF transcripts at distinct cellular locations. In addition, neurotransmitters and membrane depolarization are also involved in the regulation of BDNF expression.

BDNF has at least 18 transcripts produced by alternative promoters, splicing, and polyadenylation sites, but each encodes an identical BDNF protein product. This complex transcriptional organization seems to provide multiple layers of regulation through alternative promoter usage, differential mRNA stability, and differential subcellular localization of either mRNA or protein. For example, it has been shown that the expression of the BDNF gene is regulated by neural activity through calcium-mediated pathways and that different BDNF mRNA transcripts driven by different promoters are differentially regulated. BDNF exon I and exon IV transcripts have previously been characterized as the most highly induced BDNF mRNAs, in response to excitatory treatment and KCl-mediated membrane depolarization in embryonic cortical neuron cultures. Several calcium-responsive elements and transcription factors binding to these elements in the promoter regions have been characterized. BDNF exon V, exon VII, exon VIII, and exon IXA transcripts have also been shown to be regulated by kainic acid and that the induction magnitude is comparable to that of BDNF exon I and IV transcripts. Importantly, differential regulation of BDNF mRNAs is found in mood disorder, exercise, and learning. Thus, it is suggested that the differential regulation of BDNF exon mRNAs might be involved in different neurodegenerative diseases in which BDNF levels are altered.

Regulation of the BDNF gene has been well studied. In addition to the transcriptional regulation, different BDNF messenger RNA (mRNA) transcripts are found at different subcellular locations in hippocampal and cortical neurons. BDNF expression was remarkably increased in the neocortex following experimentally induced seizures (Ernfors et al. 1991; Isackson et al. 1991). Several other studies also reported regulation of BDNF in vivo by sensory stimulation and in vitro by neuronal activity (Castren et al. 1992; Bozzi et al. 1995; Ghosh and Greenberg 1995; Lauterborn et al. 1996), suggesting that this is an important mechanism by which levels of BDNF are normally regulated. The transcription of BDNF can be regulated by neuron activity in hippocampal neurons (Tongiorgi et al. 1997). A recent study also showed that strong depolarizing stimuli, both in vitro and in vivo, cause accumulation of BDNF mRNA and protein in the distal dendrites,

through the activation of the *N*-methyl-D-aspartate and tyrosine kinase receptor B (TrkB) (Tongiorgi et al. 2006). Indeed, neuron activity can modulate the expression of many genes that affect the synaptic plasticity (Prakash et al. 2008). Therefore, knowledge regarding gene regulation of BDNF is important, in order for us to understand how altered BDNF expression and release can have an impact on the function of the neuronal circuit. Future characterization of the regulatory sequences and transcription factors mediating regulation of novel BDNF transcripts in different disease models is important for understanding BDNF gene regulation and its contribution to pathology.

3 BDNF Processing

BDNF, like other neuropeptide, is initially synthesized as a precursor containing a signal peptide in the endoplasmic reticulum. The signal peptide is cleaved off immediately, yielding so-called proproteins which undergo further posttranslational modifications in Golgi apparatus. Following the cleavage of the signal peptide, proBDNF is packaged in trans-Golgi network (TGN) for sorting into secretory vesicles. However, proBDNF may be converted into mature BDNF via both intracellular and extracellular enzymes. Studies show that the cleavage of proBDNF on the RVRR motif occurs in the trans-Golgi, by members of the subtilisin-kexin family of endoproteases such as furin, or in the immature secretory granules by proprotein convertases. It is also observed that tissue-plasminogen activator (tPA) converts proBDNF to the mature BDNF by activating the extracellular protease plasmin, which is critical for long-term potentiation (LTP) of the mouse hippocampus. Whether the conversion of proBDNF to mature BDNF is regulated by extracellular signals is not yet known. To understand the mechanisms that regulate the conversion of proBDNF to mBDNF, some experiments were undertaken to investigate how the physiologically relevant condition is associated with the secretion of specific isoforms of endogenous BDNF. It was demonstrated that under conditions that induce long-term depression (LTD), proBDNF is mainly secreted.

A stable status of endogenous proBDNF is important for its efficient axonal transport. It has been identified that sortilin binds to the prodomain of BDNF, which modulates its stabilization and regulates it into the secretory pathway. This regulation is dependent on the function of sortilin domains. Deletion of sortilin intracellular domain causes BDNF missorting to the constitutive secretory pathway, while overexpression of sortilin ectodomain impairs lysosomal targeting and leads to degradation of BDNF. Our recent data shows that co-expression of transfected proBDNF, HAP1, and sortilin in HEK293 cells can prevent proBDNF degradation, whereas the absence of sortilin significantly increased proBDNF degradation. The effect of sortilin on stabilization of proBDNF protein is dose dependent. These results suggest that normal BDNF trafficking and stabilization in neurons is dependent on the intact function of sortilin, in particular the complex of proBDNF/HAP1 and sortilin (Fig. 1).

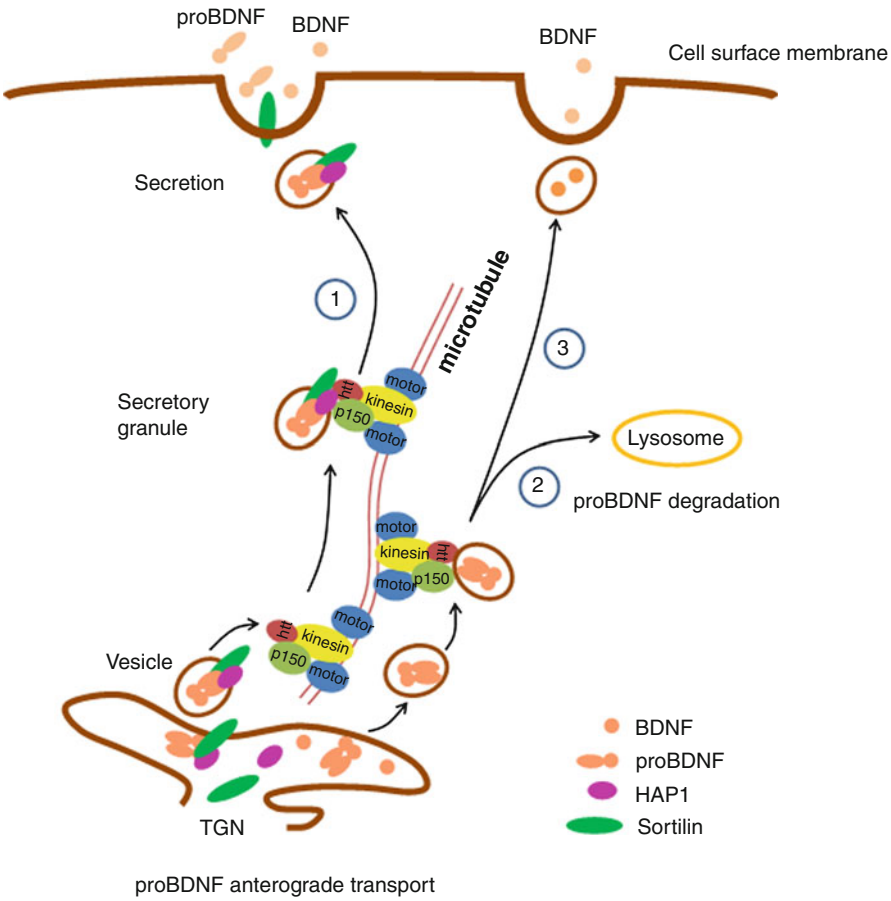

Fig. 1 proBDNF anterograde transport. New synthesized proBDNF proteins exist from endoplasmic reticulum (ER) and move to trans-Golgi network (*TGN*) for packaging and sorting. During the process, proBDNF can form a complex with HAP1 and sortilin and interacts with kinesin motor proteins through Htt and p150Glued. This proBDNF/kinesin complex is transported along with the microtubule to axonal terminal for secretion (*1*). When proBDNF is packaged itself in a vesicle without the participation of HAP1 and sortilin and is carried by the kinesin complex, some proBDNF proteins can be sent to lysosome for degradation (*2*), or a part of proBDNF can be converted to BDNF, which is sent to axonal terminal for secretion (*3*)

4 Transport of BDNF

The classic neurotrophic hypothesis is that neurotrophins are synthesized in peripheral target tissues, uptaken by the innervating nerve terminals, and retrogradely transported to the neuronal cell body, subsequently exerting their biological function by binding and activating their specific receptors. Like other neurotrophic factors,

BDNF was initially considered to be a retrogradely transported neurotrophic factor. ^{125}I-labelled BDNF injected into peripheral target tissues was found retrogradely transported into the subpopulation of peripheral and central neuronal cells. A study by intrastriatal infusion of BDNF revealed its retrograde transport in neurons in the brain. Furthermore, intraparenchymal injections of BDNF resulted in widespread retrograde transport throughout the central nervous system (CNS). However, the retrograde mode of BDNF transport cannot explain some of its functional roles. Moreover, it has been demonstrated that BDNF is produced in the sensory neurons because its mRNA and protein are both expressed in sensory neurons. Conversely, BDNF mRNA is not expressed in peripheral axonal terminals, whereas BDNF protein is detectable in terminals, suggesting that BDNF might be anterogradely transported in neurons.

We are among the first few groups that discovered the anterograde transport of BDNF in sensory neurons. We demonstrated that BDNF is transported anterogradely via both peripheral and central processes of spinal sensory neurons. Using specific antisera, we examined the distribution of BDNF immunoreactivity and found it to be present within a subpopulation of sensory neurons, primarily those with a small-to-medium diameter. The immunoreactivity was accumulated on both the distal and proximal sides of a ligature on the sciatic nerve (Fig. 2). The accumulation on the distal side, but not on the proximal side, was substantially reduced by pretreatment with BDNF antibodies in vivo. In contrast to the periphery, the immunoreactivity was only accumulated on the proximal side of a lesion of the dorsal root. In the spinal cord, most nerve terminals immunoreactive for BDNF were identified in lamina II. Lesion of the dorsal root led to a reduction of these nerve terminals. These studies have provided clear evidence that the factor is transported not only retrogradely, but also anterogradely from the spinal ganglia to terminals in the periphery and spinal cord. Consistently, studies from other research groups also confirmed anterograde transport of BDNF.

It was shown that BDNF is widely distributed in nerve terminals, even in brain areas such as the striatum that lack BDNF messenger RNA, suggesting this factor is anterogradely supplied to some areas of the brain. Interestingly, by using the same method, a study in our laboratory also showed that proBDNF is both anterogradely and retrogradely transported in sensory neurons. Similar to the mature BDNF, after ligation of the sciatic nerve, increased proBDNF expression can be detected both proximal and distal to the site of ligation. In CNS, analysis of distribution of BDNF and mRNA revealed more detailed transport patterns of BDNF in some neural structures. BDNF is also anterogradely transported in hippocampus mossy fibers and nerve terminals in other brain structures. Furthermore, BDNF is also found to be anterogradely transported in retina cells. Retinal ganglion cells (RGCs) also anterogradely transport exogenous neurotrophins including BDNF to the midbrain tectum/superior colliculus with significant downstream effects.

Consistent with in vivo studies, in vitro studies also showed BDNF is transported in an anterograde mode. Several neuronal types can internalize exogenous neurotrophic factors which then can be anterogradely transported to axonal

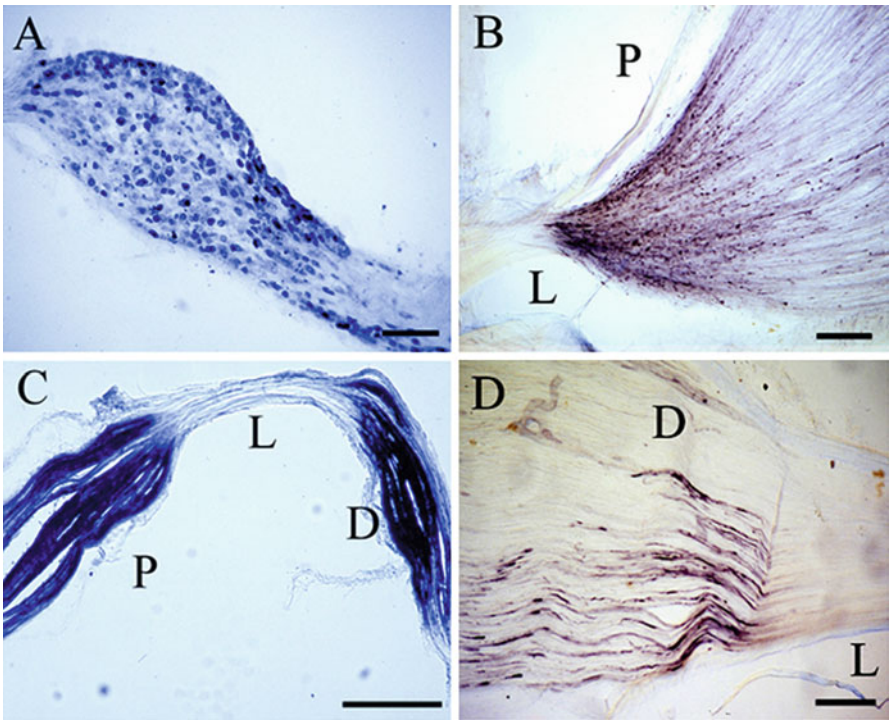

Fig. 2 Microphotographs showing the immunoactivity and transport of BDNF in the peripheral nervous system of adult rats. Panel (**a**): nodose ganglion; panel (**b**): proximal segments of ligated sciatic nerve; panel (**c**): ligated vagal nerve; (**d**): distal segment of ligated sciatic nerve. Abbreviations: *L* ligation site, *P* proximal to the ligation site, *D* distal to the ligation site. Scale bars in (**a**), (**b**), and (**d**) = 100 μm, in (**c**) = 1 mm

pathways. Neuronal bodies and dendrites possess receptors for the ligands, and a significant amount of internalized ligands are targeted for anterograde axonal transport, apparently with subsequent axonal release. While numerous studies demonstrate anterograde transport of trophic factors in axons, only few provide direct evidence of transcytosis of endogenous or exogenous trophic factors. Interestingly, a study comparing the trafficking pattern of BDNF in axons and dendrites demonstrated that axonal anterograde transport is the major form of BDNF transport. However, the axonal transport pattern is different from the dendritic transport pattern. The pattern and velocity of the trafficking of fluorescent protein-tagged BDNF is different between axons and dendrites. The movement of BDNF-GFP puncta in axons is faster than in dendrites, suggesting that the anterograde transport in axons may be the dominant site of BDNF release.

The anterograde transport of BDNF appears to be a universal phenomenon in the mammal nervous system. However, the real-time in vivo record of endogenous BDNF transport is lacking because of technique difficulties and a relatively small amount of detectable endogenous BDNF. The anterograde mode of BDNF

transport is critical for the neurotropism, which is not merely an alternative way for retrograde transport. Numerous studies have demonstrated that the bidirectional transfer and trafficking of BDNF through the neural network is a fundamental mechanism for its trophic regulation of neuronal survival, differentiation of phenotypes and dendritic morphology, synaptic plasticity, as well as excitatory neurotransmission. It is now clear that newly synthesized BDNF is packaged into vesicles and anterogradely transported to neuronal terminals for secretion, via interaction with kinesin motor protein complex along the microtubules. The molecular mechanism underlying BDNF intracellular trafficking still remains to be elucidated. Nevertheless, recent studies have provided some evidence of HAP1 and sortilin playing critical roles in BDNF trafficking and processing.

In search of the potential molecules involved in BDNF intracellular trafficking, we found that HAP1 and sortilin are ideal candidates. HAP1 is a cytoplasmic protein enriched in the brain and spinal cord. HAP1 was identified as a Huntingtin (Htt)-binding partner by yeast two-hybrid screens. It also associates with microtubules in addition to several vesicular organelles. Htt is a scaffold protein predominantly found in the cytoplasm, where it associates with various vesicular structures and molecular motors to form a cargo complex and play a role in intracellular trafficking. Despite the important role of HAP1 in neurons, the precise function of HAP1 in neurons remains to be seen. Some recent studies have shown that HAP1 is involved in endocytosis and vesicular trafficking. The expansion of polyglutamine (polyQ) repeats in Htt and the deletion of either HAP1 or p150Glued can reduce BDNF axonal transport and lead to the degeneration of striatal neurons. It is suggested that a molecular bridging of HAP1 with BDNF and kinesin motor complex may be involved in BDNF axonal transport. For example, HAP1 interacts with the p150Glued subunit of dynactin and regulates axonal trafficking. HAP1 also mediates the anterograde transport of the GABAA receptor to synapses and of neurotrophic factor receptors for neurite outgrowth.

Sortilin is a member of the Vps10p domain receptor family including SorLA and SorCS1, SorCS2, and SorCS3-3. Sortilin is highly expressed in the cortex and hippocampus. It can function as a cell surface receptor for proneurotrophins, neurotensin, and lipoprotein lipase. Sortilin is mainly distributed in intracellular compartments where it binds to diverse ligands including hydrolases. Sortilin is a sorting molecule which sorts lysosomal hydrolytic enzymes to lysosomes and BDNF into secretory pathways. Its short (53 aa) cytosolic tail contains a motif that is similar to that of the sorting receptor mannose 6-phosphate receptors (MPR) and low-density lipoprotein (LDL) receptors, which interact with the family of Golgi-localizing, γ-adaptin ear homologous ADP-ribosylation factor (ARF)-binding proteins (GGA) that shuttle between the Golgi and endosomes/lysosomes. The C-terminal acidic cluster-dileucine motif mediates endocytosis of the receptor. The cytosolic domain of sortilin also interacts with the retromer complex, which recycles sortilin back to Golgi for further trafficking and sorting of proteins. The retromer complex is composed of 3–4 VPS molecules VPS35, VPS29, and VPS26.

There are other factors which may directly or indirectly affect the BDNF trafficking. A Val66Met substitution of BDNF led to a significant decrease

of BDNF trafficking and secretion and was found to be associated with increased susceptibility to neuro-disorders including Alzheimer's disease, Parkinson's disease, depression, and bipolar disorder. Since sortilin interacts with the prodomain of BDNF in a discrete region containing the Met substitution, the altered BDNF trafficking and secretion is likely due to the disruption of the interaction between BDNF and sortilin. This concept is also supported by the evidence that sortilin is involved in anterograde transport of BDNF. These studies reveal a role of sortilin in mediating trkB receptors and BDNF anterograde transport, which is important for neurotrophin-induced neuronal survival. In addition, Jun NH (2)-terminal kinase-interacting protein 3 (JIP3) mediates trkB axonal anterograde transport and enhances BDNF signaling by directly interacting with trkB juxtamembrane region and linking trkB to kinesin-1. Given that both JIP3 and sortilin bind to trkB, their effect on enhancing BDNF signaling may rely on functions of both proteins. Nonetheless, these findings provide a novel mechanism of how the TrkB anterograde transport can be coupled to BDNF signaling in axons.

Our recent studies demonstrate that HAP1 and sortilin may play an important role in BDNF trafficking and processing. We investigated the HAP1 and BDNF interaction and the role of HAP1 in BDNF trafficking by using cell transfection and HAP1 knockout mice. Our mapping studies demonstrate that the region of HAP1 between amino acids 371 and 445 contains proBDNF-binding sites. Pull-down assays show that the region between amino acids 65 and 90 of the BDNF prodomain has a key structure for HAP1 binding. Our results also show that HAP1 regulates axonal transport and the activity-dependent release of BDNF, through HAP1 interaction with proBDNF but not mature BDNF. Reduced association of HAP1 with proBDNF by polyQ-expanded Huntingtin or Val66Met BDNF variant can negatively impact on proBDNF trafficking. We also observed a similar reduction in HAP1 and proBDNF interaction using the brain homogenate of Huntington's disease patients, suggesting that this reduced association of HAP1 with proBDNF can occur in vivo and may cause a deficiency of BDNF anterograde axonal transport. This hypothesis is supported by the evidence that ligation of the sciatic nerve did not show increased accumulation of BDNF in the proximal and distal segments of the ligation site and the deficit in proBDNF trafficking in HAP1−/− neurons was rescued by introduction of HAP1 cDNA in these neurons. Therefore, our data suggests that HAP1 may play a regulatory role in axonal transport of BDNF. Recently we also found that HAP1 can interact with proBDNF and form a complex with sortilin. This complex is found in most cellular organelles involved in protein sorting and trafficking such as ER, TGN, and Golgi complex. The complex can stabilize the proBDNF. In co-transfected HEK 293 cells, the complex helps to prevent proBDNF degradation. Furthermore, the complex facilitates furin cleavage to release mature BDNF.

5 BDNF Release and Secretion

There are two separate types of secretion pathways for BDNF: the regulated pathway and the constitutive pathway. The former employs Ca^{2+}-dependent exocytosis of

secretory granules, while the latter employs a distinct type of vesicles which releases the cargo by default when reaching the plasma membrane. It is suggested that the subcellular location of BDNF is important for understanding the function of BDNF and its role in synaptic plasticity. Since the level of endogenous BDNF is very low in most neuronal tissues, overexpression of BDNF-GFP has been used to study the transport and release of BDNF in neuronal culture. Although the expression of tagged protein may have altered binding to sorting partners, the distribution of BDNF-GFP is similar to that observed for endogenous BDNF. In dendrites, BDNF-GFP is packaged into secretory granules for the regulated secretion pathway, suggesting a dendritic secretion of BDNF. This finding is further supported by the evidence that local synthesis of dendritic BDNF and dendritic BDNF mRNA transcript has been detected in cultured hippocampal neurons. Also, alteration of dendritic BDNF mRNA can cause the change of the local BDNF level. However, a recent study, using specific monoclonal antibodies to label proBDNF and mature BDNF, has shown that proBDNF and mature BDNF exist in presynaptic dense core vesicles but not in dendrites. Hippocampal neurons from mutant mice with overexpressed BDNF also show that BDNF is only present in presynaptic dense core vesicles. The finding challenges the previous concept of activity-dependent dendritic synthesis and release of BDNF. However, the finding supports the conclusion that BDNF is transported in an anterograde mode, which is in contrast to the previous concept that BDNF is retrogradely transported in target cells. This in vivo study appears to provide convincing evidence that BDNF is located in presynaptic dense core vesicles.

There are controversial views about the neuronal release of proBDNF. Barde's group reported that proBDNF is only an intermediate metabolic product of precursor BDNF, which only presents transiently and does not have biological functions. In contrast, a similar study claims that proBDNF is present and is released from neurons exerting biological functions. In this study, different culture conditions were applied to avoid possible glial contamination, and alpha 2 antiplasmin was used to reduce the cleavage of secreted proBDNF. The secretion of proBDNF from cultured hippocampus neurons was convincingly demonstrated. The conclusion drawn from this study appears to be consistent with that from a previous study, reporting that the cleavage of proBDNF by tPA/plasma is essential for long-term hippocampus plasticity. tPA converts proBDNF to mature BDNF by activating the extracellular protease plasmin, and such conversion is critical for L-LTP expression in mouse hippocampus. Moreover, application of mBDNF is sufficient to rescue L-LTP when protein synthesis is inhibited, which suggests that mBDNF is a key protein synthesis product for L-LTP expression.

To reveal which isoform of BDNF is secreted under physiological stimulation, frequency stimulation of neuronal cells was performed. Studies have shown that proBDNF is mainly secreted from neuronal terminals during low-frequency stimulation (LFS). In contrast, mature BDNF is the major secreted isoform when neurons were stimulated with high-frequency stimulation (HFS, a condition that induces LTP). Thus, the secretion of either proBDNF or mature BDNF is dependent on low- or high-frequency neuronal activity. These findings suggest that high-frequency neuronal activity can enhance the function of mature BDNF.

In addition, the secretion of proBDNF or mature BDNF is also controlled by activity-dependent synaptic competition/elimination. ProBDNF can trigger synaptic depression and subsequent retraction of the axonal terminal through p75 receptors in Xenopus neuromuscular synapses. Active axonal terminals can cleave proBDNF to mature BDNF which protects the active terminal from depression and retraction.

BDNF release is a regulated response to its functional requirement. The dendritic BDNF synthesis and the release of BDNF-GFP under high-frequency electric stimulation provide evidence that BDNF release is in an activity-dependent manner from dendrites and axons. Although the importance of BDNF in synaptic plasticity has been clearly established, direct evidence for the activity-dependent dendritic secretion of BDNF has not been obtained. In the cerebellum, the depolarization-dependent release of BDNF from granule cells was reported to be regulated by the CAPS2 molecule, which leads to cell differentiation and survival during cerebellar development. Furthermore, exercise can increase the serum level of BDNF in human subjects. The molecular interaction has been determined to be responsible for BDNF-regulated release.

6 BDNF Transport and Release in Neurological Diseases and Neurotoxicity

BDNF is associated with the development of many neurological and psychiatric disease conditions because of its wide distribution and great functional diversities. Deficiencies in its synthesis, trafficking, and release play important roles in the pathogenesis of a wide range of neurodegenerative diseases and cognitive disorders. However, the molecular dissection of the trafficking and release of BDNF in diseased condition is limited, although the axonal transport in these disease conditions has been extensively studied. Axonal transport defects have been described as an early pathological feature in a variety of animal models of Alzheimer's disease (AD) and tauopathies. It is clear that BDNF axonal transport of BDNF is impaired in AD. In Huntington disease, there is reduced expression of BDNF and impaired axonal transport in the striatum. Huntingtin enhances retrograde transport of BDNF, which involves HAP1 and dynactin, but mutant Huntingtin is defective in this process (Gauthier et al. 2004). In Parkinson's disease, there is also reduced axonal transport of BDNF in dopaminergic neurons. The understanding of synthesis, transport, trafficking, and the release of BDNF in diseased conditions will provide useful knowledge for understanding the pathogenesis and underlying molecular mechanism and will help in developing novel therapeutic strategies.

BDNF, a powerful trophic factor with diverse functions, can act as a protector against some cellular damages caused by the neurotoxicity induced by physical and chemical insults. BDNF is a survival factor for cultured rat cerebellar granule neurons and protects them against glutamate-induced neurotoxicity. Restoring BDNF level CEP-1347 reduces mutant Huntingtin-associated neurotoxicity. Overexpression of BDNF is shown to firmly regulate cell survival and differentiation of neuron stem cells (NSC) and protects differentiated NSC against neurotoxicant trimethyltin

(TMT)-induced neurotoxicity through the PI3K/Akt and MAPK signaling pathways. BDNF can also protect against beta-amyloid-induced neurotoxicity both in vitro and in vivo in rats. How BDNF trafficking and release is affected in neurotoxicity would be an interesting area to explore.

7 Conclusion

BDNF is important for neuronal survival and synaptic plasticity. Its synthesis, transport, and release have been intensively investigated since its discovery. It is clear now that BDNF is both anterogradely and retrogradely transported in axonal terminals and it is released in an activity-dependent manner. Although controversies exist regarding the subcellular distribution of BDNF and its exact location of release, more convincing evidence shows that BDNF is stored in presynaptic dense core vesicles, suggesting that BDNF is predominantly transported in an anterograde mode. However, the molecular mechanism underlying intracellular trafficking has not been completely understood, particularly in diseased conditions. Our recent studies provide new evidence that HAP1 and sortilin play an important role in intracellular BDNF trafficking and processing. Future studies should focus on better understanding of more profound molecular mechanisms involved in the trafficking, release, and signaling of BDNF in diseased conditions. Insight into the molecular mechanism of BDNF would help in developing therapeutic treatment for different neurological disorders by targeting mature BDNF and proBDNF.

References

Adachi, N., Kohara, K., & Tsumoto, T. (2005). Difference in trafficking of brain-derived neurotrophic factor between axons and dendrites of cortical neurons, revealed by live-cell imaging. *BMC Neuroscience, 6*, 42.

Aid, T., Kazantseva, A., Piirsoo, M., Palm, K., & Timmusk, T. (2007). Mouse and rat BDNF gene structure and expression revisited. *Journal of Neuroscience Research, 85*, 525–535.

Altar, C. A., Cai, N., Bliven, T., Juhasz, M., Conner, J. M., Acheson, A. L., Lindsay, R. M., & Wiegand, S. J. (1997). Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature, 389*, 856–860.

An, J. J., Gharami, K., Liao, G. Y., Woo, N. H., Lau, A. G., Vanevski, F., Torre, E. R., Jones, K. R., Feng, Y., Lu, B., & Xu, B. (2008). Distinct role of long 3′ UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell, 134*, 175–187.

Apostol, B. L., Simmons, D. A., Zuccato, C., Illes, K., Pallos, J., Casale, M., Conforti, P., Ramos, C., Roarke, M., Kathuria, S., Cattaneo, E., Marsh, J. L., & Thompson, L. M. (2008). CEP-1347 reduces mutant huntingtin-associated neurotoxicity and restores BDNF levels in R6/2 mice. *Molecular and Cellular Neurosciences, 39*, 8–20.

Arancibia, S., Silhol, M., Mouliere, F., Meffre, J., Hollinger, I., Maurice, T., & Tapia-Arancibia, L. (2008). Protective effect of BDNF against beta-amyloid induced neurotoxicity in vitro and in vivo in rats. *Neurobiology of Disease, 31*, 316–326.

Barker, P. A., & Murphy, R. A. (1992). The nerve growth factor receptor: A multicomponent system that mediates the actions of the neurotrophin family of proteins. *Molecular and Cellular Biochemistry, 110*, 1–15.

Bibel, M., & Barde, Y. A. (2000). Neurotrophins: Key regulators of cell fate and cell shape in the vertebrate nervous system. *Genes & Development, 14*, 2919–2937.

Bonifacino, J. S., & Hurley, J. H. (2008). Retromer. *Current Opinion in Cell Biology, 20*, 427–436.

Bramham, C. R., & Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. *Progress in Neurobiology, 76*, 99–125.

Butowt, R., & von Bartheld, C. S. (2005). Anterograde axonal transport of BDNF and NT-3 by retinal ganglion cells: Roles of neurotrophin receptors. *Molecular and Cellular Neurosciences, 29*, 11–25.

Canuel, M., Libin, Y., & Morales, C. R. (2009). The interactomics of sortilin: An ancient lysosomal receptor evolving new functions. *Histology and Histopathology, 24*, 481–492.

Casalbore, P., Barone, I., Felsani, A., D'Agnano, I., Michetti, F., Maira, G., & Cenciarelli, C. (2010). Neural stem cells modified to express BDNF antagonize trimethyltin-induced neurotoxicity through PI3K/Akt and MAP kinase pathways. *Journal of Cellular Physiology, 224*, 710–721.

Chao, M. V. (1992). Neurotrophin receptors: A window into neuronal differentiation. *Neuron, 9*, 583–593.

Chen, W. G., West, A. E., Tao, X., Corfas, G., Szentirmay, M. N., Sawadogo, M., Vinson, C., & Greenberg, M. E. (2003). Upstream stimulatory factors are mediators of Ca^{2+}–responsive transcription in neurons. *The Journal of Neuroscience, 23*, 2572–2581.

Chen, Z. Y., Ieraci, A., Teng, H., Dall, H., Meng, C. X., Herrera, D. G., Nykjaer, A., Hempstead, B. L., & Lee, F. S. (2005). Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. *The Journal of Neuroscience, 25*, 6156–6166.

Chiaruttini, C., Sonego, M., Baj, G., Simonato, M., & Tongiorgi, E. (2008). BDNF mRNA splice variants display activity-dependent targeting to distinct hippocampal laminae. *Molecular and Cellular Neuroscience, 37*, 11–19.

Conner, J. M., Lauterborn, J. C., Yan, Q., Gall, C. M., & Varon, S. (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: Evidence for anterograde axonal transport. *The Journal of Neuroscience, 17*, 2295–2313.

Cotman, C. W., & Berchtold, N. C. (2002). Exercise: A behavioral intervention to enhance brain health and plasticity. *Trends in Neurosciences, 25*, 295–301.

Dieni, S., Matsumoto, T., Dekkers, M., Rauskolb, S., Ionescu, M. S., Deogracias, R., Gundelfinger, E. D., Kojima, M., Nestel, S., Frotscher, M., & Barde, Y. A. (2012). BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. *Journal of Cell Biology, 196*, 775–788.

DiStefano, P. S., Friedman, B., Radziejewski, C., Alexander, C., Boland, P., Schick, C. M., Lindsay, R. M., & Wiegand, S. J. (1992). The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. *Neuron, 8*, 983–993.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Weinberger, D. R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257–269.

Ernfors, P., Ibanez, C. F., Ebendal, T., Olson, L., & Persson, H. (1990). Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: Developmental and topographical expression in the brain. *Proceedings of the National Academy of Sciences of the United States of America, 87*, 5454–5458.

Evans, S. F., Irmady, K., Ostrow, K., Kim, T., Nykjaer, A., Saftig, P., Blobel, C., & Hempstead, B. L. (2011). Neuronal brain-derived neurotrophic factor is synthesized in excess, with levels regulated

by sortilin-mediated trafficking and lysosomal degradation. *Journal of Biological Chemistry, 286*, 29556–29567.

Fabricant, R. N., De Larco, J. E., & Todaro, G. J. (1977). Nerve growth factor receptors on human melanoma cells in culture. *Proceedings of the National Academy of Sciences of the United States of America, 74*, 565–569.

Gauthier, L. R., Charrin, B. C., Borrell-Pages, M., Dompierre, J. P., Rangone, H., Cordelieres, F. P., De Mey, J., MacDonald, M. E., Lessmann, V., Humbert, S., & Saudou, F. (2004). Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell, 118*, 127–138.

Ghosh, A., Carnahan, J., & Greenberg, M. E. (1994). Requirement for BDNF in activity-dependent survival of cortical neurons. *Science, 263*, 1618–1623.

Greenberg, M. E., Xu, B., Lu, B., & Hempstead, B. L. (2009). New insights in the biology of BDNF synthesis and release: Implications in CNS function. *Journal of Neuroscience, 29*, 12764–12767.

Gunawardena, S., & Goldstein, L. S. (2004). Cargo-carrying motor vehicles on the neuronal highway: Transport pathways and neurodegenerative disease. *Journal of Neurobiology, 58*, 258–271.

Gutekunst, C. A., Li, S. H., Yi, H., Ferrante, R. J., Li, X. J., & Hersch, S. M. (1998). The cellular and subcellular localization of huntingtin-associated protein 1 (HAP1): Comparison with huntingtin in rat and human. *Journal of Neuroscience, 18*, 7674–7686.

Hariri, A. R., Goldberg, T. E., Mattay, V. S., Kolachana, B. S., Callicott, J. H., Egan, M. F., & Weinberger, D. R. (2003). Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *Journal of Neuroscience, 23*, 6690–6694.

Hartmann, M., Heumann, R., & Lessmann, V. (2001). Synaptic secretion of BDNF after high-frequency stimulation of glutamatergic synapses. *EMBO Journal, 20*, 5887–5897.

Hashimoto, K., Shimizu, E., & Iyo, M. (2004). Critical role of brain-derived neurotrophic factor in mood disorders. *Brain Research. Brain Research Reviews, 45*, 104–114.

Hefti, F. (1994). Neurotrophic factor therapy for nervous system degenerative diseases. *Journal of Neurobiology, 25*, 1418–1435.

Hofer, M., Pagliusi, S. R., Hohn, A., Leibrock, J., & Barde, Y. A. (1990). Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *EMBO Journal, 9*, 2459–2464.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: Roles in neuronal development and function. *Annual Review of Neuroscience, 24*, 677–736.

Huang, S. H., Duan, S., Sun, T., Wang, J., Zhao, L., Geng, Z., Yan, J., Sun, H. J., & Chen, Z. Y. (2011). JIP3 mediates TrkB axonal anterograde transport and enhances BDNF signaling by directly bridging TrkB with kinesin-1. *Journal of Neuroscience, 31*, 10602–10614.

Isackson, P. J., Huntsman, M. M., Murray, K. D., & Gall, C. M. (1991). BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. *Neuron 6*(6), 937–948.

Jacobsen, L., Madsen, P., Nielsen, M. S., Geraerts, W. P., Gliemann, J., Smit, A. B., & Petersen, C. M. (2002). The sorLA cytoplasmic domain interacts with GGA1 and −2 and defines minimum requirements for GGA binding. *FEBS Letters, 511*, 155–158.

Je, H. S., Yang, F., Ji, Y., Nagappan, G., Hempstead, B. L., & Lu, B. (2012). Role of pro-brain-derived neurotrophic factor (proBDNF) to mature BDNF conversion in activity-dependent competition at developing neuromuscular synapses. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 15924–15929.

Jones, K. R., & Reichardt, L. F. (1990). Molecular cloning of a human gene that is a member of the nerve growth factor family. *Proceedings of the National Academy of Sciences of the United States of America, 87*, 8060–8064.

Kittler, J. T., Thomas, P., Tretter, V., Bogdanov, Y. D., Haucke, V., Smart, T. G., & Moss, S. J. (2004). Huntingtin-associated protein 1 regulates inhibitory synaptic transmission by

modulating gamma-aminobutyric acid type A receptor membrane trafficking. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 12736–12741.

Kuczewski, N., Porcher, C., Ferrand, N., Fiorentino, H., Pellegrino, C., Kolarow, R., Lessmann, V., Medina, I., & Gaiarsa, J. L. (2008). Backpropagating action potentials trigger dendritic release of BDNF during spontaneous network activity. *Journal of Neuroscience, 28*, 7013–7023.

Kuczewski, N., Porcher, C., & Gaiarsa, J. L. (2010). Activity-dependent dendritic secretion of brain-derived neurotrophic factor modulates synaptic plasticity. *European Journal of Neuroscience, 32*, 1239–1244.

Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*, 1945–1948.

Lauterborn, J. C., Rivera, S., Stinis, C. T., Hayes, V. Y., Isackson, P. J., & Gall, C. M. (1996). Differential effects of protein synthesis inhibition on the activity-dependent expression of BDNF transcripts: evidence for immediate-early gene responses from specific promoters. *J Neurosci 16*(23), 7428–7436.

Li, X. J., & Li, S. H. (2005). HAP1 and intracellular trafficking. *Trends in Pharmacological Sciences, 26*, 1–3.

Li, X. J., Li, S. H., Sharp, A. H., Nucifora, F. C., Jr., Schilling, G., Lanahan, A., Worley, P., Snyder, S. H., & Ross, C. A. (1995). A huntingtin-associated protein enriched in brain with implications for pathology. *Nature, 378*, 398–402.

Lindholm, D., Dechant, G., Heisenberg, C. P., & Thoenen, H. (1993). Brain-derived neurotrophic factor is a survival factor for cultured rat cerebellar granule neurons and protects them against glutamate-induced neurotoxicity. *European Journal of Neuroscience, 5*, 1455–1464.

Lou, H., Kim, S. K., Zaitsev, E., Snell, C. R., Lu, B., & Loh, Y. P. (2005). Sorting and activity-dependent secretion of BDNF require interaction of a specific motif with the sorting receptor carboxypeptidase e. *Neuron, 45*, 245–255.

Maisonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M., & Yancopoulos, G. D. (1990). Neurotrophin-3: A neurotrophic factor related to NGF and BDNF. *Science, 247*, 1446–1451.

Martin, E. J., Kim, M., Velier, J., Sapp, E., Lee, H. S., Laforet, G., Won, L., Chase, K., Bhide, P. G., Heller, A., Aronin, N., & Difiglia, M. (1999). Analysis of huntingtin-associated protein 1 in mouse brain and immortalized striatal neurons. *The Journal of Comparative Neurology, 403*, 421–430.

Matsumoto, T., Rauskolb, S., Polack, M., Klose, J., Kolbeck, R., Korte, M., & Barde, Y. A. (2008). Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. *Nature Neuroscience, 11*, 131–133.

Mazella, J., Zsurger, N., Navarro, V., Chabry, J., Kaghad, M., Caput, D., Ferrara, P., Vita, N., Gully, D., Maffrand, J. P., & Vincent, J. P. (1998). The 100-kDa neurotensin receptor is gp95/sortilin, a non-G-protein-coupled receptor. *Journal of Biological Chemistry, 273*, 26273–26276.

Mellstrom, B., Torres, B., Link, W. A., & Naranjo, J. R. (2004). The BDNF gene: Exemplifying complexity in Ca^{2+}−dependent gene expression. *Critical Reviews in Neurobiology, 16*, 43–49.

Mogi, M., Togari, A., Kondo, T., Mizuno, Y., Komure, O., Kuno, S., Ichinose, H., & Nagatsu, T. (1999). Brain-derived growth factor and nerve growth factor concentrations are decreased in the substantia nigra in Parkinson's disease. *Neuroscience Letters, 270*, 45–48.

Mowla, S. J., Pareek, S., Farhadi, H. F., Petrecca, K., Fawcett, J. P., Seidah, N. G., Morris, S. J., Sossin, W. S., & Murphy, R. A. (1999). Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. *Journal of Neuroscience, 19*, 2069–2080.

Mufson, E. J., Kroin, J. S., Sobreviela, T., Burke, M. A., Kordower, J. H., Penn, R. D., & Miller, J. A. (1994). Intrastriatal infusions of brain-derived neurotrophic factor: Retrograde transport and colocalization with dopamine containing substantia nigra neurons in rat. *Experimental Neurology, 129*, 15–26.

Nagappan, G., Zaitsev, E., Senatorov, V. V., Jr., Yang, J., Hempstead, B. L., & Lu, B. (2009). Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 1267–1272.

Nielsen, M. S., Jacobsen, C., Olivecrona, G., Gliemann, J., & Petersen, C. M. (1999). Sortilin/neurotensin receptor-3 binds and mediates degradation of lipoprotein lipase. *Journal of Biological Chemistry, 274*, 8832–8836.

Pang, P. T., Teng, H. K., Zaitsev, E., Woo, N. T., Sakata, K., Zhen, S., Teng, K. K., Yung, W. H., Hempstead, B. L., & Lu, B. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science, 306*, 487–491.

Parain, K., Murer, M. G., Yan, Q., Faucheux, B., Agid, Y., Hirsch, E., & Raisman-Vozari, R. (1999). Reduced expression of brain-derived neurotrophic factor protein in Parkinson's disease substantia nigra. *Neuroreport, 10*, 557–561.

Petersen, C. M., Nielsen, M. S., Nykjaer, A., Jacobsen, L., Tommerup, N., Rasmussen, H. H., Roigaard, H., Gliemann, J., Madsen, P., & Moestrup, S. K. (1997). Molecular identification of a novel candidate sorting receptor purified from human brain by receptor-associated protein affinity chromatography. *Journal of Biological Chemistry, 272*, 3599–3605.

Phillips, H. S., Hains, J. M., Armanini, M., Laramee, G. R., Johnson, S. A., & Winslow, J. W. (1991). BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron, 7*, 695–702.

Poo, M. M. (2001). Neurotrophins as synaptic modulators. *Nature Review Neuroscience, 2*, 24–32.

Rong, J., McGuire, J. R., Fang, Z. H., Sheng, G., Shin, J. Y., Li, S. H., & Li, X. J. (2006). Regulation of intracellular trafficking of huntingtin-associated protein-1 is critical for TrkA protein levels and neurite outgrowth. *Journal of Neuroscience, 26*, 6019–6030.

Russo-Neustadt, A. A., & Chen, M. J. (2005). Brain-derived neurotrophic factor and antidepressant activity. *Current Pharmaceutical Design, 11*, 1495–1510.

Sadakata, T., Mizoguchi, A., Sato, Y., Katoh-Semba, R., Fukuda, M., Mikoshiba, K., & Furuichi, T. (2004). The secretory granule-associated protein CAPS2 regulates neurotrophin release and cell survival. *Journal of Neuroscience, 24*, 43–52.

Schindowski, K., Belarbi, K., & Buee, L. (2008). Neurotrophic factors in Alzheimer's disease: Role of axonal transport. *Genes, Brain, and Behavior, 7*(Suppl 1), 43–56.

Shieh, P. B., & Ghosh, A. (1999). Molecular mechanisms underlying activity-dependent regulation of BDNF expression. *Journal of Neurobiology, 41*, 127–134.

Smith, M. A., Zhang, L. X., Lyons, W. E., & Mamounas, L. A. (1997). Anterograde transport of endogenous brain-derived neurotrophic factor in hippocampal mossy fibers. *Neuroreport, 8*, 1829–1834.

Tabuchi, A., Sakaya, H., Kisukeda, T., Fushiki, H., & Tsuda, M. (2002). Involvement of an upstream stimulatory factor as well as cAMP-responsive element-binding protein in the activation of brain-derived neurotrophic factor gene promoter I. *Journal of Biological Chemistry, 277*, 35920–35931.

Tao, X., Finkbeiner, S., Arnold, D. B., Shaywitz, A. J., & Greenberg, M. E. (1998). Ca^{2+} influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron, 20*, 709–726.

Tao, X., West, A. E., Chen, W. G., Corfas, G., & Greenberg, M. E. (2002). A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF. *Neuron, 33*, 383–395.

Teng, H. K., Teng, K. K., Lee, R., Wright, S., Tevar, S., Almeida, R. D., Kermani, P., Torkin, R., Chen, Z. Y., Lee, F. S., Kraemer, R. T., Nykjaer, A., & Hempstead, B. L. (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *Journal of Neuroscience, 25*, 5455–5463.

Timmusk, T., Lendahl, U., Funakoshi, H., Arenas, E., Persson, H., & Metsis, M. (1995). Identification of brain-derived neurotrophic factor promoter regions mediating tissue-specific, axotomy-, and neuronal activity-induced expression in transgenic mice. *Journal of Cell Biology, 128*, 185–199.

Tongiorgi, E. (2008). Activity-dependent expression of brain-derived neurotrophic factor in dendrites: Facts and open questions. *Neuroscience Research, 61*, 335–346.

Tongiorgi, E., Domenici, L., & Simonato, M. (2006). What is the biological significance of BDNF mRNA targeting in the dendrites? Clues from epilepsy and cortical development. *Molecular Neurobiology, 33*, 17–32.

Tonra, J. R. (1999). Classical and novel directions in neurotrophin transport and research: Anterograde transport of brain-derived neurotrophic factor by sensory neurons. *Microscopy Research and Technique, 45*, 225–232.

Twelvetrees, A. E., Yuen, E. Y., Arancibia-Carcamo, I. L., MacAskill, A. F., Rostaing, P., Lumb, M. J., Humbert, S., Triller, A., Saudou, F., Yan, Z., & Kittler, J. T. (2010). Delivery of GABAARs to synapses is mediated by HAP1-KIF5 and disrupted by mutant huntingtin. *Neuron, 65*, 53–65.

Tyler, W. J., Alonso, M., Bramham, C. R., & Pozzo-Miller, L. D. (2002). From acquisition to consolidation: On the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learning & Memory, 9*, 224–237.

Vaegter, C. B., Jansen, P., Fjorback, A. W., Glerup, S., Skeldal, S., Kjolby, M., Richner, M., Erdmann, B., Nyengaard, J. R., Tessarollo, L., Lewin, G. R., Willnow, T. E., Chao, M. V., & Nykjaer, A. (2011). Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. *Nature Neuroscience, 14*, 54–61.

Vantini, G., & Skaper, S. D. (1992). Neurotrophic factors: From physiology to pharmacology? *Pharmacological Research, 26*, 1–15.

West, A. E., Chen, W. G., Dalva, M. B., Dolmetsch, R. E., Kornhauser, J. M., Shaywitz, A. J., Takasu, M. A., Tao, X., & Greenberg, M. E. (2001). Calcium regulation of neuronal gene expression. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 11024–11031.

Wetmore, C., Olson, L., & Bean, A. J. (1994). Regulation of brain-derived neurotrophic factor (BDNF) expression and release from hippocampal neurons is mediated by non-NMDA type glutamate receptors. *Journal of Neuroscience, 14*, 1688–1700.

Wu, L. L., & Zhou, X. F. (2009). Huntingtin associated protein 1 and its functions. *Cell Adhesion & Migration, 3*, 71–76.

Wu, L. L., Fan, Y., Li, S., Li, X. J., & Zhou, X. F. (2010). Huntingtin-associated protein-1 interacts with pro-brain-derived neurotrophic factor and mediates its transport and release. *Journal of Biological Chemistry, 285*, 5614–5623.

Yan, Q., Radeke, M. J., Matheson, C. R., Talvenheimo, J., Welcher, A. A., & Feinstein, S. C. (1997a). Immunocytochemical localization of TrkB in the central nervous system of the adult rat. *The Journal of Comparative Neurology, 378*, 135–157.

Yan, Q., Rosenfeld, R. D., Matheson, C. R., Hawkins, N., Lopez, O. T., Bennett, L., & Welcher, A. A. (1997b). Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. *Neuroscience, 78*, 431–448.

Yang, F., Je, H. S., Ji, Y., Nagappan, G., Hempstead, B., & Lu, B. (2009a). Pro-BDNF-induced synaptic depression and retraction at developing neuromuscular synapses. *Journal of Cell Biology, 185*, 727–741.

Yang, J., Siao, C. J., Nagappan, G., Marinic, T., Jing, D., McGrath, K., Chen, Z. Y., Mark, W., Tessarollo, L., Lee, F. S., Lu, B., & Hempstead, B. L. (2009b). Neuronal release of proBDNF. *Nature Neuroscience, 12*, 113–115.

Yang, M., Lim, Y., Li, X., Zhong, J. H., & Zhou, X. F. (2011). Precursor of brain-derived neurotrophic factor (proBDNF) forms a complex with huntingtin-associated protein-1 (HAP1) and sortilin that modulates proBDNF trafficking, degradation, and processing. *Journal of Biological Chemistry, 286*, 16272–16284.

Zafra, F., Hengerer, B., Leibrock, J., Thoenen, H., & Lindholm, D. (1990). Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. *EMBO Journal, 9*, 3545–3550.

Zhou, X. F., & Rush, R. A. (1996). Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. *Neuroscience, 74*, 945–953.

Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B. R., Goffredo, D., Conti, L., MacDonald, M. E., Friedlander, R. M., Silani, V., Hayden, M. R., Timmusk, T., Sipione, S., & Cattaneo, E. (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science, 293*, 493–498.

Trafficking of Neurotrophins and Their Receptors and Pathological Significance

Christopher S. von Bartheld

Contents

Abstract

To survive and function properly, innervating neurons need to communicate with their targets. This task is accomplished, in part, by trafficking of neurotrophic molecules, in particular the family of nerve growth factor-like molecules, the neurotrophins (NTs). This chapter reviews the intra- and intercellular trafficking of NTs and their receptors with a focus on axonal transport and synaptic transfer, methodology, current concepts, and the relevance for pathological neurological conditions. Neurotrophins are transported by retrograde axonal transport from the neuronal or non-neuronal target cells to the innervating neuronal cell body. The neurotrophic hypothesis postulates that targets produce limiting amounts of trophic substances, including NTs, for

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which innervating neurons compete. NTs bind to NT receptors on axonal terminals and are internalized, presumably in vesicles or endosomes (signaling endosome hypothesis), and are transported by dynein-mediated retrograde axonal transport along microtubules to the cell body where the NT-specific receptors (trkA, B, and C) elicit signal transduction cascades that result in increased neuronal survival, differentiation, and/or plasticity. In addition to the retrograde route, NTs can also be transported anterogradely along axons and can be released at the axon terminal to influence postsynaptic cells. Thus, NTs can transfer in both directions across the synaptic cleft. NT receptors are expressed on the presynaptic as well as postsynaptic membrane, allowing for the activation of signal cascades at pre- as well as postsynaptic sites. NTs such as BDNF and NT-3 are particularly important messengers in synaptic plasticity. In addition to mature NTs, uncleaved precursors (pro-NTs) can also be trafficked and released, and their actions can oppose the functions of mature NTs by activating death signaling through the common NT receptor, p75. Several neurological disorders are currently being investigated for defects in NT trafficking, including Alzheimer's disease, Huntington's disease, Parkinson's disease, neurodevelopmental disorders, epilepsy, glaucoma, hyperalgesia, neuropathies, depression, and eating disorders. Progress in the understanding of physiological and pathological mechanisms of NT trafficking therefore may lead to improved therapies for neurological diseases.

Keywords

Axonal transport • Neurotrophin • Neurotrophin receptor • Neurotrophic hypothesis • Neurodegeneration • Synaptic plasticity

List of Abbreviations

BDNF	Brain-derived neurotrophic factor
CREB	cAMP response element-binding protein
DRG	Dorsal root ganglion
EM	Electron microscopy
ER	Endoplasmic reticulum
GFP	Green fluorescent protein
LDCV	Large dense-core vesicle
LTP	Long-term potentiation
MVB	Multivesicular body
NGF	Nerve growth factor
NT	Neurotrophin
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4
P75	Common neurotrophin receptor
TrkA	Tropomyosin receptor kinase A
TrkB	Tropomyosin receptor kinase B
TrkC	Tropomyosin receptor kinase C

1 Introduction

Intra- and intercellular trafficking of neurotrophic factors is fundamentally important for the neurotrophic hypothesis. This wider framework includes the initial overproduction of neurons during development, followed by programmed (developmental) neuronal death, and the regulation of survival or death of neurons by neurotrophic factors, including the NTs. Besides survival functions, NTs have additional maintenance and plasticity functions in the adult nervous system. Nerve growth factor (NGF) served as the first, prototypical neurotrophic factor. NGF trafficking was closely linked to a paradigm shift in the later half of the 1900s that provided a plausible explanation of how the final number of neurons is matched to the size of their target (systems matching) and how neurons receive feedback from their target. This concept also involves competition for limited trophic supply in the target and trafficking of the NT from the target to deliver the trophic signal to the cell body of the innervating neuron. Early NGF research benefited from some remarkably favorable circumstances that have been reviewed on multiple occasions (Levi-Montalcini 1987; Purves 1988; Oppenheim 1996).

The discovery of the specific and saturable axonal transport of NGF has had a major impact in the field (Hendry et al. 1974; Stoeckel et al. 1975; reviewed from a historical perspective by Oppenheim 1996). Probing neurons with radiolabeled NGF in both in vivo and in vitro settings provided major insights due to high specificity and sensitivity of a quantifiable signal. These studies provided crucial experimental support for the neurotrophic hypothesis. NTs were studied in "Campenot chambers" (Campenot 1977), an experimental approach that allowed to examine NT trafficking in vitro combined with biochemical and molecular analyses (Bhattacharyya et al. 1997; Watson et al. 1999, 2001; MacInnis and Campenot 2002). The 1980s and 1990s saw further seminal progress in NT research with Yves Barde's identification, purification, and sequencing of the second NT, BDNF (Leibrock et al. 1989), and the subsequent expansion of the NT family (NT-3, NT-4). Not only was the entire family of NTs identified but also two types of NT receptors – first p75, then trkA, and other trks (Barbacid 1994; Huang and Reichardt 2003). At the same time, it was realized that signaling and endocytosis are intertwined in important ways (Di Fiore and de Camilli 2001; Sorkin and von Zastrow 2002), and progress was made in the identification of the molecular motors that move organelles and molecules along microtubules: dynein and kinesins and associated motor proteins (Hirokawa 1998; Goldstein and Yang 2000; Vallee et al. 2004). The early 2000s saw the distinction of the roles of mature NTs vs. pro-NTs and increased attention to pro-NT's death signaling capacities (Lee et al. 2001; Lu et al. 2005). The relevance of NTs – especially BDNF – for synaptic plasticity and LTP was realized; this added an entire new dimension in NT function and with that, also new questions and insights into NT trafficking (Poo 2001; Lu et al. 2005).

The scope and focus of this review is the intracellular and intercellular trafficking of NTs and their receptors; the review will consider primarily axonal but also dendritic, synaptic, and somal forms of trafficking. The chapter will also review current knowledge of how NTs are released from neurons and their target cells, how

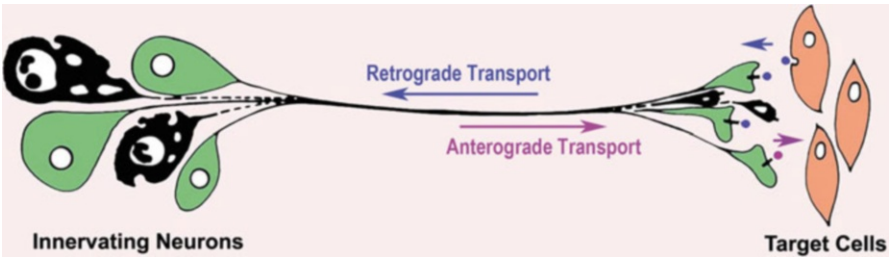

Fig. 1 Two major trafficking modes of neurotrophins: retrograde (*blue arrow*) and anterograde axonal transport (*purple arrow*). The neurotrophic hypothesis postulates that developing neurons survive (*green neurons*) only when they successfully compete for target-derived trophic molecules (*blue arrow*) that are internalized at the nerve terminal and are transported retrogradely along the axon (*long blue arrow*) to the cell body. Neurons that fail to obtain a sufficient flow of trophic molecules from the target die by programmed cell death (*black neurons* with condensed chromatin). Neurons also synthesize and send neurotrophins anterogradely along the axon (*long purple arrow*), from the cell body to the nerve terminal, where the neurotrophin is released (*purple*) (Modified and reproduced with permission from the publisher, Elsevier (Barde 1989, *Neuron* **2**, 1525–1534))

they are internalized and transported retrogradely and anterogradely (Fig. 1), and the type of methodology that is used with a discussion of technical challenges and limitations, and finally will examine the relevance of NT trafficking for neurological diseases. For other aspects of NT physiology and pathology, the reader is referred to previous reviews, e.g., on NT history (Oppenheim 1996), NT signaling (Huang and Reichardt 2001, 2003), synaptic plasticity/LTP and synaptic transfer of NTs (Poo 2001; Lessmann et al. 2003), functions of pro- vs. mature NTs (Lee et al. 2001; Lu et al. 2005), internalization of NT receptors in cell lines (Bronfman et al. 2007), retrograde transport of NTs (Reynolds et al. 2000), and retrograde NT signaling (Zweifel et al. 2005).

2 Release of Neurotrophins (NTs)

According to the neurotrophic hypothesis, NTs need to be released from target cells that can be neurons or other, non-neuronal cells, such as muscle. NTs can also be released from glial cells. NTs are extremely potent signaling molecules (Thoenen and Barde 1980), and therefore, cells release frustratingly low amounts of endogenous NTs (Gärtner et al. 2000; Dieni et al. 2012, but see Rush 2001, for an alternative view). Low amounts of released NTs are consistent with the neurotrophic hypothesis that postulates that trophic material be released in limiting amounts from target cells and must be competed for by innervating neurons. The apparent low abundance of NTs for release poses a considerable technical challenge, as will be further discussed in Sect. 5. Nevertheless, studies have shown that NTs can be released from different neuronal compartments, including dendrites (Hartmann et al. 2001; Kojima et al. 2001) and axon terminals (von Bartheld et al. 1996a; Fawcett et al. 1997; Kohara et al. 2001; Lever et al. 2001; Wang et al. 2002), while release from cell bodies is less

clear (Blöchl and Thoenen 1995; Balkowiec and Katz 2000). How are NTs or pro-NTs trafficked to sites of release? This is presumably accomplished by trafficking along routes previously established for other peptides, namely, through rough endoplasmic reticulum (ER), transport through the Golgi system, glycosylation, and cleavage of the pro-form in the Golgi system (Conner et al. 1998; Mowla et al. 1999, 2001; Farhadi et al. 2000; Lessmann et al. 2003), while the role of smooth endoplasmic reticulum is less clear (Gärtner et al. 2000). Distinct vesicles containing BDNF can be targeted to either dendritic or axonal compartments (Adachi et al. 2005; Dean et al. 2012).

Cells release peptides via two principal types of release, constitutive and regulated (activity-dependent) modes (Burgess and Kelly 1987). Release of NTs has been reported to be accomplished by both, constitutive and regulated pathways (Blöchl and Thoenen 1995; Goodman et al. 1996; Gärtner and Staiger 2002; Lessmann et al. 2003), although some investigators question the dichotomy and the existence of a true activity-independent baseline of release (Poo 2001). Several groups showed activity-dependent release (Ghosh et al. 1994; Blöchl and Thoenen 1995). Furthermore, the pattern of stimulation frequency is important (Balkowiec and Katz 2000), and release appears to depend on the status of internal calcium stores (Blöchl and Thoenen 1995; Gärtner and Staiger 2002; reviewed by Lessmann et al. 2003). Such release modes are highly relevant for mechanisms of LTP. In this context, properties of a val-met mutation of BDNF are of interest – this mutation does not affect constitutive release, but it impairs regulated BDNF release, apparently due to disruption of a trafficking signal in the BDNF prodomain region (Egan et al. 2003). The resulting defect in BDNF sorting to the regulated secretory pathway has consequences for several neurodegenerative and psychiatric disorders, including depression, eating, and bipolar disorders (Chao et al. 2006), as discussed in Sect. 6 of this review. Which compartments and organelles are involved in NT release? Ultrastructural studies designed to reveal the compartments and organelles involved in NT release from neurites have been inconclusive (Gärtner et al. 2000). Smooth ER membranes were labeled as well as microtubules, but there was no accumulation at synaptic vs. extrasynaptic sites, and large dense-core vesicles (LDCVs) were not labeled. The examination of the ultrastructural distribution in axon terminals has consistently shown NTs to be associated at least to some degree with LDCVs (Michael et al. 1997; Fawcett et al. 1997; Luo et al. 2001; Salio et al. 2007; Dieni et al. 2012). It has been proposed that the apparent lack or paucity of NT immunoreactivity in dendrites (Drake et al. 1999) is due to NTs being "obscured" by trks, so that NT antibodies fail to recognize NTs when they are bound by trk receptors (Rush 2001).

Release at synaptic/extrasynaptic sites – wiring or volume transmission? Being neuropeptides, NTs are supposed to be released primarily at extrasynaptic locations via "volume transmission" (Merighi 2002; Torrealba and Carrasco 2004; Feng et al. 2011), but not directly at the synaptic density. Consistent with such volume transmission, NTs appear to be released from neurites without preference for synaptic sites (Gärtner et al. 2000; Hartmann et al. 2001; Gärtner and Staiger 2002). On the other hand, NTs can be released from axon terminals,

as indicated by studies examining synaptosomes (Androutsellis-Theotokis et al. 1996; Fawcett et al. 1997), DRG terminals (Lever et al. 2001), cultured neuronal networks (Kohara et al. 2001), and retinal ganglion cell axon terminals (Wang et al. 2002). This suggests synaptic release after anterograde axonal transport, but primarily extra- (or iuxta-)synaptic release from postsynaptic sites in dendrites. Recent work indicates that synaptotagmin (Syt-IV) is involved in targeting BDNF-containing vesicles to either dendritic or axonal synaptic sites where they are released by activity-dependent mechanisms (Dean et al. 2012).

Are release mechanisms different between different NTs? Some studies have reported that BDNF release is regulated (or that regulated release occurs over a baseline constitutive component, Blöchl and Thoenen 1995; Lessmann et al. 2003), while NGF (and NT-3) release was reported to always be constitutive (Mowla et al. 1999; Farhadi et al. 2000). However, such differences may be ascribed to differences in experimental protocols rather than true differences between NTs (Griesbeck et al. 1999; Neet and Campenot 2001; Wu et al. 2004), and, as mentioned, some investigators question whether constitutive release truly exists for NTs (Poo 2001).

Initially, only release of mature NTs was envisioned, based on the assumption that all pro-NTs were cleaved intracellularly prior to release (Mowla et al. 1999, 2001; Farhadi et al. 2000). It is now thought that cells differ in the degree of such cleaving, resulting in release of both pro-NTs and mature NTs. If cleavage can occur both intra- and extracellularly, then this provides a novel level of complexity in the regulation of NT availability and function (Lee et al. 2001; Lu et al. 2005). However, because of the low abundance of released endogenous NTs, it is still controversial whether NTs and specifically BDNF are released by neurons in vivo as the pro-NT form or mostly as mature BDNF (Matsumoto et al. 2008; Yang et al. 2009; Greenberg et al. 2009; Dieni et al. 2012). This is an important question that has resulted in an ongoing controversy. Endogenous proBDNF can be transported anterogradely and retrogradely along axons (Wang et al. 2006), but this does not inform about the released form of BDNF. BDNF is easier to measure when overexpressed or tagged, and in vitro, but these conditions may change the release mode, the cleavage properties, and capacity of NT trafficking, and the in vitro condition may not reflect the in vivo situation (see Sect. 5).

Another question is whether the release of newly synthesized NTs is different from that of recycled (internalized) NTs. Based on evidence from other growth factors and examination of exogenous (radiolabeled) NTs, a substantial fraction of released and internalized trophic factors are not immediately degraded, but shuttled into a recycling pathway in neurons (von Bartheld et al. 2001; Rind et al. 2005). Depending on how much NT may be recycled rather than degraded, a major fraction of NTs in neurons may not comprise newly synthesized NT, but rather recycled NT after its initial release and internalization (von Bartheld et al. 2001; Lessmann et al. 2003). When recycled, what is the storage compartment? Multivesicular bodies (MVBs) are strong candidates for NT storage – NT-filled MVBs are often localized adjacent to synapses on the postsynaptic side (Rind et al. 2005; von Bartheld and Altick 2011). It has been shown that the recycled pool of BDNF indeed is relevant for LTP (Santi et al. 2006).

What is the role of NT receptors in release? NT receptors need to be targeted to pre- as well as postsynaptic membranes (Ascaño et al. 2009), indicating that NTs and/or pro-NTs can be released at synapses and may bind to pre- as well as postsynaptic NT receptors. Truncated trkB receptors have a role for BDNF release from glial cells (Alderson et al. 2000) and possibly neurons as well (Biffo et al. 1995).

3 Retrograde Axonal Transport

This section will follow the pathway of NTs when they are internalized by axon terminals and sent onto a trafficking route along the axon (a distance that may exceed 1–2 m) and that culminates with the delivery of the NT (and NT-induced signals) to the neuronal cell body. The evidence for receptors and organelles involved in this process will be reviewed, and the significance of the prevailing "endosome signaling hypothesis" and its alternatives discussed.

Internalization. It has been appreciated for a long time that the internalization of NTs is receptor mediated (Hendry et al. 1974; Stoeckel et al. 1975; Reynolds et al. 2000). The NT-receptor complex can be endocytosed by either clathrin-mediated or non-clathrin-mediated pathways (Howe and Mobley 2004; Bronfman et al. 2007). The so-called caveosomes may concentrate lipid-raft-derived NT signals (reviewed in Howe and Mobley 2004). Thus, in order to achieve internalization of NTs at the axon terminus, NT receptors need to be targeted first to, and incorporated into, the axon terminal plasma membrane. Which NT receptors mediate internalization and retrograde axonal transport of NTs? This has been examined extensively in the 1990s, and various lines of evidence indicate that NGF is internalized and transported in neurons primarily by trkA, while for other NTs (BDNF, NT-3 and NT-4) both trk receptors and p75 receptors contribute, and NT-4 appears to rely the most on p75 as a transporter (Curtis et al. 1995; von Bartheld et al. 1996b). p75 is believed to be expressed in vivo in a ratio of about five-to-tenfold higher than trk receptors (Chao 1994), and the relationship between p75 and trk receptors and the functional significance of co-expression of these two receptor types has been intensely studied (Bothwell 1995; Bronfman et al. 2007). Co-expression of p75 and trk receptors appears to create a higher-affinity binding site for NTs (Chao 1994; Bothwell 1995; Esposito et al. 2001). Initial models suggested a simultaneous binding of p75, trk, and the NT in a trimeric complex, but this is not consistent with more recent molecular modeling data (Wehrman et al. 2007; Barker 2007). It is possible that p75 receptors "present" the NT to the less common trk receptors (Biffo et al. 1995; Barker 2007). It has been suggested that the NT is initially captured by the more abundant p75 receptors and over time (during axonal transport) accumulates on trk receptors in the signaling endosome (Butowt and von Bartheld 2009; Chowdary et al. 2012). This proposed mechanism is consistent with the known differences in on-rates and off-rates (the p75 receptor has a faster off-rate than the trk receptors – Rodriguez-Tébar and Barde 1988; Bothwell 1995). NT-containing endosomes have been reported to co-localize p75 and trk receptors to varying degrees, and some studies suggest that p75-containing endosomes are routed differently than trk-containing endosomes (Hibbert et al. 2006; Bronfman et al. 2007). It has been known for a long time that

NTs injected directly into the cell body have no trophic effect, demonstrating that the binding of NTs to the cell surface NT receptor and the internalization of the ligand-receptor complex are essential for mediating the trophic signal (Claude et al. 1982; critically reviewed by Neet and Campenot 2001). Several molecules involved in the internalization and transport of NTs and NT receptors have been identified; these include pincher and Rabs (Rab5 and Rab7) (Heerssen et al. 2004; Saxena et al. 2005; Valdez et al. 2005; Deinhardt et al. 2006; Bronfman et al. 2007). The p75 receptor can be cleaved and the C-terminal p75 fragment may be trafficked; this fragment has been shown to retain signaling capacity (Kanning et al. 2003). In summary, there is abundant evidence for retrograde axonal transport of NTs (reviewed in Reynolds et al. 2000) as well as the NT receptors, p75 and trks (Johnson et al. 1987; Ross et al. 1994; Ehlers et al. 1995; Mufson et al. 1999; Moises et al. 2007).

The endosome signaling hypothesis and two alternatives. Evidence for the receptor-mediated internalization of NTs and emerging knowledge about endocytosis of growth factor receptors and their ligands prompted the so-called "endosome signaling" concept: Pinching off NT receptor containing plasma membrane results in vesicles with the NT bound on the inside and the tyrosine kinase domain of the NT receptor on the outside of the vesicle, available for docking of additional signaling molecules (Fig. 2). A large number of studies have accumulated supporting evidence for the validity of this model: Phosphorylated (activated) trkA is retrogradely transported (Ehlers et al. 1995; Bhattacharyya et al. 1997); signaling endosomes can be purified from nerves (Delcroix et al. 2003); they contain the relevant signaling molecules depicted in Fig. 2; some of the NT receptors possess moieties that directly attach to the molecular motor (dynein) that moves the endosome retrogradely along microtubules (Yano et al. 2001; Yano and Chao 2004); these vesicles retain signaling capacity, as reviewed by multiple groups (Zweifel et al. 2005; Wu et al. 2009; Oppenheim and von Bartheld 2008). Some of the signaling may occur already during transit, since sufficient signal transducers are attached to the NT- and NT-receptor-containing vesicle. However, most of the signaling is believed to occur once the signaling vesicle is delivered to the cell body. At the cell body, signaling cascades (via CREB and other pathways) regulate gene expression in the nucleus (Riccio et al. 1997); after delivery of the signal, the signaling endosome is believed to be dismantled, possibly by transition to a MVB and eventual to a lysosome (Mukherjee et al. 1997; von Bartheld and Altick 2011). Alternatively, the endosome or components of the signaling endosome are recycled by targeting to other cellular compartments (e.g., synapses on dendrites, von Bartheld et al. 2001; Rind et al. 2005). Currently, there is widespread support among investigators in the field for the signaling endosome hypothesis.

One alternative to the signaling endosome hypothesis is the concept that NTs may signal locally within the axon terminus and that this generates one or more second messengers in the axon terminal that is moved retrogradely along the axon. There is some evidence that second messengers other than axonally transported NTs or NT receptors contribute to the retrograde signal; these include G proteins as well as MEK kinase, MAP kinases, and PI3 kinases and/or their subunits

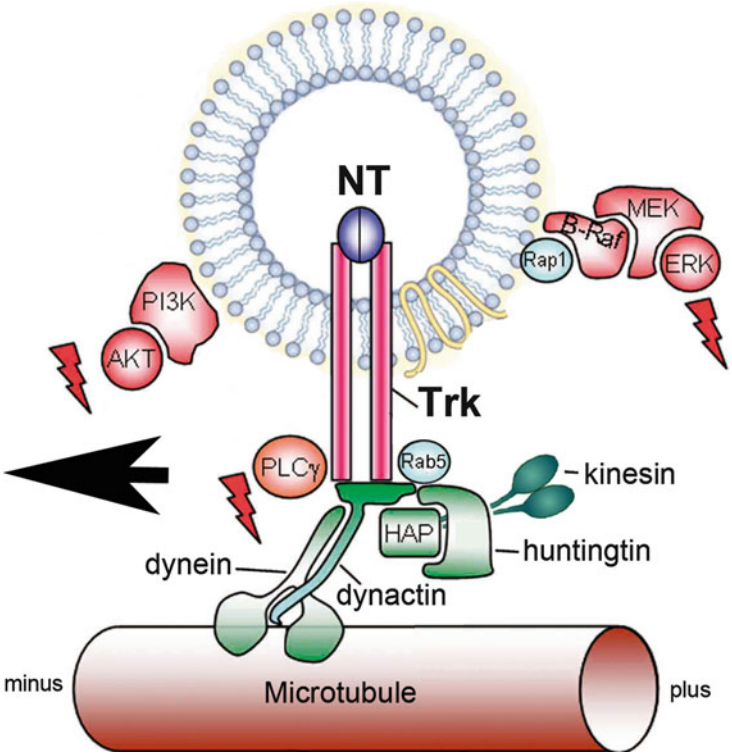

Fig. 2 The signaling endosome. The internalized neurotrophin (*NT*)-receptor (*Trk*) complex is transported retrogradely from the nerve terminal to the cell body. The internalized vesicle serves as a platform for docking of proteins that elicit signal transduction cascades (*red*): via the phospholipase C-gamma (*PLCγ*), Raf-MAPK-ERK, and PI3K/AKT signaling pathways (*red lightning symbol*); molecular motors and regulators (*green*) attach for transport along microtubules (*brown*). The *arrow* indicates the direction of transport toward the minus end of microtubules. For details and references, see Zweifel et al. 2005 (*Nature Reviews Neuroscience* **6**, 615–625) and Gauthier et al. 2004 (*Cell* **118**, 127–138) (Material adapted, with permission, from: Oppenheim and von Bartheld 2008 (Fundamental Neuroscience, 2nd edition, Academic Press))

(Johanson et al. 1996; Reynolds et al. 2000; Weible and Hendry 2004). It is unclear to what extent the transported NT-containing vesicle or endosome may contribute to the signaling beyond the initial signaling at the axon terminus; in the strongest form of the second messenger concept, the retrograde axonal transport of the NT may simply serve to remove the NT from the region of the axon terminus ("vacuum cleaner model", Bothwell 1995), so that the local NT signal can be terminated and does not continue to elicit signals.

A third possibility is the "wave model," which is based on the surprising observation that at least in some cases, a trophic signal arrives at the level of the cell body prior to the time needed for the NT-containing endosome to arrive there (Senger and Campenot 1997) and that consequently some signaling occurs that is independent of the retrograde axonal transport of the NT-containing vesicle that

initiated the signal (Miller and Kaplan 2001; Neet and Campenot 2001; MacInnis and Campenot 2002). The major weakness of this model is that there is no compelling physical mechanism of how the signal could spread as a lateral "wave" along the required distances (Ginty and Segal 2002; Howe and Mobley 2004; Wu et al. 2009). Furthermore, current evidence is based on a small set of in vitro experiments, and some assumptions are made about identical properties of radiolabeled vs. native NTs.

Transport organelle. Considering the long distance from the axon terminus to the cell body, it is surprising that there is very little direct evidence for the type of organelle that carries the NT signal retrogradely along the axon. Two major models can be distinguished: The signaling organelle is a multivesicular body (MVB, Parton et al. 1992; Valdez et al. 2005; Weible and Hendry 2004; Philippidou et al. 2011), or it is a small vesicle or endosome (Delcroix et al. 2003; Altick et al. 2009; Wu et al. 2009; von Bartheld and Altick 2011). The former notion is based primarily on in vitro experiments performed with large tags of the NT, which recent work has shown to generate abnormal organelles or quantities of organelles (von Bartheld and Altick 2011). The alternative view is that the endosome is a small vesicle and remains such a structure up to the level of the cell body. Purification experiments support this view (Delcroix et al. 2003), as do experiments with quantum dot-labeled NTs (Cui et al. 2007). MVBs were ruled out as the "normal" carrier based on their low frequency in normal nerves in vivo and lack of labeling when the ultrastructural distribution of radiolabeled NTs was determined within nerves (Altick et al. 2009). While there is no doubt that retrogradely transported NTs eventually accumulate in MVBs within the cell body (Claude et al. 1982; Rind et al. 2005; von Bartheld and Altick 2011), NTs do not appear to travel within this type of organelle while en route within the axon in vivo.

Arrival of the NT signal in cell body. The signal transduction pathways activated by NTs in the cell body have been reviewed elsewhere and will not be repeated here (Huang and Reichardt 2001, 2003). There has been one major aspect that has puzzled the investigators: How is it that the signal within the signaling endosome is so long-lived? When the same signaling cascades are activated by other ligands and other types of tyrosine kinase receptors, the MAP kinase activation is measured within minutes, not hours (Kao et al. 2001; Wu et al. 2001). Prolonged activation appears essential, since it can take hours to move a signaling endosome (or the NT) from the axon terminus to the cell body, e.g., in the motor neurons of the spinal cord. Several solutions have been offered why NT binding to trks in neurons may induce longer-lasting signal duration (Kao et al. 2001; Wu et al. 2001; Saxena et al. 2005). The concentration of trk receptors in the endosome is sufficiently high to dimerize and autophosphorylate (Miller and Kaplan 2001). Another mechanism in play could be that the NT comes off one NT receptor and binds another NT receptor within the same vesicle (Butowt and von Bartheld 2009; Chowdary et al. 2012). Does the cell body somehow know where the trophic signal was generated (axon target vs. dendritic afferents)? This question was examined by Segal and her colleagues,

and it appears that some signal transduction pathways (ERK) have certain specificity for where the NT signal was generated (Watson et al. 2001; Heerssen and Segal 2002). However, another study found that the same ERK was contained in signaling endosomes (Delcroix et al. 2003), indicating that the type of ERK activation may be variable between different cell types or other conditions (Bronfman et al. 2007).

Fate of NTs after arrival in the cell body. How is the NT signal "turned off"? One solution that has been proposed is that the NT endosome is incorporated into the internal vesicles of a MVB, essentially preventing access of the NT receptor to the cytosol (Sorkin and von Zastrow 2002; von Bartheld and Altick 2011). Initially, it was assumed that NTs are degraded immediately or within a few hours after signaling (Claude et al. 1982; Reynolds et al. 2000; Neet and Campenot 2001). Some neurotrophic factors such as cardiotrophin-1 indeed appear to be rapidly degraded in lysosomes (Rind et al. 2005). However, other trophic molecules are used multiple times prior to degradation, and NTs appear to belong to this group (von Bartheld et al. 2001; Rind et al. 2005; Sharma et al. 2010). Thus, there are two pools of NTs: newly synthesized NTs and internalized NTs that can be recycled. In fact, recycled, internalized NTs have been shown to serve important functions in LTP (Santi et al. 2006). One of the molecular mechanisms that decides between degradation and recycling of retrogradely transported NTs appears to involve phosphorylation of the p75 receptor (Butowt and von Bartheld 2009). While there has been progress in elucidating some of the mechanisms of retrograde neurotrophin signaling, major gaps in understanding of the transport and arrival of the signaling complex remain to be filled. The major steps of retrograde and anterograde trafficking of NTs are summarized in the schematic of Fig. 3.

4 Anterograde Axonal Transport

Based on the neurotrophic hypothesis and the early discovery of retrograde axonal transport of NGF and other NTs (Hendry et al. 1974; DiStefano et al. 1992), trafficking of NTs was for several decades thought to occur primarily if not exclusively in the direction toward the cell body. Despite abundant evidence for trophic effects of afferents on neurons (Linden 1994), it came as a surprise when it was discovered in 1996 that both exogenous and endogenous NTs can move anterogradely along axons (von Bartheld et al. 1996a; Zhou and Rush 1996) and that NTs can be transferred from presynaptic to postsynaptic sites (von Bartheld et al. 1996a). These first reports were rapidly confirmed (Altar et al. 1997; Johnson et al. 1997; Altar and DiStefano 1998; Conner et al. 1998). The discovery of anterograde axonal transport of NTs opened a new venue of NT research: Can NTs behave like neurotransmitters? Are they released at the axon terminus by activity-dependent mechanisms? In which organelles are they stored there, and how are they released? What are the functions of this new trophic pathway?

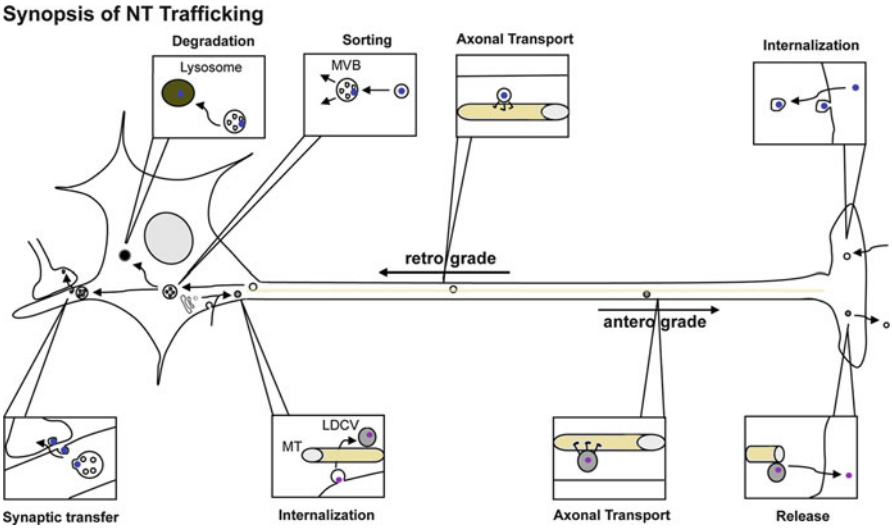

Fig. 3 Synopsis of retrograde and anterograde trafficking of neurotrophins (*NTs*). The upper half of the schematic illustrates the major steps of retrograde traffic, from internalization at the axon terminus, via microtubule-mediated axonal transport along the axon, sorting in the cell body and eventual degradation in lysosomes. The lower half of the schematic shows the major steps of synthesis or internalization of NTs in the cell body, microtubule-mediated anterograde axonal transport, and release at the axon terminus. In addition, synaptic transfer of NTs via MVBs is indicated. Abbreviations: *LDCV* large dense-core vesicle, *MT* microtubule, *MVB* multivesicular body

Some investigators now believe that anterograde axonal transport of NTs is quantitatively more significant than the "conventional" retrograde axonal transport (Conner et al. 1998; Adachi et al. 2005). Despite its moment of fame in the late 1990s, anterograde axonal transport of NTs has been studied much less than retrograde transport, and therefore, less is known about its mechanism. Anterogradely transported NTs are now believed to be mostly stored within LDCVs in axon terminals (Michael et al. 1997; Fawcett et al. 1997; Luo et al. 2001; Salio et al. 2007; Dieni et al. 2012), although some controversy remains. The amounts of both endogenous and exogenous NTs that are transported anterogradely appear to be lower, on a per neuron basis, than those that can be transported retrogradely (von Bartheld et al. 1996b). Anterogradely transported neurotrophins have been shown to have multiple effects on their afferents, not only with regard to neuronal survival but also synaptic transmission and dendritic morphology (Caleo et al. 2003; Caleo and Cenni 2004; von Bartheld 2004).

It should be noted that anterograde axonal transport of NTs and NT receptors serves two very different functions: NT anterograde transport appears to mediate trophic signals that arise from afferents (Linden 1994), as opposed to the well-established roles of efferent targets, while anterograde transport of NT receptors primarily delivers the NT receptors that are needed in the axon terminus for

internalization and retrograde transport of target-derived trophic signals (Ascaño et al. 2009). While it is known that the anterograde axonal transport of NTs in adults does not require p75 receptors, the role of trk receptors is less clear, although they seem to contribute to anterograde axonal transport of BDNF (Butowt and von Bartheld 2005). Anterograde axonal transport of NTs is mediated by conventional kinesins (Butowt and von Bartheld 2007), while anterograde axonal transport of trk receptors is facilitated by sortilin (Vaegter et al. 2011), and the association between trkB receptors and kinesin-1 is accomplished by the protein JIP3 (Huang et al. 2011).

Release mechanisms of anterogradely transported NTs have to be studied specifically at the axon terminus, which is not an easy task. Such release was first examined in synaptosomal preparations (Androutsellis-Theotokis et al. 1996; Fawcett et al. 1997), and subsequently by utilizing advantageous model systems such as the DRG central projection into the spinal cord (Lever et al. 2001), retinal ganglion cell axons in the optic tectum (Wang et al. 2002). In addition, one can culture neurons and let them form synaptic connections, engineered to express BDNF-GFP in the presynaptic neuron and observe labeled puncta to transfer from presynaptic to postsynaptic sites (Kohara et al. 2001). These studies showed that such pre- to postsynaptic transfer of NTs was activity dependent and modulated by numerous compounds, including intracellular calcium, cAMP, NMDA receptors, and stimulation pattern (Kohara et al. 2001; Lever et al. 2001; Wang et al. 2002).

Anterograde axonal transport of internalized NTs. While much of the anterograde axonal transport of NTs presumably involves newly synthesized NTs, exogenous NTs can also be targeted to an anterograde axonal pathway (Butowt and von Bartheld 2001). Such exogenous NTs are internalized by retinal ganglion cells, traverse the Golgi system, and thereby join what is presumably the "normal" anterograde axonal route. Some of the NTs in this pathway are packaged in LDCVs (Wang et al. 2002), and they are released from axon terminals in the optic tectum in an activity-dependent fashion (Wang et al. 2002). One exogenous NT, NGF, was shown to be immunoprecipitated by p75 receptor antibodies in this pathway to the optic tectum where the same NGF subsequently (after release) binds primarily to trkA receptors and embarks on a new route to the locus ceruleus (Feng et al. 2011). Thus, NT itineraries can be linked in neural networks in different anterograde and retrograde trafficking combinations (Figs. 1 and 3).

5 Methodology

This section reviews the types of techniques that researchers use to investigate trafficking of NTs. It will examine limitations and pitfalls, as well as strategies to overcome challenges, and discuss some current controversies in the field.

When endogenous NTs are examined, they can be visualized with NT-specific antibodies (Conner 2001). Another approach is to express a specific tag (such as GFP or a myc tag) with the NT of interest. Obviously, all NTs recognized by the antibody or the tag signal itself will be visualized (as in a snap shot), and therefore,

this approach alone does not reveal the trafficking itself (either qualitative or quantitative). For example, the presence of the NT in the axon suggests axonal transport, but it does not indicate the direction (anterograde or retrograde) or the velocity and capacity. Some hints may be given by the lack of synthesis (by in situ hybridization) of the NT in the cell body or the target cells (Conner et al. 1997), but if expression levels are very low, no firm conclusions can be made about trafficking. A useful and common technique is to apply a ligature to a nerve and to determine whether the NT accumulates distally or proximally to the ligature or both (Johnson et al. 1987; Ross et al. 1994; Ehlers et al. 1995; Mufson et al. 1999). This gives information about the direction of axonal transport and a rough estimate of the relative amount. Caveats are that glial cells surrounding the site of ligature may be induced to express NTs, and they may thus accumulate in response to such nerve injury, resulting in false-positives.

A major problem with the study of endogenous NTs is the extremely low abundance of the endogenous NTs in vivo. This is a challenge, because the methods used to visualize the NT may not have sufficient sensitivity. In addition, NTs may simply be present in concentrations too low to be detected, yet high enough to have significant trophic effects. Therefore, lack of label cannot be interpreted as evidence for a lack of NTs. Furthermore, based on the localization of NTs, antibodies may fail to reveal the NT, for example, if the antibody does not recognize the NT when it is bound to certain receptors (Rush 2001). One solution to the problem of low abundance is to overexpress the NT of interest. However, this approach may saturate normal trafficking capacities and thus lead to false conclusions about trafficking. Another approach is to transfect tissues or cells with a molecular tag on the NT – this, however, may change NT trafficking, receptor binding, sorting and degradation, as well as kinetics. This is especially important to consider when the tag is large relative to the size of the NT protein (Möller et al. 1998; Parak et al. 2005; Chowdary et al. 2012).

When exogenous NTs are examined, some of the abovementioned problems (such as not knowing the direction and quantity of trafficking) are minimized when the NT is introduced into a well-defined compartment. However, the exogenous NT has to be different or tagged so that it can be distinguished from the endogenous NT already present in the system. There are several different options of how NTs can be tagged (Fig. 4). Particularly important, historically, has been the tagging of NTs with the isotope 125I (radioiodination). This procedure only minimally modifies the NT, so the radiolabeled NT's binding properties are unaffected and the NT maintains bioactivity (Johnson et al. 1978; Vale and Shooter 1985; Rosenfeld et al. 1993; von Bartheld 2001). The probe is highly sensitive and quantifiable and can be localized at the ultrastructural level without compromising tissue quality, since probe penetration through tissue is not an issue as it is with antibody visualization. Drawbacks are that the probe cannot be tracked in real time and that the resolution at the EM level is poor, allowing only localization of the NT (silver grain) with major organelles and large enough structures such as synapses (Claude et al. 1982; von Bartheld 2001; Rind et al. 2005). Other options for tagging NTs include biotinylation (Rosenberg et al. 1986), conjugation with GFP (Gerdes and

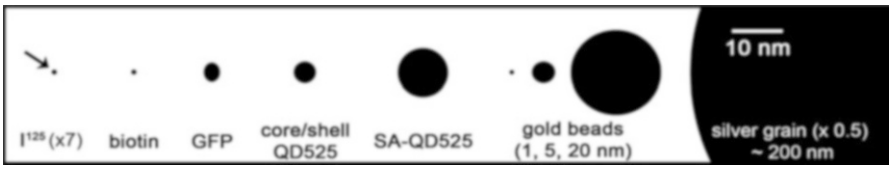

Fig. 4 Comparison of the sizes of commonly used protein tags. I125 is the smallest tag (seven times smaller than indicated). The core/shell of quantum dots (*QDs*) is similar in size to green fluorescent protein (*GFP*). Gold particles come in various sizes, as indicated. After autoradiographic development of radiolabel in monolayer emulsion, silver grains are the largest with diameters of 100–200 nm, thereby relinquishing the small size of the radiolabeled tag. Abbreviation: *SA* streptavidin

Kaether 1996; Haubensak et al. 1998; Watson et al. 1999; Kohara et al. 2001; Adachi et al. 2005; Guillemot et al. 2007), myc tagging (Möller et al. 1998), gold particle tagging (Sandow et al. 2000; Salio et al. 2007), and conjugation with quantum dots (Giepmans et al. 2005; Vu et al. 2005; Cui et al. 2007; Rajan et al. 2008; Altick et al. 2009; Chowdary et al. 2012; Xie et al. 2012). With the exception of biotinylation and myc tagging, these procedures result in a NT-tag complex that is considerably larger than the original, native NT, and the obvious concern is that such a protein modification may change trafficking properties of NTs. Typical controls include verification that the tagged NT still binds to its receptors, has bioactivity, and that it is internalized (Vu et al. 2005; Cui et al. 2007; Rajan et al. 2008; Xie et al. 2012). However, investigators rarely test tagged NTs for any trafficking changes, including capacity, speed, sorting, intracellular aggregation, organelle transition, and degradation kinetics (Neet and Campenot 2001). Indeed, it is not known how such tags affect the NT itineraries, sorting, recycling, and degradation and whether and at what point the tag may dissociate from its target protein (Tekle et al. 2008; Wiggins et al. 2012).

Another problem with expression of either tagged NTs or overexpression of tagged or native NTs is that this may result in much higher levels of the NT than normal, and this may saturate the normal capacity of NT processing, cleavage, trafficking, sorting, and release and accordingly may lead to false-positives, because the tagged and/or overexpressed NT may partially or entirely be directed to abnormal trafficking routes and sites of accumulation (Hartmann et al. 2012; Wiggins et al. 2012). Some of these issues may explain discrepant findings and current controversies, for example, the question whether neurons release BDNF mostly as a mature BDNF form or whether also the uncleaved proBDNF is released by neurons in vivo (Matsumoto et al. 2008; Yang et al. 2009; Greenberg et al. 2009; Dieni et al. 2012). In this context, it is important to remind the reader that caution is also advised when experiments are performed in vitro as opposed to in vivo. For example, extracellular BDNF is rapidly cleaved in vivo by glial cells; when neurons are maintained in an artificial environment lacking glial cells, extracellular proBDNF can persist for a longer time (Yang et al. 2009). Also, neurites (neuronal processes) grown in vitro tend to express many more MVBs than they would in vivo, and application of exogenous NTs conjugated to particles such as gold

can induce formation of multivesicular bodies and incorporation of NTs into such organelles, when this would not normally happen (von Bartheld and Altick 2011). Nevertheless, trafficking studies in Campenot chambers and microfluidic chambers have considerably extended knowledge and concepts of NT trafficking (MacInnis and Campenot 2002; Cohen et al. 2011). Examination of NT receptors rather than NT ligands is technically more robust, since receptors are expressed at higher levels than NTs and therefore are easier to visualize, both by immunological and GFP-expression methods. Furthermore, NT receptors stay confined to the producing cells and typically are not released from cells and do not transfer from one cell to another cell (with the possible exception of transfer via exosomes, von Bartheld and Altick 2011). Taken together, results obtained from in vitro studies, and results obtained by observing trafficking of grossly-modified NTs, need to be interpreted with considerable caution.

6 Diseases and Disorders Related to NT Trafficking

With the emergence of the neurotrophic hypothesis, it was suggested that the neurotrophic concept may not only apply to the development and differentiation of neurons but also to their maintenance. Accordingly, neurodegeneration may be the result of a failed supply of neurotrophic signals (Appel 1981; Saper et al. 1987). Based on this concept, neurodegenerative diseases may result from a reduced production, reduced axonal transport, or reduced efficacy of neurotrophic signaling. Many neurodegenerative diseases manifest with axonal transport problems (Krüttgen et al. 2003; Bronfman et al. 2007; Perlson et al. 2010; de Vos et al. 2008), and some neurological disorders have been associated with specific NTs (Shu and Mendell 1999; Bender 2004; Chao et al. 2006; Perlson et al. 2010; Hartmann et al. 2012). Abnormalities in neurotrophin signaling during formation of neural circuits may lead to disorganization of those circuits and predispose to neuropsychiatric disease during later life (Angelucci et al. 2005; Autry and Monteggia 2012). This section reviews current evidence for defects or abnormalities in NT or NT receptor trafficking for several key neurological disorders, including Alzheimer's disease, Huntington's disease, Parkinson's disease, Rett and fragile X syndromes, epilepsy, glaucoma, hyperalgesia, neuropathies, as well as psychiatric disorders such as schizophrenia and eating disorders.

6.1 Neurodegenerative Diseases

Alzheimer's disease. The hallmark of Alzheimer's disease is the degeneration of cholinergic basal forebrain neurons, among other cell types. These neurons are NGF dependent, and mouse models of Alzheimer's disease suggest that abnormalities in the retrograde axonal transport of NGF play a pathophysiological role (Cooper et al. 2001; Salehi et al. 2009). Increased levels of amyloid precursor protein (APP) appear to cause a specific NGF trafficking defect in cholinergic

basal forebrains in a mouse model (Salehi et al. 2006). Enhancing levels of NGF has been tested as a therapeutic approach in patients with early stage Alzheimer's and showed potential effectiveness (Tuszynski et al. 2005), but this phase 1 clinical trial also revealed some of the challenges in NT delivery to the brain (Tuszynski 2007).

Huntington's disease. In the heritable Huntington's disease, neurons degenerate in the basal ganglia, primarily the caudate/putamen. The key mutation of this disease is huntingtin, a protein that attaches to the molecular motor that moves cargo along microtubules, among other functions (Gauthier et al. 2004; Ross and Tabrizi 2011). Cortical neurons produce BDNF and transport it and other cargo molecules by anterograde axonal transport to the striatum (Charrin et al. 2005), but when huntingtin is mutated, the anterograde trafficking of BDNF to striatal synapses is abnormally reduced. Indeed, supply of exogenous BDNF can improve symptoms of Huntington's in animal models of the disease (Zuccato and Cattaneo 2007). Accordingly, this is a prime example of the physiological significance of a trafficked NT for disease progression and therapeutic improvement using targeted NTs. In other motor degenerative disease such as amyotrophic lateral sclerosis, trafficking of neurotrophic factors does not appear to be central to disease pathology (Marinkovic et al. 2012; Redler and Dokholyan 2012).

Parkinson's disease. The cause of Parkinson's disease is the degeneration of dopaminergic neurons in the substantia nigra, pars compacta. It is suspected that neurotrophic factors are involved, including NTs, but the precise cause and problem (expression, transport, receptors), susceptibility to toxic factors and cytokines, reduced levels of neurotrophins, and thus potential therapeutic strategies are not yet clear (Siegel and Chauhan 2000; Rangasamy et al. 2010).

6.2 Neurodevelopmental Disorders

Rett syndrome is a severe neurodevelopmental disorder with regressive cognitive and language skills, caused by a mutation in the MeCP gene. Brains of patients with Rett syndrome have reduced dendritic growth and spine densities in cortical neurons. Not only BDNF expression, but possibly also BDNF trafficking and secretion are affected (Zeev et al. 2009). Fragile X syndrome is another neurodevelopmental disorder, with impairment of BDNF vesicle docking and fusion (dense-core vesicle docking). Thus in both syndromes, BDNF expression, trafficking, and/or release are suspected to play key roles in the disease process (reviewed in Hartmann et al. 2012).

6.3 Other Disorders

Glaucoma. Glaucoma is characterized by progressive optic nerve degeneration and retinal ganglion cell death due to increased intraocular pressure. It has been shown that a major factor causing retinal ganglion cell death is the reduction in BDNF

retrograde axonal transport along the optic nerve fibers from central targets (Quigley et al. 2000; Almasieh et al. 2012). Thus, the sustained retrograde axonal trafficking of an NT is a major determinant of neuronal survival in the adult nervous system.

Epilepsy. Seizures induce BDNF mRNA upregulation that leads to increased BDNF production by the dentate granule cells, enhanced anterograde transport and release of BDNF from mossy fiber axons, and activation of either pre- or postsynaptic trkB receptors in the hilus and CA3 stratum lucidum (reviewed in Bender 2004).

Hyperalgesia and Charcot-Marie-Tooth neuropathy. The anterograde axonal transport of BDNF in DRG neurons affects pain thresholds (Shu and Mendell 1999). Several proteins controlling endosomal traffic have recently been shown to be affected in peripheral neuropathies. The small GTPase Rab7 regulates trkA trafficking (Saxena et al. 2005). Dynamin and Rabs affect the traffic of several different types of endosomes, but do not appear to be specific for endosomes containing NTs (Bronfman et al. 2007). Therefore, these traffic regulatory proteins likely affect multiple signaling systems.

Psychiatric diseases. Bipolar disorder, schizophrenia, eating disorders, and depression have been associated with BDNF (Angelucci et al. 2005). The BDNF hypothesis of neuropsychiatric diseases postulates that BDNF is required developmentally for normal circuit formation and also in the mature CNS to maintain plasticity and function. Diseases specifically linked to BDNF included eating disorder, depression, bipolar disorder, and schizophrenia (reviewed in Chao et al. 2006). A mouse model with a BDNF mutant (Egan et al. 2003) suggested that a relatively common BDNF mutation in humans causes anxiety and depression-like behavior (Chen et al. 2006). However, this simple hypothesis that a traffic-impaired BDNF mutant caused depression or anxiety did not hold up with further scrutiny and meta-analyses (Groves 2007; Frustaci et al. 2008; Verhagen et al. 2010). This does not call into question a fundamentally important role of BDNF in the pathophysiology and/or treatment of these diseases. BDNF's role appears well supported for eating disorders and bipolar disorder/schizophrenia, but the precise roles of BDNF need further clarification.

7 Conclusion

Trafficking of NTs is important in neuronal development, for neuronal plasticity, and in the adult nervous system. Ideal tagging of NTs is still hypothetical, and limitations of current tagging methods need to be taken into account. It has major consequences that NT trafficking can be altered by impairments of the molecular motors that move NTs along axons and in endosome traffic. Although the details of the pathophysiological mechanisms are still to be worked out, there is hope that therapeutic interventions can be designed to remove the toxic insult, halt the degenerative process, and preserve the remaining neurons in neurodegenerative disorders.

References

Adachi, N., Kohara, K., & Tsumoto, T. (2005). Difference in trafficking of brain-derived neurotrophic factor between axons and dendrites of cortical neurons, revealed by live-cell imaging. *BMC Neuroscience, 6*, 42.

Alderson, R. F., Curtis, R., Alterman, A. L., Lindsay, R. M., & DiStefano, P. S. (2000). Truncated TrkB mediates the endocytosis and release of BDNF and neurotrophin-4/5 by rat astrocytes and Schwann cells in vitro. *Brain Research, 871*, 210–222.

Almasieh, M., Wilson, A. M., Morquette, B., Cueva Vargas, J. L., & Di Polo, A. (2012). The molecular basis of retinal ganglion cell death in glaucoma. *Progress in Retinal and Eye Research, 31*, 152–181.

Altar, C. A., & DiStefano, P. S. (1998). Neurotrophin trafficking by anterograde transport. *Trends in Neurosciences, 21*, 433–437.

Altar, C. A., Cai, N., Bliven, T., Juhasz, M., Conner, J. M., Acheson, A. L., Lindsay, R. M., & Wiegand, S. J. (1997). Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature, 389*, 856–860.

Altick, A. L., Baryshnikova, L. M., Vu, T. Q., & von Bartheld, C. S. (2009). Quantitative analysis of multivesicular bodies (MVBs) in the hypoglossal nerve: evidence that neurotrophic factors do not use MVBs for retrograde axonal transport. *The Journal of Comparative Neurology, 514*, 641–657.

Androutsellis-Theotokis, A., McCormack, W. J., Bradford, H. F., Stern, G. M., & Pliego-Rivero, F. B. (1996). The depolarisation-induced release of [^{125}I]BDNF from brain tissue. *Brain Research, 743*, 40–48.

Angelucci, F., Brenè, S., & Mathé, A.A. (2005). BDNF in schizophrenia, depression and corresponding animal models. *Molecular Psychiatry, 10*, 345–352.

Appel, S. H. (1981). A unifying hypothesis for the cause of amyotrophic lateral sclerosis, parkinsonism, and Alzheimer disease. *Annals of Neurology, 10*, 499–505.

Ascaño, M., Richmond, A., Borden, P., & Kuruvilla, R. (2009). Axonal targeting of Trk receptors via transcytosis regulates sensitivity to neurotrophin responses. *Journal of Neuroscience, 29*, 11674–11685.

Autry, A. E., & Monteggia, L. M. (2012). Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacological Reviews, 64*, 238–258.

Balkowiec, A., & Katz, D. M. (2000). Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. *Journal of Neuroscience, 20*, 7417–7423.

Barbacid, M. (1994). The Trk family of neurotrophin receptors. *Journal of Neurobiology, 25*, 1386–1403.

Barker, P. A. (2007). High affinity not in the vicinity? *Neuron, 53*, 1–4.

Bender, D. K. (2004). The role of BDNF in epilepsy and other diseases of the mature nervous system. In D. K. Bender & H. E. Scharfman (Eds.), *Recent advances in epilepsy research* (pp. 34–56). New York: Kluwer Academic/Plenum Publishers.

Bhattacharyya, A., Watson, F. L., Bradlee, T. A., Pomeroy, S. L., Stiles, C. D., & Segal, R. A. (1997). Trk receptors function as rapid retrograde signal carriers in the adult nervous system. *Journal of Neuroscience, 17*, 7007–7016.

Biffo, S., Offenhäuser, N., Carter, B. D., & Barde, Y. A. (1995). Selective binding and internalisation by truncated receptors restrict the availability of BDNF during development. *Development, 121*, 2461–2470.

Blöchl, A., & Thoenen, H. (1995). Characterization of nerve growth factor (NGF) release from hippocampal neurons: evidence for a constitutive and an unconventional sodium-dependent regulated pathway. *European Journal of Neuroscience, 7*, 1220–1228.

Bothwell, M. (1995). Functional interactions of neurotrophins and neurotrophin receptors. *Annual Review of Neuroscience, 18*, 223–253.

Bronfman, F. C., Escudero, C. A., Weis, J., & Krüttgen, A. (2007). Endosomal transport of neurotrophins: roles in signaling and neurodegenerative diseases. *Developmental Neurobiology, 67*, 1183–1203.

Burgess, T. L., & Kelly, R. B. (1987). Constitutive and regulated secretion of proteins. *Annual Review of Cell Biology, 3*, 243–293.

Butowt, R., & von Bartheld, C. S. (2001). Sorting of internalized neurotrophins into an endocytic transcytosis pathway via the Golgi system: ultrastructural analysis in retinal ganglion cells. *Journal of Neuroscience, 21*, 8915–8930.

Butowt, R., & von Bartheld, C. S. (2005). Anterograde axonal transport of BDNF and NT-3 by retinal ganglion cells: roles of neurotrophin receptors. *Molecular and Cellular Neuroscience, 29*, 11–25.

Butowt, R., & von Bartheld, C. S. (2007). Conventional kinesin-I motors participate in the anterograde axonal transport of neurotrophins in the visual system. *Journal of Neuroscience Research, 85*, 2546–2556.

Butowt, R., & von Bartheld, C. S. (2009). Fates of neurotrophins after retrograde axonal transport: phosphorylation of p75NTR is a sorting signal for delayed degradation. *Journal of Neuroscience, 29*, 10715–10729.

Caleo, M., & Cenni, M. C. (2004). Anterograde transport of neurotrophic factors: possible therapeutic implications. *Molecular Neurobiology, 29*, 179–196.

Caleo, M., Medini, P., von Bartheld, C. S., & Maffei, L. (2003). Provision of brain-derived neurotrophic factor via anterograde transport from the eye preserves the physiological responses of axotomized geniculate neurons. *Journal of Neuroscience, 23*, 287–296.

Campenot, R. B. (1977). Local control of neurite development by nerve growth factor. *Proceedings of the National Academy of Sciences USA, 74*, 4516–4519.

Chao, M. V. (1994). The p75 neurotrophin receptor. *Journal of Neurobiology, 25*, 1373–1385.

Chao, M. V., Rajagopal, R., & Lee, F. S. (2006). Neurotrophin signalling in health and disease. *Clinical Science (London), 110*, 167–173.

Charrin, B. C., Saudou, F., & Humbert, S. (2005). Axonal transport failure in neurodegenerative disorders: the case of Huntington's disease. *Pathology Biology (Paris), 53*, 189–192.

Chen, Z. Y., Jing, D., Bath, K. G., Ieraci, A., Khan, T., Siao, C. J., Herrera, D. G., Toth, M., Yang, C., McEwen, B. S., Hempstead, B. L., & Lee, F. S. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science, 314*, 140–143.

Chowdary, P. D., Che, D. L., & Cui, B. (2012). Neurotrophin signaling via long-distance axonal transport. *Annual Review of Physical Chemistry, 63*, 571–594.

Claude, P., Hawrot, E., Dunis, D. A., & Campenot, R. B. (1982). Binding, internalization, and retrograde transport of ^{125}I-nerve growth factor in cultured rat sympathetic neurons. *Journal of Neuroscience, 2*, 431–442.

Cohen, M. S., Carlos, B. O., Kim, H. J., Jeon, N. L., & Jaffrey, S. R. (2011). Neurotrophin-mediated dendrite-to-nucleus signaling revealed by microfluidic compartmentalization of dendrites. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 11246–11251.

Conner, J. M. (2001). Localization of neurotrophin proteins within the central nervous system by immunohistochemistry. *Methods in Molecular Biology, 169*, 3–19.

Conner, J. M., Lauterborn, J. C., Yan, Q., Gall, C. M., & Varon, S. (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *Journal of Neuroscience, 17*, 2295–2313.

Conner, J. M., Lauterborn, J. C., & Gall, C. M. (1998). Anterograde transport of neurotrophin proteins in the CNS–a reassessment of the neurotrophic hypothesis. *Reviews in the Neurosciences, 9*, 91–103.

Cooper, J. D., Salehi, A., Delcroix, J. D., Howe, C. L., Belichenko, P. V., Chua-Couzens, J., Kilbridge, J. F., Carlson, E. J., Epstein, C. J., & Mobley, W. C. (2001). Failed retrograde transport of NGF in a mouse model of Down's syndrome: reversal of cholinergic

neurodegenerative phenotypes following NGF infusion. *Proceedings of the National Academy of Sciences USA, 98*, 10439–10444.

Cui, B., Wu, C., Chen, L., Ramirez, A., Bearer, E. L., Li, W. P., Mobley, W. C., & Chu, S. (2007). One at a time, live tracking of NGF axonal transport using quantum dots. *Proceedings of the National Academy of Sciences USA, 104*, 13666–13671.

Curtis, R., Adryan, K. M., Stark, J. L., Park, J. S., Compton, D. L., Weskamp, G., Huber, L. J., Chao, M. V., Jaenisch, R., Lee, K. F., Lindsay, R. M., & DiStefano, P. S. (1995). Differential role of the low affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins. *Neuron, 14*, 1201–1211.

De Vos, K. J., Grierson, A. J., Ackerley, S., & Miller, C. C. (2008). Role of axonal transport in neurodegenerative diseases. *Annual Review of Neuroscience, 31*, 151–173.

Dean, C., Liu, H., Staudt, T., Stahlberg, M. A., Vingill, S., Bückers, J., Kamin, D., Engelhardt, J., Jackson, M. B., Hell, S. W., & Chapman, E. R. (2012). Distinct subsets of Syt-IV/BDNF vesicles are sorted to axons versus dendrites and recruited to synapses by activity. *Journal of Neuroscience, 32*, 5398–5413.

Deinhardt, K., Salinas, S., Verastegui, C., Watson, R., Worth, D., Hanrahan, S., Bucci, C., & Schiavo, G. (2006). Rab5 and Rab7 control endocytic sorting along the axonal retrograde transport pathway. *Neuron, 52*, 293–305.

Delcroix, J. D., Valletta, J. S., Wu, C., Hunt, S. J., Kowal, A. S., & Mobley, W. C. (2003). NGF signaling in sensory neurons: evidence that early endosomes carry NGF retrograde signals. *Neuron, 39*, 69–84.

Di Fiore, P. P., & De Camilli, P. (2001). Endocytosis and signaling. An inseparable partnership. *Cell, 106*, 1–4.

Dieni, S., Matsumoto, T., Dekkers, M., Rauskolb, S., Ionescu, M. S., Deogracias, R., Gundelfinger, E. D., Kojima, M., Nestel, S., Frotscher, M., & Barde, Y. A. (2012). BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. *The Journal of Cell Biology, 196*, 775–788.

DiStefano, P. S., Friedman, B., Radziejewski, C., Alexander, C., Boland, P., Schick, C. M., Lindsay, R. M., & Wiegand, S. J. (1992). The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. *Neuron, 8*, 983–993.

Drake, C. T., Milner, T. A., & Patterson, S. L. (1999). Ultrastructural localization of full-length trkB immunoreactivity in rat hippocampus suggests multiple roles in modulating activity-dependent synaptic plasticity. *Journal of Neuroscience, 19*, 8009–8026.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Weinberger, D. R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257–269.

Ehlers, M. D., Kaplan, D. R., Price, D. L., & Koliatsos, V. E. (1995). NGF-stimulated retrograde transport of trkA in the mammalian nervous system. *The Journal of Cell Biology, 130*, 149–156.

Esposito, D., Patel, P., Stephens, R. M., Perez, P., Chao, M. V., Kaplan, D. R., & Hempstead, B. L. (2001). The cytoplasmic and transmembrane domains of the p75 and Trk A receptors regulate high affinity binding to nerve growth factor. *Journal of Biological Chemistry, 276*, 32687–32695.

Farhadi, H. F., Mowla, S. J., Petrecca, K., Morris, S. J., Seidah, N. G., & Murphy, R. A. (2000). Neurotrophin-3 sorts to the constitutive secretory pathway of hippocampal neurons and is diverted to the regulated secretory pathway by coexpression with brain-derived neurotrophic factor. *Journal of Neuroscience, 20*, 4059–4068.

Fawcett, J. P., Aloyz, R., McLean, J. H., Pareek, S., Miller, F. D., McPherson, P. S., & Murphy, R. A. (1997). Detection of brain-derived neurotrophic factor in a vesicular fraction of brain synaptosomes. *Journal of Biological Chemistry, 272*, 8837–8840.

Feng, C. Y., Wiggins, L. M., & von Bartheld, C. S. (2011). The locus ceruleus responds to signaling molecules obtained from the CSF by transfer through tanycytes. *Journal of Neuroscience, 31*, 9147–9158.

Frustaci, A., Pozzi, G., Gianfagna, F., Manzoli, L., & Boccia, S. (2008). Meta-analysis of the brain-derived neurotrophic factor gene (BDNF) Val66Met polymorphism in anxiety disorders and anxiety-related personality traits. *Neuropsychobiology, 58*, 163–170.

Gärtner, A., & Staiger, V. (2002). Neurotrophin secretion from hippocampal neurons evoked by long-term-potentiation-inducing electrical stimulation patterns. *Proceedings of the National Academy of Sciences USA, 99*, 6386–6391.

Gärtner, A., Shostak, Y., Hackel, N., Ethell, I. M., & Thoenen, H. (2000). Ultrastructural identification of storage compartments and localization of activity-dependent secretion of neurotrophin 6 in hippocampal neurons. *Molecular and Cellular Neuroscience, 15*, 215–234.

Gauthier, L. R., Charrin, B. C., Borrell-Pagès, M., Dompierre, J. P., Rangone, H., Cordelières, F. P., De Mey, J., MacDonald, M. E., Lessmann, V., Humbert, S., & Saudou, F. (2004). Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell, 118*, 127–138.

Gerdes, H. H., & Kaether, C. (1996). Green fluorescent protein: applications in cell biology. *FEBS Letters, 389*, 44–47.

Ghosh, A., Carnahan, J., & Greenberg, M. E. (1994). Requirement for BDNF in activity-dependent for BDNF in activity-dependent survival of cortical neurons. *Science, 263*, 1618–1623.

Giepmans, B. N., Deerinck, T. J., Smarr, B. L., Jones, Y. Z., & Ellisman, M. H. (2005). Correlated light and electron microscopic imaging of multiple endogenous proteins using Quantum dots. *Nature Methods, 2*, 743–749.

Ginty, D. D., & Segal, R. A. (2002). Retrograde neurotrophin signaling: Trk-ing along the axon. *Current Opinion in Neurobiology, 12*, 268–274.

Goldstein, L. S., & Yang, Z. (2000). Microtubule-based transport systems in neurons: the roles of kinesins and dyneins. *Annual Review of Neuroscience, 23*, 39–71.

Goodman, L. J., Valverde, J., Lim, F., Geschwind, M. D., Federoff, H. J., Geller, A. I., & Hefti, F. (1996). Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. *Molecular and Cellular Neuroscience, 7*, 222–238.

Greenberg, M. E., Xu, B., Lu, B., & Hempstead, B. L. (2009). New insights in the biology of BDNF synthesis and release: implications in CNS function. *Journal of Neuroscience, 29*, 12764–12767.

Griesbeck, O., Canossa, M., Campana, G., Gärtner, A., Hoener, M. C., Nawa, H., Kolbeck, R., & Thoenen, H. (1999). Are there differences between the secretion characteristics of NGF and BDNF? Implications for the modulatory role of neurotrophins in activity-dependent neuronal plasticity. *Microscopy Research and Technique, 45*, 262–275.

Groves, J. O. (2007). Is it time to reassess the BDNF hypothesis of depression? *Molecular Psychiatry, 12*, 1079–1088.

Guillemot, F., Cerutti, I., Auffray, C., & Devignes, M. D. (2007). A transgenic mouse model engineered to investigate human brain-derived neurotrophic factor in vivo. *Transgenic Research, 16*, 223–237.

Hartmann, M., Heumann, R., & Lessmann, V. (2001). Synaptic secretion of BDNF after high-frequency stimulation of glutamatergic synapses. *EMBO Journal, 20*, 5887–5897.

Hartmann, D., Drummond, J., Handberg, E., Ewell, S., & Pozzo-Miller, L. (2012). Multiple approaches to investigate the transport and activity-dependent release of BDNF and their application in neurogenetic disorders. *Neural Plasticity, 2012*, 203734.

Haubensak, W., Narz, F., Heumann, R., & Lessmann, V. (1998). BDNF-GFP containing secretory granules are localized in the vicinity of synaptic junctions of cultured cortical neurons. *Journal of Cell Science, 111*, 1483–1493.

Heerssen, H. M., Pazyra, M. F., & Segal, R. A. (2004). Dynein motors transport activated Trks to promote survival of target-dependent neurons. *Nature Neuroscience, 7*, 596–604.

Heerssen, H.M., & Segal, R.A. (2002). Location, location, location: a spatial view of neurotrophin signal transduction. *Trends in Neuroscience, 25*, 160–165.

Hendry, I. A., Stöckel, K., Thoenen, H., & Iversen, L. L. (1974). The retrograde axonal transport of nerve growth factor. *Brain Research, 68*, 103–121.

Hibbert, A. P., Kramer, B. M., Miller, F. D., & Kaplan, D. R. (2006). The localization, trafficking and retrograde transport of BDNF bound to p75NTR in sympathetic neurons. *Molecular and Cellular Neuroscience, 32*, 387–402.

Hirokawa, N. (1998). Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science, 279*, 519–526.

Howe, C. L., & Mobley, W. C. (2004). Signaling endosome hypothesis: a cellular mechanism for long distance communication. *Journal of Neurobiology, 58*, 207–216.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. *Annual Review of Neuroscience, 24*, 677–736.

Huang, E. J., & Reichardt, L. F. (2003). Trk receptors: roles in neuronal signal transduction. *Annual Review of Biochemistry, 72*, 609–642.

Huang, S. H., Duan, S., Sun, T., Wang, J., Zhao, L., Geng, Z., Yan, J., Sun, H. J., & Chen, Z. Y. (2011). JIP3 mediates TrkB axonal anterograde transport and enhances BDNF signaling by directly bridging TrkB with kinesin-1. *Journal of Neuroscience, 31*, 10602–10614.

Johnson, E. M., Jr., Andres, R. Y., & Bradshaw, R. A. (1978). Characterization of the retrograde transport of nerve growth factor (NGF) using high specific activity [^{125}I]NGF. *Brain Research, 150*, 319–331.

Johnson, E. M., Jr., Taniuchi, M., Clark, H. B., Springer, J. E., Koh, S., Tayrien, M. W., & Loy, R. (1987). Demonstration of the retrograde transport of nerve growth factor receptor in the peripheral and central nervous system. *Journal of Neuroscience, 7*, 923–929.

Johnson, F., Hohmann, S. E., DiStefano, P. S., & Bottjer, S. W. (1997). Neurotrophins suppress apoptosis induced by deafferentation of an avian motor-cortical region. *Journal of Neuroscience, 17*, 2101–2111.

Johanson, S. O., Crouch, M. F., & Hendry, I. A. (1996). Signal transduction from membrane to nucleus: the special case for neurons. *Neurochemical Research, 21*, 779–785.

Kanning, K. C., Hudson, M., Amieux, P. S., Wiley, J. C., Bothwell, M., & Schecterson, L. C. (2003). Proteolytic processing of the p75 neurotrophin receptor and two homologs generates C-terminal fragments with signaling capability. *Journal of Neuroscience, 23*, 5425–5436.

Kao, S., Jaiswal, R. K., Kolch, W., & Landreth, G. E. (2001). Identification of the mechanisms regulating the differential activation of the mapk cascade by epidermal growth factor and nerve growth factor in PC12 cells. *Journal of Biological Chemistry, 276*, 18169–18177.

Kohara, K., Kitamura, A., Morishima, M., & Tsumoto, T. (2001). Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. *Science, 291*, 2419–2423.

Kojima, M., Takei, N., Numakawa, T., Ishikawa, Y., Suzuki, S., Matsumoto, T., Katoh-Semba, R., Nawa, H., & Hatanaka, H. (2001). Biological characterization and optical imaging of brain-derived neurotrophic factor-green fluorescent protein suggest an activity-dependent local release of brain-derived neurotrophic factor in neurites of cultured hippocampal neurons. *Journal of Neuroscience Research, 64*, 1–10.

Krüttgen, A., Saxena, S., Evangelopoulos, M. E., & Weis, J. (2003). Neurotrophins and neurodegenerative diseases: receptors stuck in traffic? *Journal of Neuropathology and Experimental Neurology, 62*, 340–350.

Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*, 1945–1948.

Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H., & Barde, Y. A. (1989). Molecular cloning and expression of brain-derived neurotrophic factor. *Nature, 341*, 149–152.

Lessmann, V., Gottmann, K., & Malcangio, M. (2003). Neurotrophin secretion: Current facts and future prospects. *Progress in Neurobiology, 69*, 341–374.

Lever, I. J., Bradbury, E. J., Cunningham, J. R., Adelson, D. W., Jones, M. G., McMahon, S. B., Marvizón, J. C., & Malcangio, M. (2001). Brain-derived neurotrophic factor is released in the dorsal horn by distinctive patterns of afferent fiber stimulation. *Journal of Neuroscience, 21*, 4469–4477.

Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. *Science, 237*, 1154–1162.
Linden, R. (1994). The survival of developing neurons: A review of afferent control. *Neuroscience, 58*, 671–682.
Lu, B., Pang, P. T., & Woo, N. H. (2005). The yin and yang of neurotrophin action. *Nature Reviews Neuroscience, 6*, 603–614.
Luo, X. G., Rush, R. A., & Zhou, X. F. (2001). Ultrastructural localization of brain-derived neurotrophic factor in rat primary sensory neurons. *Neuroscience Research, 39*, 377–384.
MacInnis, B. L., & Campenot, R. B. (2002). Retrograde support of neuronal survival without retrograde transport of nerve growth factor. *Science, 295*, 1536–1539.
Marinkovic, P., Reuter, M. S., Brill, M. S., Godinho, L., Kerschensteiner, M., & Misgeld, T. (2012). Axonal transport deficits and degeneration can evolve independently in mouse models of amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences USA, 109*, 4296–4301.
Matsumoto, T., Rauskolb, S., Polack, M., Klose, J., Kolbeck, R., Korte, M., & Barde, Y. A. (2008). Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. *Nature Neuroscience, 11*, 131–133.
Merighi, A. (2002). Costorage and coexistence of neuropeptides in the mammalian CNS. *Progress in Neurobiology, 66*, 161–190.
Michael, G. J., Averill, S., Nitkunan, A., Rattray, M., Bennett, D. L., Yan, Q., & Priestley, J. V. (1997). Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. *Journal of Neuroscience, 17*, 8476–8490.
Miller, F. D., & Kaplan, D. R. (2001). On Trk for retrograde signaling. *Neuron, 32*, 767–770.
Moises, T., Dreier, A., Flohr, S., Esser, M., Brauers, E., Reiss, K., Merken, D., Weis, J., & Krüttgen, A. (2007). Tracking TrkA's trafficking: NGF receptor trafficking controls NGF receptor signaling. *Molecular Neurobiology, 35*, 151–159.
Möller, J. C., Krüttgen, A., Heymach, J. V., Jr., Ghori, N., & Shooter, E. M. (1998). Subcellular localization of epitope-tagged neurotrophins in neuroendocrine cells. *Journal of Neuroscience Research, 51*, 463–472.
Mowla, S. J., Pareek, S., Farhadi, H. F., Petrecca, K., Fawcett, J. P., Seidah, N. G., Morris, S. J., Sossin, W. S., & Murphy, R. A. (1999). Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. *Journal of Neuroscience, 19*, 2069–2080.
Mowla, S. J., Farhadi, H. F., Pareek, S., Atwal, J. K., Morris, S. J., Seidah, N. G., & Murphy, R. A. (2001). Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *Journal of Biological Chemistry, 276*, 12660–12666.
Mufson, E. J., Kroin, J. S., Sendera, T. J., & Sobreviela, T. (1999). Distribution and retrograde transport of trophic factors in the central nervous system: Functional implications for the treatment of neurodegenerative diseases. *Progress in Neurobiology, 57*, 451–484.
Mukherjee, S., Ghosh, R. N., & Maxfield, F. R. (1997). Endocytosis. *Physiological Reviews, 77*, 759–803.
Neet, K. E., & Campenot, R. B. (2001). Receptor binding, internalization, and retrograde transport of neurotrophic factors. *Cellular and Molecular Life Sciences, 58*, 1021–1035.
Oppenheim, R. W. (1996). The concept of uptake and retrograde transport of neurotrophic molecules during development: History and present status. *Neurochemical Research, 21*, 769–777.
Oppenheim, R. W., & von Bartheld, C. S. (2008). Programmed cell death and neurotrophic factors. In L. Squire (Ed.), *Fundamental neuroscience* (3rd ed., pp. 437–467). San Diego: Elsevier.
Parak, W. J., Pellegrino, T., & Plank, C. (2005). Labelling of cells with quantum dots. *Nanotechnology, 16*, R9–R25.
Parton, R. G., Simons, K., & Dotti, C. G. (1992). Axonal and dendritic endocytic pathways in cultured neurons. *The Journal of Cell Biology, 119*, 123–137.
Perlson, E., Maday, S., Fu, M. M., Moughamian, A. J., & Holzbaur, E. L. (2010). Retrograde axonal transport: Pathways to cell death? *Trends in Neurosciences, 33*, 335–344.

Philippidou, P., Valdez, G., Akmentin, W., Bowers, W. J., Federoff, H. J., & Halegoua, S. (2011). Trk retrograde signaling requires persistent, Pincher-directed endosomes. *Proceedings of the National Academy of Sciences USA, 108*, 852–857.

Poo, M. M. (2001). Neurotrophins as synaptic modulators. *Nature Reviews Neuroscience, 2*, 24–32.

Purves, D. (1988). *Body and brain. A trophic theory of neural connections* (pp. 1–231). Cambridge: Harvard University Press.

Quigley, H. A., McKinnon, S. J., Zack, D. J., Pease, M. E., Kerrigan-Baumrind, L. A., Kerrigan, D. F., & Mitchell, R. S. (2000). Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. *Investigative Ophthalmology and Visual Science, 41*, 3460–3466.

Rajan, S. S., Liu, H. Y., & Vu, T. Q. (2008). Ligand-bound quantum dot probes for studying the molecular scale dynamics of receptor endocytic trafficking in live cells. *ACS Nano, 2*, 1153–1166.

Rangasamy, S. B., Soderstrom, K., Bakay, R. A., & Kordower, J. H. (2010). Neurotrophic factor therapy for Parkinson's disease. *Progress in Brain Research, 184*, 237–264.

Redler, R. L., & Dokholyan, N. V. (2012). The complex molecular biology of amyotrophic lateral sclerosis (ALS). *Progress in Molecular Biology and Translational Science, 107*, 215–262.

Reynolds, A. J., Bartlett, S. E., & Hendry, I. A. (2000). Molecular mechanisms regulating the retrograde axonal transport of neurotrophins. *Brain Research Reviews, 33*, 169–178.

Riccio, A., Pierchala, B. A., Ciarallo, C. L., & Ginty, D. D. (1997). An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. *Science, 277*, 1097–1100.

Rind, H. B., Butowt, R., & von Bartheld, C. S. (2005). Synaptic targeting of retrogradely transported trophic factors in motoneurons: Comparison of glial cell line-derived neurotrophic factor, brain-derived neurotrophic factor, and cardiotrophin-1 with tetanus toxin. *Journal of Neuroscience, 25*, 539–549.

Rodriguez-Tébar, A., & Barde, Y. A. (1988). Binding characteristics of brain-derived neurotrophic factor to its receptors on neurons from the chick embryo. *Journal of Neuroscience, 8*, 3337–3342.

Rosenberg, M. B., Hawrot, E., & Breakefield, X. O. (1986). Receptor binding activities of biotinylated derivatives of beta-nerve growth factor. *Journal of Neurochemistry, 46*, 641–648.

Rosenfeld, R., Philo, J. S., Haniu, M., Stoney, K., Rohde, M. F., Wu, G. M., Narhi, L. O., Wong, C., Boone, T., Hawkins, N. N., Miller, J. M., & Arakawa, T. (1993). Sites of iodination in recombinant human brain-derived neurotrophic factor and its effect on neurotrophic activity. *Protein Science, 2*, 1664–1674.

Ross, C. A., & Tabrizi, S. J. (2011). Huntington's disease: From molecular pathogenesis to clinical treatment. *Lancet Neurology, 10*, 83–98.

Ross, A. H., Lachyankar, M. B., Poluha, D. K., & Loy, R. (1994). Axonal transport of the trkA high-affinity NGF receptor. *Progress in Brain Research, 103*, 15–21.

Rush, R. A. (2001). Neurotrophin immuno-histochemistry: an unrealized potential. In I. Mocchetti (Ed.), *Neurobiology of the neurotrophins* (pp. 101–117). Johnson City: FP Graham Publishing.

Salehi, A., Delcroix, J. D., Belichenko, P. V., Zhan, K., Wu, C., Valletta, J. S., Takimoto-Kimura, R., Kleschevnikov, A. M., Sambamurti, K., Chung, P. P., Xia, W., Villar, A., Campbell, W. A., Kulnane, L. S., Nixon, R. A., Lamb, B. T., Epstein, C. J., Stokin, G. B., Goldstein, L. S., & Mobley, W. C. (2006). Increased App expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. *Neuron, 51*, 29–42.

Salehi, A., Wu, C., Zhan, K., & Mobley, W. C. (2009). Axonal transport of neurotrophic signals: An Achilles' heel for neurodegeneration? *Intracellular Traffic and Neurodegenerative Disorders, 2009*, 87–101.

Salio, C., Averill, S., Priestley, J. V., & Merighi, A. (2007). Costorage of BDNF and neuropeptides within individual dense-core vesicles in central and peripheral neurons. *Developmental Neurobiology, 67*, 326–338.

Sandow, S. L., Heydon, K., Weible, M. W., 2nd, Reynolds, A. J., Bartlett, S. E., & Hendry, I. A. (2000). Signalling organelle for retrograde axonal transport of internalized neurotrophins from the nerve terminal. *Immunology and Cell Biology, 78*, 430–435.

Santi, S., Cappello, S., Riccio, M., Bergami, M., Aicardi, G., Schenk, U., Matteoli, M., & Canossa, M. (2006). Hippocampal neurons recycle BDNF for activity-dependent secretion and LTP maintenance. *EMBO Journal, 25*, 4372–4380.

Saper, C. B., Wainer, B. H., & German, D. C. (1987). Axonal and transneuronal transport in the transmission of neurological disease: Potential role in system degenerations, including Alzheimer's disease. *Neuroscience, 23*, 389–398.

Saxena, S., Bucci, C., Weis, J., & Krüttgen, A. (2005). The small GTPase Rab7 controls the endosomal trafficking and neuritogenic signaling of the nerve growth factor receptor TrkA. *Journal of Neuroscience, 25*, 10930–10940.

Senger, D. L., & Campenot, R. B. (1997). Rapid retrograde tyrosine phosphorylation of trkA and other proteins in rat sympathetic neurons in compartmented cultures. *The Journal of Cell Biology, 138*, 411–421.

Sharma, N., Deppmann, C. D., Harrington, A. W., St Hillaire, C., Chen, Z. Y., Lee, F. S., & Ginty, D. D. (2010). Long-distance control of synapse assembly by target-derived NGF. *Neuron, 67*, 422–434.

Shu, X. Q., & Mendell, L. M. (1999). Neurotrophins and hyperalgesia. *Proceedings of the National Academy of Sciences USA, 96*, 7693–7696.

Siegel, G. J., & Chauhan, N. B. (2000). Neurotrophic factors in Alzheimer's and Parkinson's disease brain. *Brain Research Reviews, 33*, 199–227.

Sorkin, A., & Von Zastrow, M. (2002). Signal transduction and endocytosis: Close encounters of many kinds. *Nature Reviews Molecular Cell Biology, 3*, 600–614.

Stoeckel, K., Schwab, M., & Thoenen, H. (1975). Specificity of retrograde transport of nerve growth factor (NGF) in sensory neurons: A biochemical and morphological study. *Brain Research, 89*, 1–14.

Tekle, C., Deurs, B., Sandvig, K., & Iversen, T. G. (2008). Cellular trafficking of quantum dot-ligand bioconjugates and their induction of changes in normal routing of unconjugated ligands. *Nano Letters, 8*, 1858–1865.

Thoenen, H., & Barde, Y. A. (1980). Physiology of nerve growth factor. *Physiological Reviews, 60*, 1284–1335.

Torrealba, F., & Carrasco, M. A. (2004). A review on electron microscopy and neurotransmitter systems. *Brain Research Reviews, 47*, 5–17.

Tuszynski, M. H. (2007). Nerve growth factor gene therapy in Alzheimer disease. *Alzheimer Disease and Associated Disorders, 21*, 179–189.

Tuszynski, M. H., Thal, L., Pay, M., Salmon, D. P., U, H. S., Bakay, R., Patel, P., Blesch, A., Vahlsing, H. L., Ho, G., Tong, G., Potkin, S. G., Fallon, J., Hansen, L., Mufson, E. J., Kordower, J. H., Gall, C., & Conner, J. (2005). A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nature Medicine, 11*, 551–555.

Vaegter, C. B., Jansen, P., Fjorback, A. W., Glerup, S., Skeldal, S., Kjolby, M., Richner, M., Erdmann, B., Nyengaard, J. R., Tessarollo, L., Lewin, G. R., Willnow, T. E., Chao, M. V., & Nykjaer, A. (2011). Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. *Nature Neuroscience, 14*, 54–61.

Valdez, G., Akmentin, W., Philippidou, P., Kuruvilla, R., Ginty, D. D., & Halegoua, S. (2005). Pincher-mediated macroendocytosis underlies retrograde signaling by neurotrophin receptors. *Journal of Neuroscience, 25*, 5236–5247.

Vale, R. D., & Shooter, E. M. (1985). Assaying binding of nerve growth factor to cell surface receptors. *Methods in Enzymology, 109*, 21–39.

Vallee, R. B., Williams, J. C., Varma, D., & Barnhart, L. E. (2004). Dynein: An ancient motor protein involved in multiple modes of transport. *Journal of Neurobiology, 58*, 189–200.

Verhagen, M., van der Meij, A., van Deurzen, P. A., Janzing, J. G., Arias-Vásquez, A., Buitelaar, J. K., & Franke, B. (2010). Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: Effects of gender and ethnicity. *Molecular Psychiatry, 15*, 260–271.

von Bartheld, C. S. (2001). Tracing with radiolabeled neurotrophins. *Methods in Molecular Biology, 169*, 195–216.

von Bartheld, C. S. (2004). Axonal transport and neuronal transcytosis of trophic factors, tracers, and pathogens. *Journal of Neurobiology, 58*, 295–314.

von Bartheld, C. S., & Altick, A. L. (2011). Multivesicular bodies in neurons: Distribution, protein content, and trafficking functions. *Progress in Neurobiology, 93*, 313–340.

von Bartheld, C. S., Williams, R., Lefcort, F., Clary, D. O., Reichardt, L. F., & Bothwell, M. (1996a). Retrograde transport of neurotrophins from the eye to the brain in chick embryos: Roles of the p75NTR and trkB receptors. *Journal of Neuroscience, 16*, 2995–3008.

von Bartheld, C. S., Byers, M. R., Williams, R., & Bothwell, M. (1996b). Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system. *Nature, 379*, 830–833.

von Bartheld, C. S., Wang, X. X., & Butowt, R. (2001). Anterograde axonal transport, transcytosis, and recycling of neurotrophic factors: The concept of trophic currencies in neural networks. *Molecular Neurobiology, 24*, 1–28.

Vu, T. Q., Maddipati, R., Blute, T. A., Nehilla, B. J., Nusblat, L., & Desai, T. A. (2005). Peptide-conjugated quantum dots activate neuronal receptors and initiate downstream signaling of neurite growth. *Nano Letters, 5*, 603–607.

Wang, X. X., Butowt, R., Vasko, M. R., & von Bartheld, C. S. (2002). Mechanisms of the release of anterogradely transported neurotrophin-3 from axon terminals. *Journal of Neuroscience, 22*, 931–945.

Wang, H., Wu, L. L., Song, X. Y., Luo, X. G., Zhong, J. H., Rush, R. A., & Zhou, X. F. (2006). Axonal transport of BDNF precursor in primary sensory neurons. *European Journal of Neuroscience, 24*, 2444–2452.

Watson, F. L., Heerssen, H. M., Moheban, D. B., Lin, M. Z., Sauvageot, C. M., Bhattacharyya, A., Pomeroy, S. L., & Segal, R. A. (1999). Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex. *Journal of Neuroscience, 19*, 7889–7900.

Watson, F. L., Heerssen, H. M., Bhattacharyya, A., Klesse, L., Lin, M. Z., & Segal, R. A. (2001). Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. *Nature Neuroscience, 4*, 981–988.

Wehrman, T., He, X., Raab, B., Dukipatti, A., Blau, H., & Garcia, K. C. (2007). Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors. *Neuron, 53*, 25–38.

Weible, M. W., 2nd, & Hendry, I. A. (2004). What is the importance of multivesicular bodies in retrograde axonal transport in vivo? *Journal of Neurobiology, 58*, 230–243.

Wiggins, L. M., Altick, A. L., Vermehren-Schmaedick, A., Vu, T. Q., & von Bartheld, C. S. (2012). Trafficking of quantum-dot-tagged neurotrophic factors in vivo: ultrastructural evidence for altered endocytic routes after axonal transport. Society for Neuroscience Abstracts, #738.06.

Wu, C., Lai, C. F., & Mobley, W. C. (2001). Nerve growth factor activates persistent Rap1 signaling in endosomes. *Journal of Neuroscience, 21*, 5406–5416.

Wu, Y. J., Krüttgen, A., Möller, J. C., Shine, D., Chan, J. R., Shooter, E. M., & Cosgaya, J. M. (2004). Nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 are sorted to dense-core vesicles and released via the regulated pathway in primary rat cortical neurons. *Journal of Neuroscience Research, 75*, 825–834.

Wu, C., Cui, B., He, L., Chen, L., & Mobley, W. C. (2009). The coming of age of axonal neurotrophin signaling endosomes. *Journal of Proteomics, 72*, 46–55.

Xie, W., Zhang, K., & Cui, B. (2012). Functional characterization and axonal transport of quantum dot labeled BDNF. *Integrative Biology (Cambridge), 4*, 953–960.

Yang, J., Siao, C. J., Nagappan, G., Marinic, T., Jing, D., McGrath, K., Chen, Z. Y., Mark, W., Tessarollo, L., Lee, F. S., Lu, B., & Hempstead, B. L. (2009). Neuronal release of proBDNF. *Nature Neuroscience, 12*, 113–115.

Yano, H., & Chao, M. V. (2004). Mechanisms of neurotrophin receptor vesicular transport. *Journal of Neurobiology, 58*, 244–257.

Yano, H., Lee, F. S., Kong, H., Chuang, J., Arevalo, J., Perez, P., Sung, C., & Chao, M. V. (2001). Association of Trk neurotrophin receptors with components of the cytoplasmic dynein motor. *Journal of Neuroscience, 21*, RC125.

Zeev, B. B., Bebbington, A., Ho, G., Leonard, H., de Klerk, N., Gak, E., Vecsler, M., & Christodoulou, J. (2009). The common BDNF polymorphism may be a modifier of disease severity in Rett syndrome. *Neurology, 72*, 1242–1247.

Zhou, X. F., & Rush, R. A. (1996). Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. *Neuroscience, 74*, 945–953.

Zuccato, C., & Cattaneo, E. (2007). Role of brain-derived neurotrophic factor in Huntington's disease. *Progress in Neurobiology, 2007*(81), 294–330.

Zweifel, L. S., Kuruvilla, R., & Ginty, D. D. (2005). Functions and mechanisms of retrograde neurotrophin signalling. *Nature Reviews Neuroscience, 6*, 615–625.

Part V

Diseases and Disorders Relevant to Neurotoxins

Drug Abuse Neurotoxicity: Alcohol and Nicotine as Developmental Stressors

Trevor Archer, Serafino Ricci, and Max Rapp-Ricciardi

Contents

Abstract

Drugs of abuse have the property of inducing adverse health complications, not least neurotoxicity under conditions where both the environmental conditions and activity states associated with their intake may strongly enhance drug toxicity, thereby causing life-threatening health complications and tragedy for relations and caregivers. While both chronic alcohol and/or nicotine abuse induce a variety of neuropathological effects, including damage to the brain, the extent of damage and disruption observed in the developing brain and CNS is a considerable affliction for the affected individuals. On the basis of laboratory and clinical studies, the potential of chemicals, including therapeutic and abused agents, to induce neurotoxic effects has been assessed, with considerations of abuse drugs neurotoxicity encompassing several factors that may accelerate and complicate prevailing conditions: the type and influence of environmental

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conditions, the presence of daily habits such as coffee breaks/smoking breaks, nutritional status, and neuroimmune system mobilization. Abuse neurotoxicity at several stages of early development, alcohol neurotoxicity, nicotine neurotoxicity, and combinations of alcohol-nicotine neurotoxicity present a threatening scenario of two compounds, benefitting from legality and availability that nevertheless have such potential for destruction over multiple domains, particularly in the undeveloped brain.

Keywords
Abuse drugs • Alcohol • Apoptosis • Development • Nicotine • Oxidative stress • Smoking

1 Introduction

The potential of chemicals, including therapeutic and abused agents, to induce neurotoxic effects may be assessed at (i) early developmental, (ii) adolescent/preadolescent, and (iii) adult stages of individuals' life cycle. In each case, the level of neurotoxicity or neurotoxic propensity remains a singularly important regulatory drug issue and a uniquely significant area of public concern, not least due to the preponderance of agents that have been characterized or are still to be characterized (Judson et al. 2009) due to logistical constraints (OECD 2007). Normal brain development follows a preprogrammed pathway, whether at neuronal, circuitry, or regional levels, that differentiates specific and distinctive regional structural characteristics, phenotypes, and functions in the infant, adolescent, and adult (Bayer et al. 1993; Herschkowitz et al. 1997). The influence of epigenetic factors (e.g., mutant strains), of evermore critical importance (Dufault et al. 2012; Marti et al. 2007), reflects the sensitivity and responsiveness of human and animal brains in constantly changing circumstances regulating gene expression profiles (Archer et al. 2011). The course of brain development may be altered by a wide range of exogenous agents thereby threatening the integrity of affected brains with permanent consequences (Stanwood and Levitt 2004) that culminate in dire neurodevelopmental (Llop et al. 2012) and neuropathophysiological (Miller et al. 2012) outcomes. The enormous range of exogenous agents capable of disrupting the integrity of the affected brain, within defined parameters and conditions, may wreak damage that is all too often permanent and irreversible (Archer 2011). In order to confront the neurotoxic propensities of abused drugs, alcohol and nicotine (smoking), and their combination, are chosen as "drug cases" for several reasons, not least the situation that both are "legal drugs" judicially in most countries. Alcohol presents the greatest social and monetary chemical risk to individuals' well-being and health, and alcoholism is a multifactorial, genetically associated disorder with major negative issues, highly frequent and a strong cause of premature death.

Considerations of abuse drugs neurotoxicity must take into account several factors that may accelerate and complicate prevailing conditions: the type and influence of environmental conditions, the presence of daily habits such as coffee

breaks/smoking breaks, nutritional status, and neuroimmune system mobilization. It ought to be borne in mind that the environmental conditions and activity states associated with their intake are likely to enhance markedly drug toxicity levels, thereby developing a situation wherein continued abuse leads not only to adverse health complications, including neurotoxicity, but also to life-threatening health complications. For example, animal experiments indicate that even moderate increases in environmental temperatures and physiological activation, the conditions typical of the so-called "rave parties," dramatically potentiate brain hyperthermic effects of psychomotor stimulants, particularly methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA). Kiyatkin and Sharma (2012) have demonstrated that methamphetamine also induces breakdown of the blood–brain barrier, acute glial activation, brain edema, and structural abnormalities of various subtypes of brain cells. These effects are also strongly enhanced when the drug is used at moderately warm environmental conditions. They implied that the mechanisms underlying environmental modulation of acute drug neurotoxicity focus on the role of brain temperature, a critical homeostatic parameter that could be affected by metabolism-enhancing drugs and environmental conditions and affect neural activity and functions.

Another important consideration is the concomitant consumption of caffeine, i.e., daily coffee intake and habitual behavior, with recreational psychostimulant drugs of abuse, may provoke varyingly severe acute adverse reactions in addition to longer-term consequences since the mechanisms by which caffeine increases the toxicity of psychostimulants include changes to body temperature regulation, cardiotoxicity, and lowering of the seizure threshold. Vanattou-Saïfoudine et al. (2012) have reviewed current notions of such caffeine-related drug interactions, placing a particular emphasis on an adverse interaction between caffeine and the substituted amphetamine MDMA which has been most recently described and characterized. The coadministration of caffeine and MDMA profoundly enhances the acute toxicity of MDMA in rats, as manifested by high core body temperature, tachycardia, and increased mortality. In addition, coadministration of caffeine enhances the long-term serotonergic neurotoxicity induced by MDMA. Their studies coincide with reports of caffeine-related drug interactions with cocaine, d-amphetamine, and ephedrine where similar mechanisms are implicated. Finally, it is an unpleasant reality that neurotoxic actions of chemical agents on humans and animals are usually studied with little consideration of the subject's nutritional status. The status of protein-calorie, vitamin, and/or mineral undernutrition levels and contingencies are linked with a range of neurodevelopmental, neurological, and psychiatric disorders, commonly with involvement of both the central and the peripheral nervous system. Undernutrition can modify risk for certain chemical-induced neurologic diseases, and in some cases, undernutrition may be a prerequisite for neurotoxicity to surface. Spencer and Palmer (2012) have observed that combined effects of undernutrition and chemical neurotoxicity are most relevant to people in disaster areas or those with low incomes who experience, and continue to experience, chronic hunger, parasitism, and infectious disease; monotonous diets of plants with neurotoxic potential (notably cassava);

environmental pollution from rapid industrial development; chronic alcohol abuse; or prolonged treatment with certain therapeutic drugs. Finally, it is increasingly clear that drug-induced activation of central immune signaling contributes markedly to the pharmacodynamic actions of the drugs of abuse, e.g., by enhancing the engagement of classical mesolimbic dopamine reward pathways and withdrawal centers. Coller and Hutchinson (2012) have reviewed critical in vivo animal, human, biological, and molecular evidence of these central immune signaling actions of opioids, alcohol, cocaine, METH, and MDMA. These emerging notions of the central immune function signaling property of drugs of abuse present unlimited possibilities for novel therapeutic strategies and eventual interventions with opportunities to identify "at-risk" individuals through the use of immunogenetics.

2 Abuse Neurotoxicity at Early Developmental Stages

Brain and CNS development encompass several critical periods of vulnerability during which the developing individual is particularly sensitive to environmental neurotoxic insult, such as alcohol or nicotine (Berger et al. 1998; Rice and Barone 2000; Taranukhin et al. 2010). The effects of these drugs have been shown at both prenatal and postnatal stages of early development with regard to disruptions of both structure and function. For instance, the prenatal effects of alcohol exposure for the developing brain and CNS have been described (Goodlett et al. 2005; Riley et al. 2001; Savage et al. 2010). Purkinje cell vulnerability, for instance, occurs during postnatal days 4–6 in rodents when peak blood ethanol concentration (BEC) determines the extent of cell loss (Pierce et al. 2010). Oxidative stress is one of several mechanisms through which drugs instigate a neurotoxic action (Crews and Nixon 2009; Haorah et al. 2008). Alcohol induces lipid peroxidation in the developing brain (Kumral et al. 2005; Wang et al. 2012). Ramezani et al. (2011) observed that ethanol, administered 6 g/kg on postnatal days 4 and 5, induced lipid peroxidation, increased thiobarbituric acid reactive substance (TBARS), and decreased glutathione peroxidase levels in the cerebellum of rat pups, as well as impairing rotarod performance and locomotor activity at adolescent age of test. They observed also the neuroprotective action of 17β-estradiol, injected 30 min before ethanol, which attenuated the motor impairments and lipid peroxidation and restored antioxidant levels. Alcohol is now generally acknowledged as the leading human teratogen and, if the estimates of prevalence of fetal alcohol spectrum disorders (FASD) are correct, is one of the leading known causes of neurodevelopmental disorders in Western society (Goodlett 2010).

3 Alcohol Neurotoxicity

Ethanol crosses the blood–brain barrier readily and is metabolized in the brain by several enzymes in processes rendering reactive oxygen species. Excessive reactive oxygen species (ROS) that disturb the normal cellular redox state induce oxidative

stress leading to cellular damage (Hampton and Orrenius 1998). Alcohol is a neuroteratogen which induces loss of neurons in the CNS that from a neurodevelopmental aspect account for several of the symptoms expressed in fetal alcohol syndrome (Chen et al. 2008; Riley and McGee 2005). Certain regions of the developing brain are selectively vulnerable to alcohol and there are peak periods of sensitivity within these regions (Rice and Barone 2000; Stoltenburg-Didinger 1994). A single ethanol exposure in rats during the first several postnatal days (P4-5) that correspond to the human third trimester induces a huge loss of neurons in brain (Bonthius and West 1991; Hamre and West 1993); slightly later periods show greater resistance to similar exposure (Goodlett and Eilers 1997; Light et al. 2002). The window of vulnerability (also termed peak sensitivity, although the latter is more reliably linked to vision) coincides with the developmental period of synaptogenesis, also known as the brain growth-spurt period, which in rodents is a postnatal event, but in humans extends from the 6th month of gestation to several years after birth. It appears likely that the alcohol-induced disruption of neurodevelopment involves apoptotic processes implicating the Bcl-2 family of survival-regulatory proteins (Ge et al. 2004; Lee et al. 2008; Ullah et al. 2012). The proapoptotic Bax protein, within the Bcl-2 gene family, is implicated in alcohol-induced cell death in developing organisms. After neonatal ethanol administration during ages of peak sensitivity, Bax migrates to the mitochondrial membrane where it participates in initiating apoptosis (Siler-Marsiglio et al. 2005; Heaton et al. 2012a). Heaton et al. (2012b) studied the interactions between Bax and proteins of the permeability transition pore (PTP), voltage-dependent anion channel (VDAC), and adenine nucleotide translocator (ANT) of the outer and inner mitochondrial membranes. They found that after alcohol treatment, Bax proapoptotic associations with VDAC and ANT were increased, especially at peak sensitivity, and sustained at least 2 h posttreatment. Ethanol neurotoxicity in cultured neuronal preparations was abolished by pharmacological inhibition of VDAC and ANT interactions but not by a Bax channel blocker. They concluded that, following ethanol treatment, BAX initiated the apoptosis cascade through interactions with proteins of the PTP complex but not channel formation independent of PTP constituents.

Generally, chronic alcohol intake is more harmful than acute intake (Eysseric et al. 1997; Lamarche et al. 2003). The brain and CNS, and not least neurons, are major targets for the uptake and pharmacological action of alcohol, and chronic consumption of ethanol is associated with several neurodegenerative disorders (Brooks 1997, 2000; Dlugos 2009) and conditions of neurodegeneration (Qin and Crews 2012). The brain damage is characterized by cerebral and cerebellar atrophy and widespread neuronal deficits in several regions in adult chronic alcohol abusers (Bleich et al. 2003; Harper 1998; Harper and Matsumoto 2008). Alcohol-related neuropathology is associated with both excitotoxic (Krystal et al. 2003) and oxidative stress (Sakaguchi et al. 2011; Tiwari et al. 2012). Ethanol exposure produces ROS which cause oxidative stress in the brain. The excessive production of ROS during ethanol catabolism or from damaged mitochondria following ethanol exposure exacerbates the neurotoxicity, particularly during brain development. Kruman et al. (2012) have outlined mechanisms involving the accumulation of DNA

damage in the absence of cellular repair that unfolds in the expression of genomic instability and neuronal death. Genomic instability involves structural alterations that modify the standard chromosomal complement; this process may lead to a situation of aneuploidy, an abnormal number of chromosomes, through which the cells present a chromosomic number that is either higher or lower than the normal complement for the species. For example, alcohol is capable of contributing toward the initiation of cancer through the induction of genomic (chromosomal) instability and aneuploidy, which may be a result of multiple indirect mechanisms (Benassi-Evans and Fenech 2011). Alcohol interacts also with different metabolic pathways. The one-carbon metabolism (OCM) is critical for the synthesis of DNA precursors. It is also a methyl donor for methylation events. Maintenance of genomic stability is dependent upon the processes of DNA repair and methylation such that the interaction of ethanol with OCM poses the threat of DNA damage and DNA repair dysfunction (Kruman et al. 2012).

Autophagy is a carefully regulated pathway that involves the lysosomal degradation of cytoplasmic organelles or cytosolic components, a cell biological process providing a central component of the integrated stress response. Since eukaryotic cells, defined by their membrane complexity, are under constant need to adapt to changing environments (or "stressors" of varying magnitude), it is the response of such cells to these fluctuating stressors that determines whether functionality and survival or cell death occurs. Incorporated in the response to stress (assumed sublethal) is the capacity for rapid changes adapting metabolism and promoting protection. Autophagy is a major pathway mediating stress-induced metabolic adaptation and protective measures that promote species survival against neurodegenerative, neuroinflammatory, and neoplastic stressors, involving abnormal cell proliferation (Levine and Kroemer 2008; Mizushima et al. 2008). A close integration between regulatory processes in response to stress, including nutrient uptake, metabolism, cell cycle and growth control, cell fate and lineage decisions, and cellular survival and death programs, and processes regulating autophagy is maintained, thereby promoting the fundamental adaptation to environmental stressors. Impairments in autophagy lead to accumulation of damaged proteins and organelles thereby eliciting increased cellular stress. Autophagy, through the elimination of potential proapoptotic sources such as damaged mitochondria, provides a cytoprotective pathway which sets a higher threshold against global apoptosis induction (Kroemer et al. 2010). The survival function of autophagy in nutrient- or growth factor-deprived cells (Klionsky 2007) is linked to cellular repair and antipathogen defense (Melendez and Neufeld 2008). The generation of oxidative stress and mitochondrial-related changes by alcohol is an important aspect of the damaging influence of the drug (McVicker et al. 2009). Nonetheless, since autophagy exerts either a pro-survival or pro-death effect, its influence by exposure to the drug is of some importance. Von Haefen et al. (2011) have shown that ethanol downregulated the autophagy proteins, Beclin-1 and LC3-II, with enhanced apoptosis through breakdown of mitochondrial potential, upregulation of cleaved caspase-3 and PARP-1, and downregulation of antiapoptotic protein Bcl-2. They concluded that ethanol inhibited autophagy thereby enhancing vulnerability for cell death.

4 Nicotine Neurotoxicity

The disrupting influences of developmental neurotoxicity by drugs of abuse presents an important regulatory issue as well as a necessary area for significant public concern (Archer and Kostrzewa 2012). Despite the plethora of evidence, the developmental neurotoxicity of a huge number of compounds has yet to be determined (Judson et al. 2009). This type of neurotoxicity involves several manners of expression, including cell proliferation (Breier et al. 2008), synaptogenesis (Harrill et al. 2011), gene expression (Hill et al. 2008), neurite formation (Radio and Mundy 2008), network formation (Robinette et al. 2011), and function and regional integrity (Lai et al. 2011). Applying methods for detecting chemical-induced alterations in proliferation, apoptosis, and viability, Culbreth et al. (2012) have described viability (status of a cell to survive, grow, and multiply) changes in human neuroprogenitor cells following nicotine exposure. Despite evidence that cigarette smoking during pregnancy is associated with fetal, obstetrical, and developmental outcomes, 15–20 % of all women smoke throughout the duration of pregnancy (Andres and Day 2000; Srisukhumbowornchai et al. 2012). Maternal smoking results in myriad physical, cognitive, and behavioral effects in offspring due to prenatal exposure to nicotine. A great proportion of the adverse physiological symptoms attributed to smoking termination (craving, irritability, restlessness, anxiety, and appetite) are related to nicotine withdrawal (Glynn et al. 2009). Through its capacity to bind with cholinergic nicotinic receptors (nAChRs), nicotine dysregulates several essential biological processes such as angiogenesis, apoptosis, and cell-mediated immunity (Zeidler et al. 2007), with proapoptotic effects, in vivo and in vitro, particularly in the developing brain (Abreu-Villaca et al. 2004; Qiao et al. 2003; Roy et al. 1998, 2002). Finally, the effects of nicotine upon permeability of the blood–brain barrier may expose brain regions to potentially serious neurotoxicity (Manda et al. 2010).

Nicotine taken in by pregnant women crosses the placenta, concentrates in fetal blood and amniotic fluid, and is detectable in breast milk during lactation (Lambers and Clark 1996). The effects of nicotine replacement therapy for offspring are linked to fetotoxicity and neuroteratogenicity (Ginzel et al. 2007), as well as adverse cardiovascular, respiratory, endocrine, and metabolic outcomes (Laitinen et al. 2012; Oken et al. 2008; Syme et al. 2010). In order to model the human maternal smoking situation in the animal laboratory, a gestational nicotine exposure has been devised (Coleman 2008; Dwyer et al. 2008, 2009; Franke et al. 2007; Prins et al. 2012). Similar to prenatal smoking exposure in humans, the gestational exposure in animals dose-dependently induces low birth weight, cognitive performance deficits, and enhanced locomotor activity (Eppolito and Smith 2006). Nicotine, a major neurotoxin in cigarette smoke, crosses the placental barrier and fetal, poorly-developed, blood–brain barrier and binds to nAChRs, thereby contributing to the pathogenesis of abnormal brainstem neurochemistry. The gestational nicotine model presented also extensive neurochemical deficits that were manifested during adolescence (Franke et al. 2008; Slotkin 2008). Nevertheless, not all the prenatal nicotine alterations seem to imply permanent deficits. Santiago and

Huffman (2012) have observed that although mouse mother nutrition, dam weight gain, and litter size were not significantly affected by nicotine treatment, prenatal nicotine exposure resulted in lower newborn birth weight and brain weight and length. Remarkably however, the reduction of body weight, brain weight, and brain length observed in newborn prenatal smoking exposure mice, compared to control mice, was no longer present at the 10th postnatal day. Wei et al. (2011) used a pathway-focused oligonucleotide microarray to analyze molecular mechanisms underlying how gestational exposure to nicotine alters brain development in five regions: amygdala, prefrontal cortex, nucleus accumbens, periventricular nucleus of the hypothalamus, and caudate putamen. They identified 24 cell death/survival-related pathways modulated significantly by gestational nicotine that were classified into three categories: (i) growth factor, (ii) death receptor, and (iii) kinase cascade, on the basis of their biological functions. Kinase cascades present a series of several protein kinases that phosphorylate and activate each other: Kinase-1 phosphorylates and activates kinase-2, in turn then phosphorylating and activating kinase 3. This cascade serves to amplify greatly a signal since each protein kinase can phosphorylate multiple substrates. Quantitative real-time PCR (polymerase chain reaction) array was applied to verify their findings through measures of 29 genes involved in cell death/survival-related pathways and indicated that gestational nicotine exposure exerted significant effects on these pathways in the brains of adolescent offspring. These effects were brain region specific and actualized through the expression of growth factors and receptors, caspases, kinases, and transcription factors. They have presented the notion that gestational nicotine exposure affects cell death and survival in adolescent offspring brains by regulating the balance between growth-factor and death-receptor pathways (Wei et al. 2011).

The apoptotic propensity of fetal and neonatal nicotine exposure and its accompanying health hazards have been described both neurodevelopmentally and pathophysiologically (Bruin et al. 2008; 2012). In this vein, it ought to be noted that, whereas there is no information on the long-term effects of developmental nicotine exposure in humans, the consensus from laboratory studies indicates that nicotine alone is the key factor underlying pathophysiology, including Type 2 diabetes, neurobehavioral defects, obesity, hypertension, impaired fertility, and respiratory dysfunction (Bruin et al. 2010). The neurotoxic agency of nicotine is not straightforward but rather complex and enigmatic. It would appear that nicotine exerts, concurrently, both positive, neuroprotective, and apoptosis-inhibiting effects (Hejmadi et al. 2003; Tizabi et al. 2005; Zhang et al. 2006) and conversely negative, neurotoxic, and apoptosis-promoting effects (Barr et al. 2007; Crowley-Weber et al. 2003; Zhao and Reece 2005) with regard to the integrity of the developing and the mature brain. Nicotine, with its specific binding to nAChR, functions as a dysregulator of essential developmental biological processes, such as cell proliferation, apoptosis, migration, invasion, angiogenesis, inflammation, and cell-mediated immunity in a wide variety of cells including fetal (regulation of development), embryonic, and adult stem cells, adult tissues, as well as invading cancer cells (Cardinale et al. 2012). In cardiomyocytes, which may offer a model cell type for examining neurotoxicity, nicotine promoted apoptosis by inducing

oxidative stress and disrupting apoptosis-related gene expression (Zhou et al. 2010). The neurodevelopmental toxicity of nicotine has been described to some extent and in comparison with other real and potential neurotoxicants using zebrafish development as the laboratory model (Muth-Köhne et al. 2012). From these propensities, it seems clear that the action of the drug is multifaceted, possibly involving different neuronal circuits influencing each other through complicated interactions. Ferrea and Winterer (2009) have summarized the most important results of experiments about nicotinic neuroprotection and neurotoxicity propensities in humans and animals. In the first place, well-known modifications of cholinergic transmission during physiological (normal aging) and pathological neurodegeneration have been outlined, and in the second place, neuroprotective and neurotoxic effects of nicotine, also mentioning the underlying molecular mechanisms, have been described.

Maternal smoking exerts critically health hazards that may through indirect means induce neurotoxic actions in brain regions leading to functional deficits. Multiple pathophysiological mechanisms, e.g., respiratory control abnormalities, contribute to these defects. Smoking/nicotine during pregnancy pressurizes the maternal metabolic system and contributes to adverse pregnancy outcomes, predicting future risks for respiratory dysfunction, neurobehavioral deficits in cognitive-emotional domains, cardiovascular disease, obesity, and Type-2 diabetes (Ellis et al. 2012; Langley et al. 2012; Mamun et al. 2012; Zhu et al. 2012). "Catch-up" growth is observed normally in children exposed to intrauterine smoke which was linked to subsequent childhood obesity (Chen et al. 2012). The abnormal hypothalamic gene expression of appetite regulators, e.g., downregulation of pro-opiomelanocortin and neuropeptide Y in the arcuate nucleus of the hypothalamus may be one expression of health hazard. Human studies indicate that maternal smoking causes changes in ventilator control, and respiratory and arousal patterns in newborn infants, decreasing ventilator drive and blunted hypoxic ventilator response (Ueda et al. 1999). There exists a dose-dependent increase in the frequency and duration of obstructive respiratory events in term infants born to mothers who smoked (Kahn et al. 1994). Prenatal exposure to cigarette smoke is associated with decreases in arousal responses after obstructive events (Tirosh et al. 1996). Preterm infants born to smoking mothers showed increased apneic events and decreased respiratory arousal (Sawnani et al. 2004). Nicotine, despite the presence of other chemicals in cigarette smoking, provides the major factor underlying alterations in respiratory control. Nicotine-exposed neonatal rats showed lower minute ventilation on room air thereby evidencing nicotine effects on eupneic, normal, good breathing, ventilation (St. John and Leiter 1999), showing lower tidal volume and higher respiratory frequency (Hafstrom et al. 2002a). Prenatal nicotine attenuates the hypoxic ventilator response (Bamford and Carroll 1999; Hafstrom et al. 2002b; Milerad et al. 1995). Prenatal nicotine exposure is accompanied by a decrease in the magnitude of hypoxic ventilator depression, the component of hypoxic ventilator response that activates the PDGF-β receptor (platelet-derived growth factor-β receptor) and its downstream antiapoptotic cascade in the caudal brainstem of developing rats (Gozal et al. 2000).

Simakajornboon et al. (2010) examined the effect of prenatal nicotine exposure on PDGF-β receptor activation and the subsequent activation of downstream antiapoptotic processes through the Akt/BAD pathway (Akt promotes cell survival through phosphorylation of BAD) which in itself is inhibited by stress (Zundel and Giacco 1998). They concluded that prenatal nicotine exposure attenuated the phosphorylation of PDGF-β receptor, Akt, and BAD-136 during hypoxia in the caudal brainstem of developing rats, suggesting the elevated apoptosis in this region and increased vulnerability of neural cells in the respiratory control area.

5 Alcohol-Nicotine Combinations

Adolescence, characterized as a period of vulnerability (Grant 1998), is a critical period with likely debut for both smoking and alcohol (National Institute on Drug Abuse 1998; Spear 2000), and adolescent "drinkers" are more likely to be "smokers" than "non-drinkers," and vice versa (Chen and Kandel 1995; Van Zundert et al. 2012; Zhang et al. 2012); the consequences are much more serious with pregnant mothers (McDonnell-Naughton et al. 2012). Nicotine, by itself, is damaging to the adolescent brain (Abreu-Villaca et al. 2003). Adolescents appear to be consuming larger quantities of cigarettes and alcohol earlier in life (Perkonnig et al. 2006) with a strong relationship between onset of tobacco use at an early age and alcohol addiction (Burns and Proctor 2012; Muñoz et al. 2012). Smoking prevalence among individuals under treatment for alcoholism is 3–4-fold that of the general population (Batel et al. 1995). It has been shown that nicotine and ethanol interact during adolescence among mice to affect cognitive performance (Abreu-Villaca et al. 2007) and affective status (Abreu-Villaca et al. 2008), during both exposure and withdrawal. Hippocampal cell death and reduced densities of neuronal and glial cells were observed during adolescent nicotine or ethanol exposure whereas the drug combination caused less significant effects (Oliveira-da-Silva et al. 2009). The effects of nicotine and/or ethanol upon several regions of the hippocampus were investigated (Oliveira-da-Silva et al. 2010); these included the granular layer of the dentate gyrus, molecular layer, CA1, CA2, and CA3 of C57/Bl6 mice, using TUNEL assay. There were increases in the numbers of TUNEL + cells in both the nicotine and ethanol groups, with less severe effects in the nicotine-ethanol combination group. Their results point toward the deleterious effects of nicotine and/or ethanol that were reversed during prolonged withdrawal.

Both animal and human studies have described tolerance, consumption, relapse, and behavioral interactions between ethanol and nicotine administration. Nevertheless, the interactions of these drugs with particular reference to developmental and adolescent neurotoxicity, ethanol withdrawal in adulthood for subjects who have an adolescent history of using these drugs, and their combined effects on neuroimmune signaling and mobilization remain neglected although attention emerges (Durazzo et al. 2007). For example, Riley et al. (2012) have investigated nicotine's influence on ethanol withdrawal seizures in two different age groups of

male C3H mice. Adolescent and adult male C3H mice, beginning at postnatal day 28 or 70, respectively, were subjected to a 7-day chronic exposure to ethanol only, ethanol plus nicotine, nicotine only, or vehicle treatment. Six weeks later, all the groups were subjected to chronic exposure to ethanol vapors and the severity of their ethanol withdrawal seizures was assessed by handling-induced convulsions. An adolescent exposure to chronic nicotine resulted in an exacerbation of ethanol withdrawal seizures in adulthood. Given this, adolescence may contain a neurophysiological critical period that is sensitive to nicotine and which may result in an altered response to ethanol dependency in adulthood. These findings have serious implications for the long-term consequences following co-abuse of these drugs during adolescence. The synergistically detrimental effects of combined alcohol and nicotine exposure upon neurodevelopment and brain and CNS integrity ought not to be underestimated (Ramlochansingh et al. 2011).

The effects of nicotine on neural mechanisms modulating respiratory and cardiovascular functioning outlined above become rather more noteworthy with the neurophysiological interactions that arise in combination with alcohol. The prenatal exposure to high levels of nicotine or alcohol separately, e.g., in tribal groups of the North American Plains Indians, has been considered to constitute a major factor contributing to sudden infant death (Gunn et al. 2000; Isayu et al. 2002). In an autopsy study of the Aberden Area Infant Mortality Study, Duncan et al. (2010) investigated the effects of cigarette smoke and alcohol upon the distribution and binding of nAChRs in the brainstem of sudden death cases (SIDs). The expression of nAChRs in the brain is widespread, primarily presynaptic. These receptors, contributing to neurotransmitter release, neurite outgrowth and differentiation, and neuronal survival (Gotti and Clementi 2004; Torrao and Britto 2002), are ligand-gated ion channels, pentameric in structure, and comprised of homomeric or heteromeric combinations of 12 genes [α2-10, β2-4] (Landgren et al. 2011). The ^{3}H-nicotine radioligand was chosen since it binds, with varying affinity, to all subunits of nAChRs but preferentially to the $\alpha_4\beta_2$ subunit to which nicotine in cigarette smoke binds (Benwell et al. 1988). ^{3}H-nicotine binding in five nuclei related to cardiorespiratory control decreased with increasing number of drinks (Duncan et al. 2008). In three mesopontine nuclei critical for arousal, there were reductions in binding for controls exposed to cigarette smoke compared with controls not exposed (ibid). The authors indicate that maternal smoking and alcohol affected ^{3}H-nicotine binding in the infant brainstem, irrespective of cause of death. Durazzo et al. (2004) used human in vivo proton magnetic resonance spectroscopic imaging to observe that chronic cigarette smoking exacerbated chronic alcohol-induced neuronal injury and cell membrane damage in the frontal lobes of recovering alcoholics (14 smokers and 10 nonsmokers) in treatment compared with 26 light-drinking controls (7 smokers and 19 nonsmokers). They found independent adverse effects on neuronal viability and cell membranes in the midbrain and on cell membranes of the cerebellar vermis, with higher smoking levels being associated with metabolite concentrations in the selected subcortical structures.

6 Conclusion

Substance abuse, as above with ethanol, nicotine, or both, is associated with levels of neurotoxicity encompassing most systems of the brain and CNS. The transition from occasional-recreational use to chronic abuse and addiction presents serious psychiatric obstacles that allow few avenues for effective and definitive treatment since most individuals relapse, even after long periods of abstinence. The elucidation of mechanisms through which these substances exert neurotoxicity and mediate addiction remains an essential requirement and mostly involves altered gene function (Kovatsi et al. 2011). Thus, the increasing evidence derived from epigenetics implicating the epigenome in situations of altered gene function may guide through to an understanding of etiopathogenesis. Nevertheless, despite the identification of several genes linked to alcoholism, the genetic basis remains a mystery; advances pursued through the development of transcriptomics, epigenomics, and proteomics may eventually explain genetic susceptibility to alcohol (Buscemi and Turchi 2011). It is expected that the eventual understanding of fetal alcohol syndrome disorders requires (1) the identification of genetic and epigenetic modifications that may be predictive of the neurobehavioral and neurobiological dysfunctions in offspring induced by gestational alcohol exposure and (2) the determination of the relationship structural brain alterations induced by gestational alcohol exposure and the functional-epigenetic outcomes in the offspring (Reynolds et al. 2011). Both the major problem of adverse drug reactions and related neurotoxicity (Kacevska et al. 2011) and the influence of chronic nicotine administration prior to cocaine abuse that exacerbates the epigenetic, gene expression, and electrophysiological and neurobehavioral effects during the transition from acute to chronic responses to cocaine (Volkow 2011) may be linked to the addictive process (Archer et al. 2011, 2012; Archer and Kostrzewa 2013; Blum et al. 2011).

References

Abreu-Villaca, Y., Seidler, F. J., Tate, C. A., & Slotkin, T. A. (2003). Nicotine is a neurotoxin in the adolescent brain: Critical periods, patterns of exposure, regional selectivity and dose thresholds for macromolecular alterations. *Brain Research, 979*, 114–128.

Abreu-Villaca, Y., Seidler, F. J., & Slotkin, T. A. (2004). Does prenatal nicotine exposure sensitize the brain to nicotine-induced neurotoxicity in adolescence? *Neuropsychopharmacology, 29*, 1440–1450.

Abreu-Villaca, Y., Medeiros, A. H., Lima, C. S., Faria, F. P., Filgueiras, C. C., & Manhaes, A. C. (2007). Combined exposure to nicotine and ethanol in adolescent mice differentially affects memory and learning during exposure and withdrawal. *Behavioural Brain Research, 181*, 136–146.

Abreu-Villaca, Y., Nunes, F., Queiroz-Gomes, F. E., Manhaes, A. C., & Filgueiras, C. C. (2008). Combined exposure to nicotine and ethanol in adolescent mice differentially affects anxiety levels during exposure, short term and long term withdrawal. *Neuropsychopharmacology, 33*, 599–610.

Andres, R. L., & Day, M. C. (2000). Perinatal complications associated with maternal tobacco use. *Seminars in Neonatology, 5*, 231–241.

Archer, T. (2011). Effects of exogenous agents on brain development: Stress, abuse and therapeutic compounds. *CNS Neuroscience & Therapeutics, 17*, 470–489.

Archer, T., Beninger, R. J., Palomo, T., & Kostrzewa, R. M. (2010). Epigenetics and biomarkers in the staging of neuropsychiatric disorders. *Neurotoxicity Research, 18*, 347–366.

Archer, T., & Kostrzewa, R. M. (2012). Physical excercise alleviates ADHD symptoms: Regional deficits and developmental trajectory. *Neurotoxicity Research*, *21*, 195–209.

Archer, T., & Kostrzewa, R. M. (2013). The inductive agency of stress: From perinatal to adolescent induction. In G. Laviola, S. Macri, (Eds.), *(Mal)adaptive aspects of developmental stress* (pp. 1–20). New York: Springer.

Archer, T., Oscar-Berman, M., & Blum, K. (2011). Epigenetics in developmental disorder: ADHD and endophenotypes. *Journal of Genetic Syndromes & Gene Therapy, 2*, 1–17. doi:org/10.4172/2157-7412.1000104.

Archer, T., Oscar-Berman, M., Blum, K., & Gold, M. (2012). Neurogenetics and epigenetics in impulsive behaviour: Impact on reward circuitry. *Journal of Genetic Syndromes & Gene Therapy, 3*, 1–15. doi:org/10.4172/2157-7412.1000.115.

Bamford, O. S., & Carroll, J. L. (1999). Dynamic ventilator responses in rats: Normal development and effects of prenatal nicotine exposure. *Respiration Physiology, 117*, 29–40.

Barr, J., Sharma, C. S., Sarkar, S., Wise, K., Dong, L., Periyakaruppan, A., & Ramesh, G. T. (2007). Nicotine induces oxidative stress and activates nuclear transcription factor kappa B in rat mesencephalic cells. *Molecular and Cellular Biochemistry, 297*, 93–99.

Batel, P., Pessione, F., Maitre, C., & Rueff, B. (1995). Relationship between alcohol and tobacco dependencies among alcoholics who smoke. *Addiction, 90*, 977–980.

Bayer, S. A., Altman, J., Russo, R. J., & Zhang, X. (1993). Timetables of neurogenesis in the human brain based on experimentally predetermined patterns in the rat. *Neurotoxicology, 14*, 83–144.

Benassi-Evans, B., & Fenech, M. (2011). Chronic alcohol exposure induces genome damage measured using the cytokinesis-block micronucleus cytome assay and aneuploidy in human B lymphoblastoid cell lines. *Mutagenesis, 26*, 421–429.

Benwell, M. E., Balfour, D. J., & Anderson, J. M. (1988). Evidence that tobacco smoking increases the density of (−)-[^{3}H]nicotine binding sites in human brain. *Journal of Neurochemistry, 50*, 1243–1247.

Berger, F., Gage, F. H., & Vijayaraghavan, S. (1998). Nicotinic receptor-induced apoptotic cell death of hippocampal progenitor cells. *The Journal of Neuroscience, 18*, 6871–6881.

Bleich, S., Wilhelm, J., Graesel, E., Degner, D., Sperling, W., Rössner, V., Javaheripour, K., & Kornhuber, J. (2003). Apolipoprotein E epsilon 4 is associated with hippocampal volume reduction in females with alcoholism. *Journal of Neural Transmission, 110*, 401–411.

Blum, K., Bailey, J., Gonzalez, A. M., Oscar-Berman, M., Liu, Y., Giordano, J., Braverman, E., & Gold, M. (2011). Neur-genetics of reward deficiency syndrome (RDS) as the root cause of "addiction transfer": A new phenomenon common after bariatric surgery. *Journal of Genetic Syndromes & Gene Therapy*. doi:org/10.4172/2157-7412.S2-001.

Bonthius, D. J., & West, J. R. (1991). Permanent neuronal deficits in rats exposed to alcohol during the brain growth spurt. *Teratology, 44*, 147–163.

Breier, J., Radio, N., Mundy, W. R., & Shafer, T. J. (2008). Development of a high throughput assay for assessing antiproliferative compounds using human neuroprogenitor cells. *Toxicological Sciences, 105*, 119–133.

Brooks, P. J. (1997). DNA damage, DNA repair, and alcohol toxicity. *Alcoholism, Clinical and Experimental Research, 21*, 1073–1082.

Brooks, P. J. (2000). Brain atrophy and neuronal loss in alcoholism: A role for DNA damage? *Neurochemistry International, 37*, 403–412.

Bruin, J. E., Gerstein, H. C., & Holloway, A. C. (2010). Long-term consequences of fetal and neonatal nicotine exposure: A critical review. *Toxicological Sciences, 116*, 364–374.

Bruin, J. E., Petre, M. A., Lehman, M. A., Raha, S., Gerstein, H. C., Morrison, K. M., & Holloway, A. C. (2008). Maternal nicotine exposure increases oxidative stress in the offspring. *Free Radical Biology & Medicine, 44*, 1919–1925.

Bruin, J. E., Woynillowicz, A. K., Hettinga, B. P., Tarnopolsky, M. A., Morrison, K. M., Gerstein, H. C., Holloway, A. C. (2012). Maternal antioxidants prevent beta cell apoptosis and promote formation of dual hormone-expressing endocrine cells in male offspring following fetal and neonatal nicotine exposure. doi:10.1111/j.1753-0407.2012.00195.x.

Burns, B. E., & Proctor, W. R. (2012). Cigarette smoke exposure greatly increases alcohol consumption in adolescent C57BL/6 mice. *Alcoholism Clinical and Experimental Research*. doi:10.1111/j.1530-0277.2012.01911.x.

Buscemi, L., & Turchi, C. (2011). An overview of the genetic susceptibility in alcoholism. *Medicine, Science, and the Law, 51*(Suppl 1), S2–S6.

Cardinale, A., Nastrucci, C., Cesario, A., & Russo, P. (2012). Nicotine: Specific role in angiogenesis, proliferation and apoptosis. *Critical Reviews in Toxicology, 42*, 68–89.

Chen, G., Ma, C., Bower, K. A., Shi, X., Ke, Z., & Luo, J. (2008). Ethanol promotes endoplasmic reticulum stress-induced neuronal death: Involvement of oxidative stress. *Journal of Neuroscience Research, 17*, 91–101.

Chen, H., Saad, S., Sandow, S. L., & Bertrand, P. (2012). Cigarette smoking and brain regulation of energy homeostasis. *Frontiers in Pharmacology, 3*, 1–8. doi:10.3389/fphar.2012.00147.

Chen, K., & Kandel, D. B. (1995). The natural history of drug use from adolescence to the mid-thirties in a general population sample. *American Journal of Public Health, 85*, 41–47.

Coleman, T. (2008). Reducing harm from tobacco smoke exposure during pregnancy. *Birth Defects Research Part C: Embryo Today: Reviews, 84*, 73–79.

Coller, J. K., & Hutchinson, M. R. (2012). Implications of central immune signaling caused by drugs of abuse: Mechanisms, mediators and new therapeutic approaches for prediction and treatment of drug dependence. *Pharmacology and Therapeutics, 134*, 219–245.

Crews, F. T., & Nixon, K. (2009). Mechanisms of neurodegeneration and regeneration in alcoholism. *Alcohol, 44*, 115–127.

Crowley-Weber, C. L., Dvorakova, K., Crowley, C., Bernstein, H., Bernstein, C., Garewal, H., & Payne, C. M. (2003). Nicotine increases oxidative stress, activates NF-kappaB and GRP78, induces apoptosis and sensitizes cell to genotoxic/xenobiotic stresses by a multiple stress inducer, deoxycholate: Relevance to colon carcinogenesis. *Chemico-Biological Interactions, 145*, 53–66.

Culbreth, M. E., Harrill, J. A., Freudenrich, T. M., Mundy, W. R., & Shafer, T. J. (2012). Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells. *Neurotoxicology*. doi:10.1016/j.neuro.2012.05.012.

Dlugos, C. A. (2009). Ethanol-related increases in degenerating bodies in the Purkinje neuron dendrites of aging rats. *Brain Research, 1221*, 98–107.

Dufault, R., Lukiw, W. J., Crider, R., Schnoll, R., Wallinga, D., & Deth, R. (2012). A macroepigenetic approach to identify factors responsible for the autism epidemic in the United States. *Clinical Epigenetics, 4*, 6.

Duncan, J. R., Paterson, D. S., & Kinney, H. C. (2008). The development of nicotinic receptors in the human medulla oblongata: Inter-relationship with the serotonergic system. *Autonomic Neuroscience, 144*, 61–75.

Duncan, J. R., Randall, L. L., Belliveau, R. A., Trachtenberg, G. L., Randall, B., Habbe, D., Mandell, F., Wekty, T. K., Isayu, S., & Kinney, H. C. (2010). The effect of maternal smoking and drinking during pregnancy upon ^{3}H-nicotine receptor brainstem binding in infants dying of the sudden infant death syndrome: Initial observations in a high risk population. *Brain Pathology, 18*, 21–31.

Durazzo, T. C., Cardenas, V. A., Studholme, C., Weiner, M. W., & Meyerhoff, D. J. (2007). Non-treatment-seeking heavy drinkers: Effects of chronic cigarette smoking on brain structure. *Drug and Alcohol Dependence, 87*, 76–82.

Dwyer, J. B., Broide, R. S., & Leslie, F. M. (2008). Nicotine and brain development. *Birth Defects Research Part C: Embryo Today: Reviews, 84*, 30–44.

Dwyer, J. B., McQuown, S. C., & Leslie, F. M. (2009). The dynamic effects of nicotine on the developing brain. *Pharmacology and Therapeutics, 122*, 125–139.

Ellis, L., Widmayer, A., & Das, S. (2012). Maternal smoking during pregnancy and self-reported delinquency by offspring. *Criminal Behaviour and Mental Health*. doi:10.1002/cbm.1828.

Eppolito, A. K., & Smith, R. F. (2006). Long-term behavioural and developmental consequences of pre- and perinatal nicotine. *Pharmacology Biochemistry and Behavior, 85*, 835–841.

Eysseric, H., Gonthier, B., Soubeyran, A., Bessard, G., Saxod, R., & Barret, L. (1997). There is no simple method to maintain a constant ethanol concentration in long-term cell culture: Keys to a solution applied to the survey of astrocytic ethanol absorption. *Alcohol, 14*, 111–115.

Ferrea, S., & Winterer, G. (2009). Neuroprotective and neurotoxic effects of nicotine. *Pharmacopsychiatry, 42*, 255–265.

Franke, R. M., Belluzzi, J. D., & Leslie, F. M. (2007). Gestational exposure to nicotine and monoamine oxidase inhibitors influences cocaine-induced locomotion in adolescent rats. *Psychopharmacology, 195*, 117–124.

Franke, R. M., Park, M., Belluzzi, J. D., & Leslie, F. M. (2008). Prenatal nicotine exposure changes natural and drug-induced reinforcement in adolescent male rats. *The European Journal of Neuroscience, 27*, 2952–2961.

Ge, Y., Belcher, S. M., Pierce, D. R., & Light, K. E. (2004). Altered expression of Bcl-2, Bad and Bax mRNA occurs in the rat cerebellum within hours after ethanol exposure on postnatal day 4 but not on postnatal day 9. *Molecular Brain Research, 129*, 124–134.

Ginzel, K. H., Maritz, G. S., Marks, D. F., Neuberger, M., Pauly, J. R., Polito, J. R., Schulte-Hermann, R., & Slotkin, T. A. (2007). Critical review: Nicotine for the fetus, the infant and the adolescent? *Journal of Health Psychology, 12*, 215–224.

Glynn, D. A., Cryan, J. F., Kent, P., Flynn, R. A., & Kennedy, M. P. (2009). Update on smoking cessation therapies. *Advances in Therapy, 26*, 369–382.

Goodlett, C. R. (2010). Fetal alcohol spectrum disorders: New perspectives on diagnosis and intervention. *Alcohol, 44*, 579–582.

Goodlett, C. R., & Eilers, A. T. (1997). Alcohol-induced Purkinje cell loss with a single binge exposure in neonatal rats: A stereological study of temporal windows of vulnerability. *Alcoholism, Clinical and Experimental Research, 2*, 738–744.

Goodlett, C. R., Horn, K. H., & Zhou, F. C. (2005). Alcohol teratogenesis: Mechanisms of damage and strategies for intervention. *Experimental Biology and Medicine, 230*, 394–406.

Gotti, C., & Clementi, F. (2004). Neuronal nicotinic receptors: From structure to pathology. *Progress in Neurobiology, 74*, 363–396.

Gozal, D., Simakajornboon, N., Czapla, M. A., Xue, Y. D., Gozal, E., Vlasic, V., Lasky, J. A., & Liu, J. Y. (2000). Brainstem activation of platelet-derived growth factor-beta receptor modulates the late phase of the hypoxic ventilatory response. *Journal of Neurochemistry, 74*, 310–319.

Grant, B. F. (1998). Age at smoking onset and its association with alcohol consumption and DSM-IV alcohol abuse and dependence: Results from the National longitudinal alcohol epidemiological survey. *Journal of Substance Abuse, 10*, 59–73.

Gunn, A. J., Gunn, T. R., & Mitchell, E. A. (2000). Clinical review article: Is changing the sleep environment enough? Current recommendations for SIDs. *Sleep Medicine Reviews, 4*, 453–469.

Hafstrom, O., Milerad, J., & Sundell, H. W. (2002a). Altered breathing pattern after prenatal nicotine exposure in the young lamb. *American Journal of Respiratory and Critical Care Medicine, 166*, 92–97.

Hafstrom, O., Milerad, J., & Sundell, H. W. (2002b). Prenatal nicotine exposure blunts the cardiorespiratory response to hypoxia in lambs. *American Journal of Respiratory and Critical Care Medicine, 166*, 1544–1549.

Hampton, M. B., & Orrenius, S. (1998). Redox regulation of apoptotic cell death. *BioFactors, 8*, 1–5.

Hamre, K. M., & West, J. R. (1993). The effects of the timing of ethanol exposure during the brain growth spurt on the number of cerebellar Purkinje and granule cell nuclear profiles. *Alcoholism, Clinical and Experimental Research, 17*, 610–622.

Haorah, J., Ramirez, S. H., Floreani, N., Gorantla, S., Morsey, B., & Persidsky, Y. (2008). Mechanism of alcohol-induced oxidative stress and neuronal injury. *Free Radical Biology & Medicine, 45*, 1542–1550.

Harper, C. (1998). The neuropathology of alcohol-specific brain damage, or does alcohol damage the brain? *Journal of Neuropathology and Experimental Neurology, 57*, 101–110.

Harper, C., & Matsumoto, I. (2008). Neuropathology of alcohol-related cognitive alterations. *Handbook of Clinical Neurology, 89*, 863–872.

Harrill, J. A., Robinette, B. L., & Mundy, W. R. (2011). Use of high content image analysis to detect chemical-induced changes in synaptogenesis in vitro. *Toxicology in Vitro, 25*, 368–387.

Heaton, M. B., Paiva, M., Kubovec, S., Kotler, A., Rogozinski, J., Swanson, E., Madorsky, V., & Posados, M. (2012a). Differential effects of ethanol on c-jun N-terminal kinase, 14-3-3 proteins, and Bax in postnatal day 4 and postnatal day 7 rat cerebellum. *Brain Research, 1432*, 15–27.

Heaton, M. B., Siler-Marsiglio, K. I., Paiva, M., Kotler, A., Rogozinski, J., Kubovec, S., Coursen, M., & Madorsky, V. (2012b). Ethanol influences on Bax associations with mitochondrial proteins in neonatal rat cerebellum. *Developmental Neurobiology*. doi:10.1002/dneu.22042.

Hejmadi, M. V., Dajas-Bailador, F., Barns, S. M., Jones, B., & Wonnacott, S. (2003). Neuroprotection by nicotine against hypoxia-induced apoptosis in cortical cultures involves activation of multiple nicotine acetylcholine receptor subtypes. *Molecular and Cellular Neuroscience, 24*, 779–786.

Herschkowitz, N., Kagan, J., & Zilles, K. (1997). Neurobiological bases of behavioural development in the first year. *Neuropediatrics, 28*, 296–306.

Hill, E. J., Woehrling, E. K., & Prince, M. (2008). Differentiating human NT2/D1 neurospheres as a versatile in vitro 3D model system for neurodevelopmental neurotoxicity testing. *Toxicology, 249*, 243–250.

Isayu, S., Randall, L. L., Welty, T. K., Hsia, J., Kinney, H. C., Mandell, F., McClain, M., Randall, B., Habbe, D., Wilson, H., & Willinger, M. (2002). Risk factors for sudden infant death syndrome among northern plains Indians. *JAMA: The Journal of the American Medical Association, 288*, 1717–2723.

Judson, R., Richard, A., Dix, D. J., Houck, K., Martin, M., Kavlock, R., Dellarco, V., Henry, T., Holderman, T., Sayre, P., Tan, S., Carpenter, T., & Smith, E. (2009). The toxicity data landscape for environmental chemicals. *Environmental Health Perspectives, 117*, 685–695.

Kacevska, M., Ivanov, M., & Ingelmann-Sundberg, M. (2011). Perspectives on epigenetics and its relevance to adverse drug reactions. *Nature, 89*, 902–907.

Kahn, A., Groswasser, J., Sottiaux, M., Kelmanson, I., Rebuffat, E., Franco, P., Dramaix, M., & Wayenberg, J. L. (1994). Prenatal exposure to cigarettes in infants with obstructive sleep apnea. *Pediatrics, 93*, 778–783.

Kiyatkin, E. A., & Sharma, H. S. (2012). Environmental conditions modulate neurotoxic effects of psychomotor stimulant drugs of abuse. *International Review of Neurobiology, 102*, 147–171.

Klionsky, D. J. (2007). Autophagy: From phenomenology to molecular understanding in less than a decade. *Nature Reviews Molecular Cell Biology, 8*, 931–937.

Kovatsi, L., Fragou, D., Samanidou, V., Njau, S., & Kouidou, S. (2011). Drugs of abuse: Epigenetic mechanisms in toxicity and addiction. *Current Medicinal Chemistry, 18*, 1765–1774.

Kroemer, G., Marino, G., & Levine, B. (2010). Autophagy and the integrated stress response. *Molecular Cell, 40*, 280–293.

Kruman, I. I., Henderson, G. I., & Bergeson, S. E. (2012). DNA damage and neurotoxicity of chronic alcohol abuse. *Experimental Biology and Medicine, 237*, 740–747.

Krystal, J. H., Petrakis, I. L., Mason, G., Trevisan, L., & D'Souza, D. C. (2003). N-methyl-D-aspartate glutamate receptors and alcoholism: Reward, dependence, treatment, and vulnerability. *Pharmacology and Therapeutics, 99*, 79–84.

Kumral, A., Tugyan, K., Gonenc, S., Genc, K., Genc, S., Sonmez, U., Yilmaz, O., Duman, N., Uysal, N., & Ozkan, H. (2005). Protective effects of erythropoietin against ethanol-induced

apoptotic neurodegeneration and oxidative stress in the developing C57BL/6 mouse brain. *Brain Research. Developmental Brain Research, 160*, 146–156.

Lai, A. Y., Dhami, K. S., Dibal, C. D., & Todd, K. G. (2011). Neonatal rat microglia derived from different brain regions have distinct activation responses. *Neuron Glia Biology, 7*, 5–16.

Laitinen, J., Jääskeläinen, A., Hartikainen, A. L., Sovio, U., Vääräsmäki, M., Pouta, A., Kaakinen, M., & Järvelin, M. R. (2012). Maternal weight gain during the first half of pregnancy and offspring obesity at 16 years: A prospective cohort study. *BJOG : An International Journal of Obstetrics and Gynaecology, 119*(6), 716–723. doi:10.1111/j.1471-0528.2012.03319.x.

Lamarche, F., Gonthier, B., Signorini, N., Eysseric, H., & Barret, L. (2003). Acute exposure of cultured neurones to ethanol results in reversible DNA single-strand breaks; whereas chronic exposure causes loss of cell viability. *Alcohol, 38*, 550–558.

Lambers, D. S., & Clark, K. E. (1996). The maternal and fetal physiologic effects of nicotine. *Seminars in Perinatology, 20*, 115–126.

Landgren, S., Berglund, K., Jerlhag, E., Fahlke, C., Balldin, J., Berggren, U., Zetterberg, H., Blennow, K., & Engel, J. A. (2011). Reward-related genes and personality traits in alcohol-dependent individuals: A pilot case control study. *Neuropsychobiology, 64*(1), 38–46.

Langley, K., Heron, J., Smith, G. D., & Thapar, A. (2012). Maternal and paternal smoking during pregnancy and risk of ADHD symptoms in offspring: Testing for intrauterine effects. *American Journal of Epidemiology, 176*, 261–268.

Lee, H. Y., Naha, N., Kim, J. H., Jo, M. J., Min, K. S., Seong, H. H., Shin, D. H., & Kim, M. O. (2008). Age- and area-dependent distinct effects of ethanol on Bax and Bcl-2 expression in prenatal rat brain. *Journal of Microbiology and Biotechnology, 18*, 1590–1598.

Levine, B., & Kroemer, G. (2008). Autophagy in the pathogenesis of disease. *Cell, 132*, 27–42.

Light, K. E., Belcher, S. M., & Pierce, D. R. (2002). Time course and manner of Purkinje neuron death following a single ethanol exposure on postnatal day 4 in the developing rat. *Neuroscience, 114*, 327–337.

Llop, S., Guxens, M., Murcia, M., Lertxundi, A., Ramon, R., Riaño, I., Rebagliato, M., Ibarluzea, J., Tardon, A., Sunyer, J., & Ballester, F. (2012). INMA Project. Prenatal exposure to mercury and infant neurodevelopment in a multicenter cohort in Spain: Study of potential modifiers. *American Journal of Epidemiology, 175*, 451–465.

Mamun, A. A., O'Callaghan, M. J., Williams, G. M., & Najman, J. M. (2012). Maternal smoking during pregnancy predicts adult offspring cardiovascular risk factors – evidence from a community-based large birth cohort study. *PLoS One, 7*(7), e41106.

Manda, V. K., Mittapalli, R. K., Bohn, K. A., Adkins, C. E., & Lockman, P. R. (2010). Nicotine and cotinine increases the brain penetration of saquinavir in rat. *Journal of Neurochemistry, 115*, 1495–1507.

Marti, J., Santa-Cruz, M. C., Bayer, S. A., Ghetti, B., & Hervas, J. P. (2007). Generation and survival of midbrain dopaminergic neurons in weaver mice. *International Journal of Developmental Neuroscience, 25*, 299–307.

McDonnell-Naughton, M., McGarvey, C., O'Regan, M., & Matthews, T. (2012). Maternal smoking and alcohol consumption during pregnancy as risk factors for sudden infant death. *Irish Medical Journal, 105*, 105–108.

McVicker, B. L., Tuma, P. L., Kharbanda, K. K., Lee, S. M., & Tuma, D. J. (2009). Relationship between oxidative stress and hepatic glutathione levels in ethanol-mediated apoptosis of polarized hepatic cells. *World Journal of Gastroenterology, 15*, 2609–2616.

Melendez, A., & Neufeld, T. P. (2008). The cell biology of autophagy in metazoans: A developing story. *Development, 135*, 2347–2360.

Milerad, J., Larsson, H., Lin, J., & Sundell, H. W. (1995). Nicotine attenuates the ventilatory response to hypoxia in the developing lamb. *Pediatric Research, 37*, 652–660.

Miller, B. J., Culpepper, N., Rapaport, M. H., & Buckley, P. (2012). Prenatal inflammation and neurodevelopment in schizophrenia: a review of human studies. *Prog Neuropsychopharmacol Biol Psychiatry, 42*, 92–100. PMID: 22510462.

Mizushima, N., Levine, B., Cuervo, A. M., & Klionsky, D. J. (2008). Autophagy fights disease through cellular self-digestion. *Nature, 451*, 1069–1075.

Muñoz, X., Amiano, P., Celorrio, D., Dorronsoro, M., Sánchez, M. J., Huerta, J. M., Barricarte, A., Arriola, L., Navarro, C., Molina-Montes, E., Chirlaque, M. D., Ardanaz, E., Rodriguez, L., Duell, E. J., Hijona, E., Herreros-Villanueva, M., Sala, N., & Bujanda, L. (2012). Association of alcohol dehydrogenase polymorphisms and life-style factors with excessive alcohol intake within the Spanish population (EPIC-Spain). *Addiction*. doi:10.1111/j.1360-0443.2012.03970.x.

Muth-Köhne, E., Wichmann, A., Delov, V., & Fenske, M. (2012). The classification of motor neuron defects in the zebrafish embryo toxicity test (ZFET) as an animal alternative approach to assess developmental neurotoxicity. *Neurotoxicology and Teratology, 34*, 413–424.

National Institute on Drug Abuse. (1998). *Nicotine addiction* (p. 7). Washington, DC: NIH. Publication Number 98–4342.

OECD. (2007). *Test guideline 426. OECD guideline for testing of chemicals. Developmental Neurotoxicity Study*. The Environment Directorate, Paris.

Oken, E., Levitan, E. B., & Gillman, M. W. (2008). Maternal smoking during pregnancy and child overweight: Systematic review and meta-analysis. *International Journal of Obesity, 32*, 201–210.

Oliveira-da-Silva, A., Vieira, F. B., Cristina-Rodrigues, F., Filgueira, C. C., Manhaes, A. C., & Abreu-Villaca, Y. (2009). Increased apoptosis and reduced neuronal and glial densities in the hippocampus due to nicotine and ethanol exposure in adolescent mice. *International Journal of Developmental Neuroscience, 27*, 539–548.

Oliveira-da-Silva, A., Manhaes, A. C., Cristina-Rodrigues, F., Filgueira, C. C., & Abreu-Villaca, Y. (2010). Hippocampal increased cell death and decreased cell density elicited by nicotine and/or ethanol during adolescence are reversed during drug withdrawal. *Neuroscience, 167*, 163–173.

Perkonnig, A., Pfister, H., Hofler, M., Frolich, C., Zimmermann, P., Lieb, R., & Wittchen, H. U. (2006). Substance use and substance use disorders in a community sample of adolescents and young adults: Incidence, age effects and patterns of use. *European Addiction Research, 12*, 187–196.

Pierce, D. R., Hayar, A., Williams, D. K., & Light, K. E. (2010). Developmental alterations in olivary climbing fiber distribution following postnatal ethanol exposure in the rat. *Neuroscience, 169*, 1438–1448.

Prins, J. R., Hylkema, M. N., Erwich, J. J., Huitema, S., Dekkema, G. J., Dijkstra, F. E., Faas, M. M., & Melgert, B. N. (2012). Smoking during pregnancy influences the maternal immune response in mice and humans. *American Journal of Obstetrics and Gynecology, 207*(1), 76. e1–76.e14.

Qiao, D., Seidler, F. J., Violin, J. D., & Slotkin, T. A. (2003). Nicotine is a developmental neurotoxicant and neuroprotectant: Stage-selective inhibition of DNA synthesis coincident with shielding from effects of chlorpyrifos. *Developmental Brain Research, 147*, 183–190.

Qin, L., & Crews, F. T. (2012). NADPH oxidase and reactive oxygen species contribute to alcohol-induced microglial activation and neurodegeneration. *Journal of Neuroinflammation*. doi:10.1186/1742-2094-9-5.

Radio, N. M., & Mundy, W. R. (2008). Developmental neurotoxicology testing in vitro: Models for assessing chemical effects on neurite growth. *Neurotoxicology, 29*, 361–376.

Ramezani, A., Goudarzi, I., Lashkarbolouki, T., Ghorbanian, M. T., Salmani, M. E., & Abrari, K. (2011). Neuroprotective effects of the 17β-estradiol against ethanol-induced neurotoxicity and oxidative stress in the developing male rat cerebellum: Biochemical, histological and behavioural changes. *Pharmacology Biochemistry and Behavior, 100*, 144–151.

Ramlochansingh, C., Taylor, R. E., & Tizabi, Y. (2011). Toxic effects of low alcohol and nicotine combinations in SH-SY5Y cells are apoptotically mediated. *Neurotoxicity Research, 20*, 263–269.

Reynolds, J. N., Weinberg, J., Clarren, S., Beaulieu, C., Rasmussen, C., Kobor, M., Dube, M. P., & Goldowitz, D. (2011). Fetal alcohol spectrum disorders: Gene-environment interaction interactions, predictive biomarkers, and the relationship between structural alterations in the brain and functional outcomes. *Seminars in Pediatric Neurology, 18*, 49–55.

Rice, D., & Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. *Environmental Health Perspectives, 108*(Suppl. 2), 511–533.

Riley, E. P., Thomas, J. D., Goodlett, C. R., Klintsova, A. Y., Greenough, W. T., Hungund, B. L., Zhou, F., Sari, Y., Powrozek, T., & Li, T. K. (2001). Fetal alcohol effects: Mechanisms and treatment. *Alcoholism, Clinical and Experimental Research, 25*(5 Suppl ISBRA), 110S–116S.

Riley, E. P., & McGee, C. L. (2005). Fetal alcohol spectrum disorders: An overview with emphasis on changes in brain and behavior. *Experimental Biology and Medicine, 230*, 357–365.

Riley, H. H., Zalud, A. W., & Diaz-Granados, J. L. (2012). The influence of a chronic adolescent nicotine exposure on ethanol withdrawal severity during adulthood in C3H mice. *Alcohol, 44*, 81–87.

Robinette, B. L., Harrill, J. A., Mundy, W. R., & Shafer, T. J. (2011). In vitro assessment developmental neurotoxicity: Use of microelectrode arrays to measure functional changes in neuronal network ontogeny. *Frontiers in Neuroengineering, 4*, 1.

Roy, T. S., Andrews, J. E., Seidler, F. J., & Slotkin, T. A. (1998). Nicotine evokes cell death in embryonic rat brain during neurulation. *The Journal of Pharmacology and Experimental Therapeutics, 287*, 1136–1144.

Roy, T. S., Seidler, F. J., & Slotkin, T. A. (2002). Prenatal nicotine evokes alterations of cell structure in the hippocampus and somatosensory cortex. *The Journal of Pharmacology and Experimental Therapeutics, 300*, 124–133.

Sakaguchi, S., Takahashi, S., Sasaki, T., Kumagai, T., & Nagata, K. (2011). Progression of alcoholic and non-alcoholic steatohepatitis: Common metabolic aspects of innate immune system and oxidative stress. *Drug Metabolism and Pharmacokinetics, 26*, 30–46.

Santiago, S. E., & Huffman, K. J. (2012). Postnatal effects of prenatal nicotine exposure on body weight, brain size and cortical connectivity in mice. *Neuroscience Research, 73*, 282–291.

Sawnani, H., Jackson, T., Murphy, T., Beckerman, R., & Simakajornboon, N. (2004). The effect of maternal smoking on respiratory and arousal patterns in preterm infants during sleep. *American Journal of Respiratory and Critical Care Medicine, 169*, 733–738.

Savage, D. D., Rosenberg, M. J., Wolff, C. R., Akers, K. G., El-Emawy, A., Staples, M. C., Varaschin, R. K., Wright, C. A., Seidel, J. L., Caldwell, K. K., & Hamilton, D. A. (2010). Effects of a novel cognition-enhancing agent on fetal ethanol-induced learning deficits. *Alcoholism, Clinical and Experimental Research, 34*, 1793–1802.

Siler-Marsiglio, K. I., Paiva, M., Madorsky, I., Pan, Q., Shaw, G., & Heaton, M. B. (2005). Functional mechanisms of apoptosis-related proteins in neonatal rat cerebellum are differentially influenced by ethanol at postnatal day 4 and 7. *Journal of Neuroscience Research, 81*, 632–643.

Simakajornboon, N., Kuptanon, T., & Jirapongsuwan, P. (2010). The effect of prenatal nicotine exposure on PDGFR-mediated anti-apoptotic mechanism in the caudal brainstem of developing rat. *Neuroscience Letters, 478*, 46–50.

Slotkin, T. A. (2008). If nicotine is a developmental neurotoxicant in animal studies, dare we recommend nicotine replacement therapy in pregnant women and adolescents. *Neurotoxicology and Teratology, 30*, 1–19.

Spear, L. P. (2000). The adolescent brain and age-related behavioural manifestations. *Neuroscience and Biobehavioral Reviews, 24*, 417–463.

Spencer, P. S., & Palmer, V. S. (2012). Interrelationships of undernutrition and neurotoxicity: Food for thought and research attention. *Neurotoxicology, 33*, 605–616.

Srisukhumbowornchai, S., Krikov, S., & Feldkamp, M. L. (2012). Self-reported maternal smoking during pregnancy by source in Utah, 2003–2007. *Birth Defects Research. Part A, Clinical and Molecular Teratology*. doi:10.1002/bdra.23058.

St. John, W. M., & Leiter, J. C. (1999). Maternal nicotine depresses eupneic ventilation of neonatal rats. *Neuroscience Letters, 267*, 206–208.

Stanwood, G. D., & Levitt, P. (2004). Drug exposure early in life: Functional repercussions of changing neuropharmacology during sensitive periods of brain development. *Current Opinion in Pharmacology, 4*, 65–71.

Stoltenburg-Didinger, G. (1994). Neuropathology of the hippocampus and its susceptibility to neurotoxic insult. *Neurotoxicology, 15*, 445–450.

Syme, C., Abrahamowicz, M., Mahboubi, A., Leonard, G. T., Perron, M., Richer, L., Veillette, S., Gaudet, D., Paus, T., & Pausova, Z. (2010). Prenatal exposure to maternal cigarette smoking and accumulation of intra-abdominal fat during adolescence. *Obesity, 18*, 1021–1025.

Taranukhin, A. G., Taranukhina, E. Y., Saransaari, P., Podkletnova, I. M., Pelto-Huikko, M., & Oja, S. S. (2010). Neuroprotection by taurine in ethanol-induced apoptosis in the developing cerebellum. *Journal of Biomedical Science, 17*(1), S12.

Tirosh, E., Libon, D., & Bader, D. (1996). The effect of maternal smoking during pregnancy on sleep respiratory and arousal patterns in neonates. *Journal of Perinatology, 16*, 435–438.

Tiwari, V., Kuhad, A., & Chopra, K. (2012). Neuroprotective effect of vitamin E isoforms against chronic alcohol-induced peripheral neurotoxicity: Possible involvement of oxidative-nitrodative stress. *Phytotherapy Research*. doi:10.1002/ptr.4635.

Tizabi, Y., Manaye, K. F., & Taylor, R. E. (2005). Nicotine blocks ethanol-induced apoptosis in rat cerebral cortical and cerebellar granule cells. *Neurotoxicity Research, 7*, 319–322.

Torrao, A. S., & Britto, L. R. (2002). Neurotransmitter regulation of neural development: Acetylcholine and nicotinic receptors. *Anais da Academia Brasileira de Ciências, 74*, 453–461.

Ueda, Y., Stick, S. M., Hall, G., & Sly, P. D. (1999). Control of breathing in infants born to smoking mothers. *The Journal of Pediatrics, 135*, 226–232.

Ullah, I., Ullah, N., Naseer, M. I., Lee, H. Y., & Kim, M. O. (2012). Neuroprotection with metformin and thymoquinone against ethanol-induced apoptotic neurodegeneration in prenatal rat cortical neurons. *BMC Neuroscience, 13*, 11.

Van Zundert, R. M., Kuntsche, E., & Engels, R. C. (2012). In the heat of the moment: Alcohol consumption and smoking lapse and relapse among adolescents who have quit smoking. *Drug and Alcohol Dependence, 126*(1–2), 200–205. PMID: 22682658.

Vanattou-Saïfoudine, N., McNamara, R., & Harkin, A. (2012). Caffeine provokes adverse interactions with MDMA ("Ecstasy") and related psychostimulants: Mechanisms and mediators. *British Journal of Pharmacology, 167*(5), 946–959. doi:10.1111/j.1476-5381.2012.02065.x.

Volkow, N. D. (2011). Epigenetics of nicotine: Another nail in the couching. *Science Translational Medicine, 3*, 1–4.

Von Haefen, C., Sifringer, M., Menk, M., & Spies, C. D. (2011). Ethanol enhances susceptibility to apoptotic cell death via down-regulation of autophagy-related proteins. *Alcoholism, Clinical and Experimental Research, 35*, 1381–1391.

Wang, X., Ke, Z., Chen, G., Xu, M., Bower, K. A., Frank, J. A., Zhang, Z., & Shi, X. (2012). Luo J (2012) Cdc42-dependent activation of NADPH oxidase is involved in ethanol-induced neuronal oxidative stress. *PLoS One, 7*(5), e38075.

Wei, J., Wang, J., Dwyer, J. B., Mangold, J., Cao, J., Leslie, F. M., & Li, M. D. (2011). Gestational nicotine treatment modulates cell death/survival-related pathways in the brains of adolescent female rats. *The International Journal of Neuropsychopharmacology, 14*, 91–106.

Zeidler, R., Albermann, K., & Lang, S. (2007). Nicotine and apoptosis. *Apoptosis, 12*, 1927–1943.

Zhang, T., Lu, H., Shang, X., Tian, Y., Zheng, C., Wang, S., Cheng, H., & Zhou, R. (2006). Nicotine prevents the apoptosis induced by menadione in human lung cancer cells. *Biochemical and Biophysical Research Communications, 342*, 928–934.

Zhang, T., Gao, W., Cao, W., Zhan, S., Lv, J., Pang, Z., Wang, S., Chen, R., Hu, Y., & Li, L. (2012). The genetic correlation between cigarette smoking and alcohol drinking among Chinese adult male twins: An ordinal bivariate genetic analysis. *Twin Research and Human Genetics, 15*, 483–490.

Zhao, Z., & Reece, E. A. (2005). Nicotine-induced embryonic malformations mediated by apoptosis from increasing intracellular calcium and oxidative stress. *Birth Defects Research. Part B, Developmental and Reproductive Toxicology, 74*, 383–391.

Zhou, X., Sheng, Y., Yang, R., & Kong, X. (2010). Nicotine promotes cardiomyocyte apoptosis via oxidative stress and altered apoptosis-related gene expression. *Cardiology, 115*, 243–250.

Zhu, J., Zhang, X., Xu, Y., Spencer, T. J., Biederman, J., & Bhide, P. G. (2012). Prenatal nicotine exposure mouse model showing hyperactivity, reduced cingulate cortex volume, reduced dopamine turnover, and responsiveness to oral methylphenidate treatment. *The Journal of Neuroscience, 32*, 9410–9418.

Zundel, W., & Giacco, A. (1998). Inhibition of anti-apoptotic PI(3)K/Akt/BAD pathway by stress. *Genes & Development, 12*, 1941–1946.

Experimental Approach to Alzheimer Disease

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Contents

Abstract

This review on current experimental models of Alzheimer disease is based on human postmortem findings showing keystone markers for a pathology within the ß-amyloid transduction cascade as well as pathology in the mechanism of phosphorylation of τau protein. Evidence for risk factors triggering this devastating disease focuses on type II diabetes. Therefore, modelling Alzheimer disease tries to get profound knowledge of these underlying mechanisms by studying experimental animal models. Here will be discussed in detail two

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pharmacological models, the one mirroring type II diabetes-induced AD pathology by streptozotocin and its influences on the insulin/insulin receptor cascade as well as ß-amyloid and τau pathologies. Behavioral studies give evidence that this model is currently the most detailed described AD model. While transgenic mouse models, like the APP Tg2576 model, demonstrate ß-amyloid plaque formation and impaired memory rather in old age, streptozotocin is able to aggravate the process of pathology so that AD pathology is seen months earlier. This indicates a profound interaction of AD pathology with the insulin/insulin receptor cascade and pathobiochemistry.

Modelling cholinergic deficits has been done by using the cholinotoxin AF64A. This model reflects changes in the acetylcholine metabolism as well as in monoaminergic transmitter systems, and behavioral studies are in line with AD-based impairment of learning and memory. However, ß-amyloid and τau pathologies as well as insulin-resistant brain state have not been studied in detail.

Keywords
AF64A model • Alzheimer disease • Dementia model • Streptozotocin • Transgenic dementia models • Type II diabetes

1 Introduction

Any model of a disease should reflect the core abnormalities of that disease in morphobiological and clinical terms. In case of Alzheimer disease (AD), it has to be taken into account that AD is not a single disorder from a nosological point of view. A very small proportion of 513 families worldwide (by August 2011) are suffering from AD caused by missense mutations: in the amyloid precursor protein (APP) gene on chromosome 21 (89 families ∼ 17.3 %), in the presenilin gene 1 on chromosome 14 (402 families ∼ 78.4 %), and in the presenilin gene 2 on chromosome 1 (22 families ∼ 4.3 %) (http://www.molgen.ua.ac.be/admutations). These currently known three mutations are involved in both the overexpression and the abnormal cleavage of APP and the increased formation of the APP derivative ß-amyloid (Aß1–40, Aß1–42/43), a keystone for the amyloid hypothesis of AD (Sandbrink et al. 1996; Hardy and Selkoe 2002; Selkoe 2003). Clinically, the dementia symptoms may become evident as early as in the third decade of life.

In contrast, in sporadic AD (sAD), which approximately more than 15 million people worldwide suffer from (Blennow et al. 2006), no such mutations were found as yet, i.e., the mutation-related stimulus is lacking, and its role in the causation of sAD remains enigmatic (Hoyer 1993; Joseph et al. 2001). Also, in longitudinal studies on aged nondemented, cognitively normal subjects, abundant neuritic plaques and neurofibrillary tangles were found in the brain (Crystal et al. 1988; Davis et al. 1999; Snowdon 1997). Moreover, the decline of the mental status starting in later life and followed over several years was demonstrated not to be

accompanied by changes in the numerical density of plaques and tangles (Bennett et al. 1993; Davies et al. 1987; Mann et al. 1985). Finally, it was the neuronal loss rather than neuritic plaques and neurofibrillary tangles that directly contributed to cognitive damage (Gomez-Isla et al. 1997). Also, in postmortem sAD brain, Aß42 immunization over 84 weeks induced a marked reduction of amyloid plaques. However, this clearance did not prevent both the progression of dementia and severe end-stage dementia before death (Holmes et al. 2008). Overall, the above findings clearly indicate that sAD is due to risk factors other than mutations in the APP gene or other genes.

Among the risk factors under discussion are, first, age as a leading one (Evans et al. 1989; Ott et al. 1995); second, a single nucleotide polymorphism found in the gene coding for 11ß-hydroxysteroid dehydrogenase I, which is associated with a sixfold increased risk for sAD (de Quervain et al. 2004); and, third, allelic abnormalities of the APO-E 4 gene on chromosome 19 (Mahley and Rall 2000; Tanzi and Bertram 2001). People carrying one copy of this allelic abnormality (around 24 % of the population) or carrying two copies (around 2–3 % of the population) were found to show drastic abnormalities in the cerebral metabolic rate of glucose (Corder et al. 1993), i.e., the nutritional state of the brain may be severely perturbed under these conditions.

The latter data may indicate that the cerebral glucose metabolism may be of pivotal significance. In the normal mature mammalian brain, oxidative glucose/energy metabolism is largely under the control of the neuronal insulin signal transduction cascade (Hoyer 2004a; Maurer and Hoyer 2006). This metabolic pathway is the source of acetyl-CoA necessary for the formation of the neurotransmitter acetylcholine and of ATP which drives nearly all cellular and molecular processes (for a review, see Salkovic-Petrisic et al. 2009). In patients suffering from very early sAD, the cerebral glucose metabolism was found to be reduced in the posterior cingulate cortex (Minoshima et al. 1997) whereby the impairment of cerebral glucose metabolism was found to be of predictive significance (Mosconi 2005). As sAD progresses, the abnormalities in cerebral glucose utilization grow more severe, whereas cerebral blood flow and oxygen consumption of the brain were found to be much less perturbed (Foster et al. 1984; Hoyer et al. 1991; Mielke et al. 1994), i.e., the abnormal glucose metabolism in the brain may be in the center of metabolic abnormalities in sAD, although the glucose concentration in arterial blood was found to be unchanged (Fig. 1).

2 Relevant Animal Models

Based on the latter, cerebral glucose metabolism may be experimentally damaged via the insulin receptor in the brain by two different ways: first, by chronic elevation of the circulating corticosterone (cort) concentration and, second, by direct functional damage by the diabetogenic substance streptozotocin (STZ) applied to the brain only.

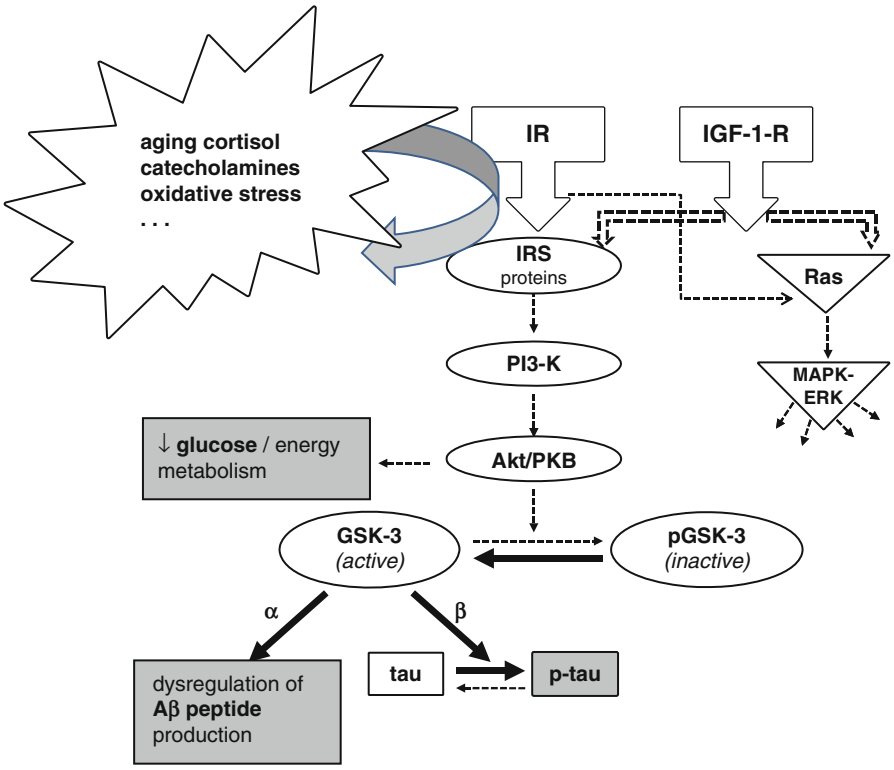

Fig. 1 *Insulin receptor signalling in the insulin-resistant brain state*. Insulin receptor (IR) signalling in the brain may be affected by various factors: physiological and pathophysiological conditions like aging, cortisol, catecholamines, and oxidative stress (as reviewed elsewhere, Hoyer 2004, Salkovic-Petrisic and Hoyer 2007). They may act at the level of the IR and its tyrosine kinase activity and/or at any other element downstream the IR signalling cascade (insulin receptor substrate/IRS) leading to its dysfunction which is finally expressed as the insulin-resistant brain state (IRBS), found in both sAD humans and respective animal models (Hoyer 2004; Salkovic-Petrisic and Hoyer 2007). Namely, the target elements downstream the IR signalling pathways, in particular the PI3K pathway parameters presented at the figure, behave like they are resistant to the influence of insulin due to the reduction/loss of physiological insulin functioning. Low activity of AKT/PKB protein kinase, induced by upstream IR signalling malfunctioning, leads to (i) a decrease in glucose/energy metabolism via glucose transporters and (ii) less phosphorylation and by that activation of glycogen synthase kinase-3 (GSK-3) isoforms α and β. Activated GSK-3α is involved in the dysregulation of β-amyloid peptide (Aβ) production, while activated GSK-3β favors hyperphosphorylation of tau protein, all being a hallmark of sAD pathology. In the STZ-icv rat model, IRBS is induced by the icv administration of low doses of STZ, a drug selectively toxic to glucose transporter-2 and IR expressing cells (Szkudelsky 2001). *Solid arrows*, function stimulated; *dashed arrows*, function inhibited

2.1 Corticosterone Model

It has been well documented that chronic elevation of cort in circulating blood induced a strong increase of cort in both cerebral cortex and hippocampus associated with both learning and memory deficits (Wüppen et al. 2010;

Hoyer and Lannert 2008; Feldmann et al. 2008). This state can be achieved by prenatal stress of (rat) dams during the last week of pregnancy and postnatal daily handling of the pups during the first 3 weeks of life. However, cognitive alterations in Y maze and radial maze were observed only in later life (Steen et al. 2005). Chronic glucocorticoid administration (as a stress model) reduced insulin-degrading enzyme (IDE) protein levels and IDE mRNA in different regions of aged macaque brain associated with increased Aß42 levels relative to Aß40 levels (Rivera et al. 2005).

Exposure of 3 T3-L1 cells to dexamethasone resulted in decreased insulin stimulation of glucose uptake (Moloney et al. 2010). This generation of an insulin-resistant brain state was found to be characterized by reduced gene expression of insulin and insulin receptor density and associated by increased tau protein gene expression (Vallee et al. 1999). Additionally, cerebral glucose metabolism was shown to be markedly reduced by corticosterone (Hoyer and Lannert 2008; Kulstad et al. 2005).

As related to human beings, a disinhibition of the HPA axis causes hypercortisolemia in sAD (Grünfeld et al. 1981), and hypercortisolemia may be supplemented by 11ß-hydroxysteroid dehydrogenase to amplify glucocorticoid action in sporadic AD (Grünfeld et al. 1981) to increase its risk.

2.2 Streptozotocin Model

With respect to insulin/insulin receptor (IR) in sAD brain, a decrease was found in both insulin concentration and tyrosine kinase activity along with an increase in IR density (Frölich et al. 1998). More recent studies demonstrated a drastic reduction of both the expression of insulin and IGF-1 and the respective receptors in different areas of the AD brain accompanied by a reduced insulin signal transduction capacity (Plaschke et al. 1996), whereby the expression of insulin, IGF-1 and IGF-2, and their receptors decreased with advancing AD (Pascualy et al. 2000). Intraneuronally, alterations were found in a predominance at internal and nuclear sites and in a diminution at cytoplasmic and dendritic sites (Frölich et al. 1998). These above findings may point to the neuronal insulin/insulin receptor system as the site of the predominant and early abnormality in sAD, i.e., an insulin-resistant brain state may be assumed.

It is difficult to establish an experimental animal model that would faithfully mimic the developmental pathology of AD in humans, in particular the pathology of the prevailing sporadic form, the causation of which is still unclear. The streptozotocin (STZ) model that will be described here has actually been reported in literature more than 20 years ago (Mayer et al. 1990), but the proposal that it could be considered as a model for the sAD came some 8 years afterwards (Lannert and Hoyer 1998).

STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose)) is a betacytotoxic drug which, following peripheral (parenteral) administration at high doses, selectively destroys insulin-producing/insulin-secreting β cells in the pancreas and causes type I diabetes mellitus in adult animals (Szkudelski 2001). Treatment with

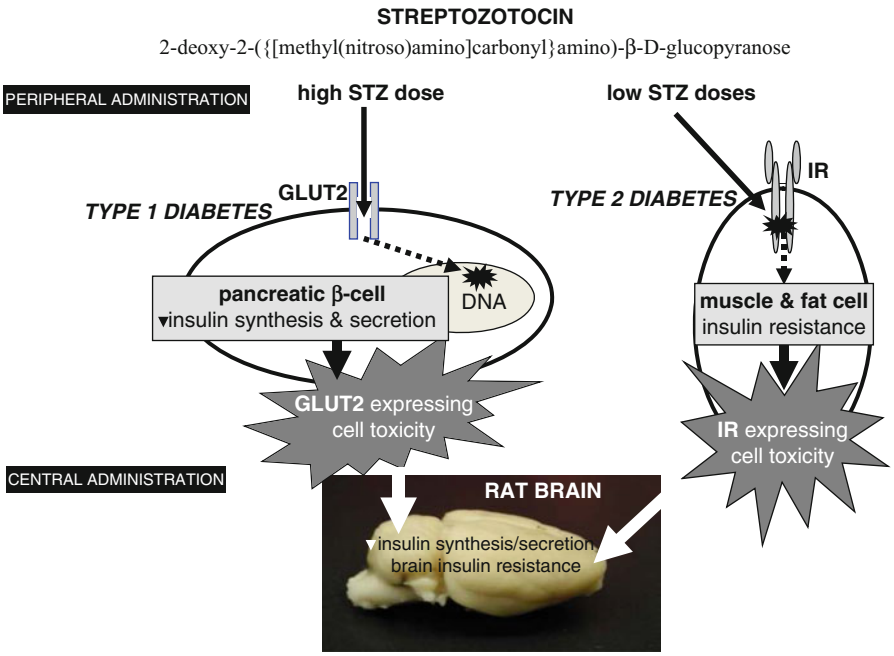

Fig. 2 *Mechanism of action of streptozotocin*. Due to its chemical structure (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose)), streptozotocin (STZ) is a selective substrate of the glucose transporter-2 (GLUT2) which at the periphery enables entry of this compound into the insulin-producing/insulin-secreting cells, the highest expression of which is on the membrane of pancreatic β cells (Szkudelsky 2001). Following peripheral (parenteral) administration at high doses, STZ selectively destroys insulin-producing/insulin-secreting β cells in the pancreas and causes type I diabetes mellitus in adult animals (Szkudelsky 2001). Treatment with low to moderate doses of STZ causes insulin resistance (Blondel and Portha 1989) via a decrease in autophosphorylation (Kadowaki et al. 1984) and an increase in total number of insulin receptor (IR), but with little change in phosphorylated IR-β subunit (Giorgino et al. 1992). The mechanism of central STZ action and its target cells/molecules in the brain have not been identified yet, but given the presence and the regionally specific distribution of GLUT2 and IR in the mammalian brain, similar mechanism may have been speculated to induce the insulin-resistant brain state following the STZ-icv administration

low to moderate doses of STZ has been demonstrated to cause insulin resistance (Blondel and Portha 1989) via a decrease in autophosphorylation (Kadowaki et al. 1984) and an increase in the total number of IRs, but with little change in phosphorylated IR-β subunit (Giorgino et al. 1992) (Fig. 2). Considering this selectivity to insulin system-related cells and a presence of insulin and IRs in the brain, the idea of the first experiments performed in the 1990s was that the central application of low STZ doses (up to 100x lower than peripheral ones) into the lateral ventricles (icv) might be associated with a kind of local, cerebral diabetes which might be reflected on behavior and brain neurochemistry. Among all changes, cognitive deficits were demonstrated first in STZ-icv-treated

rats (Mayer et al. 1990) and were followed by reports of changes in brain glucose metabolism (Nitsch and Hoyer 1991; Duelli et al. 1994). In spite of that, it has been repeatedly demonstrated that central STZ administration causes neither systemic metabolic changes nor diabetes mellitus (as reviewed by Salkovic-Petrisic and Hoyer 2007). Generally, in the research published so far, STZ has been administrated icv into one or both ventricles of adult rats, mostly in a 1–3 mg/kg dose. There are only a few reports of intracortical administration of low STZ doses (40 μg/kg) to 3-day-old rat pups (Lester-Coll et al. 2006; de la Monte et al. 2006) and recent reports of STZ-icv administration to mice in doses similar to those in rats (Plaschke et al. 2010a; Kumar et al. 2010; Pinton et al. 2011). Variation in the susceptibility of individual animals as a characteristic feature of STZ-icv treatment has been reported by some authors (Blokland and Jolles 1993, 1994; Prickaerts et al. 2000). STZ-icv-induced effects have not been related to the direct nonspecific toxic effect of STZ at the site of drug administration because identical biochemical changes were found in the left and right striatum after unilateral administration of STZ to the right lateral cerebral ventricle (Salkovic-Petrisic et al. 1995). Further support to the specific effect of STZ within the brain has come from a number of experiments which have shown that changes in neurochemistry and morphology are not generalized all over the brain but regionally specific and pronounced at most in the hippocampus (as reviewed by Salkovic-Petrisic and Hoyer 2007).

Due to its chemical structure, STZ is a selective substrate of the glucose transporter GLUT2 which at the periphery enables entry of this compound into the insulin-producing/insulin-secreting cells, the highest expression of which is on the membrane of pancreatic β cells (Szkudelsky 2001). The mechanism of central STZ action and its target cells/molecules in the brain have not been identified yet, but given the presence and the regionally specific distribution of GLUT2 in the mammalian brain as well as its expression in glucose-sensing brain neurons (Brant et al. 1993; Leloup et al. 1994; Ngarmukos et al. 2001; Arluison et al. 2004a, b), similarity to the peripheral mechanism and/or cellular targets seems likely. STZ is a cytotoxic compound, and in vitro experiments on β cells show that within the cell it causes alkylation of β-cell DNA which triggers activation of poly ADP-ribosylation, leading to depletion of cellular NAD^+ and ATP (Szkudelski 2001). Decreased levels of ATP have indeed been reported in the particular brain regions following STZ-icv treatment (Nitsch and Hoyer 1991; Lannert and Hoyer 1998), further supporting the similarity between the peripheral and the central STZ mechanisms of action.

The 20-year-long research of STZ-icv rat model has extensively documented several behavioral, neurochemical, and structural features which resemble those found in human sAD patient brain post-mortem. However, knowledge about glucose metabolism and cholinergic deficit induced by STZ-icv administration and behavioral changes has not made much progress over the years. Nevertheless a significant contribution to the characterization of this model has been provided by revealing of the alterations in the brain insulin system and tau and β-amyloid (Aβ) pathologies, which will be elaborated further on in this text.

The main clinical feature in sAD patients is progressive memory loss, and the representative experimental model of this disease should manifest similar cognitive changes. Deficits in learning and memory functions, usually recorded in the Morris water maze swimming and passive avoidance tests, have been consistently reported and observed as early as 2 weeks after the STZ-icv administration, persisting up to 12 weeks posttreatment (the longest observational period reported until now) (Lannert and Hoyer 1998; Salkovic-Petrisic et al. 2006; Shoham et al. 2007; Grünblatt et al. 2007). The former test is measuring the spatial learning and memory function, while the latter is testing the memory acquisition of the painful stimulus which should be avoided. By means of these tests, cognitive deficits have been found regardless of age in both 1–2-year- and 3-month-old rats (as reviewed by Salkovic-Petrisic and Hoyer 2007). Some correlation between the spatial discrimination performance in the Morris water maze task and the decrease in hippocampal choline acetyltransferase (ChAT) activity has been found in STZ-icv-treated rats (Blokland and Jolles 1993, 1994), although no signs of reduction in specific cholinergic markers were detected in the cortex or hippocampus prior to cognitive deficit appearance (Shoham et al. 2007). The exact mechanism by which STZ-icv treatment impairs cognitive functions has still not been elucidated, but factors like direct neurotoxicity, brain glucose/energy and cholinergic deficits, oxidative stress, and insulin-resistant brain state, all found in the STZ-icv rat model, may form the biological basis for the marked reduction in learning and memory capacities found in this model.

At the neurotransmission level, similarity between the human sAD and the STZ-icv rat model has been demonstrated in altered cholinergic neurotransmission in the brain. Significant increase in the activity of acetylcholinesterase (AChE), the enzyme responsible for a rapid acetylcholine degradation, has been found from 1 to 3 weeks following the STZ-icv treatment in adult rats (Sonkusare et al. 2005; Ishrat et al. 2006; Tota et al. 2011), rat pups (Lester-Coll et al. 2006; de la Monte et al. 2006), and mice (Kumar et al. 2010), generally associated with a decrease in the activity of ChAT, the enzyme involved in acetylcholine synthesis (Hellweg et al. 1992; Blokland and Jolles 1993, 1994; Prickaerts et al. 1999; Terwel et al. 1995).

Glucose represents the primary energy source for the brain, and brain neurons are unable to synthesize or store glucose but depend on glucose transport across the blood–brain barrier and then into the neurons. Three weeks following the STZ-icv administration, concentrations of glucose and ADP, as well as glycogen levels, were increased in the cerebral cortex (Nitsch and Hoyer 1991), and glucose metabolism was significantly decreased (44 %) (Pathan et al. 2006). Six weeks following STZ-icv treatment, reduced glucose utilization (up to 30 %) was found in 17 of 35 brain areas, particularly the frontal, parietal, sensory motor, auditory, and entorhinal cortices and in all hippocampal subfields (Duelli et al. 1994). Additionally, significant decreases in activities of glycolytic key enzymes were found in the brain cortex and hippocampus (Plaschke and Hoyer 1993). Recent data has demonstrated in vivo spatial distribution of glucose hypometabolism induced by STZ-icv administration (2 mg/kg) in cynomolgus monkeys similar to that at early

stages of sAD patients, which in the monkeys persists both 6 and 12 weeks following the STZ-icv treatment (Heo et al. 2011).

STZ generates intracellular free radicals: nitric oxide (NO) and hydrogen peroxide (Szkudelski 2001). Estimations of oxidative stress induced by STZ-icv treatment commonly utilize the measurement of malondialdehyde levels (MDA), a product of lipid peroxidation used as an indicator of free radical generation, and glutathione levels, an endogenous antioxidant that scavenges free radicals and protects against oxidative stress. Significant elevations of MDA levels and decreased glutathione levels have been found in the brain up to 8 weeks following the STZ-icv treatment (Sharma and Gupta 2001, 2002; Ishrat et al. 2006; Pathan et al. 2006; Shoham et al. 2007; Kumar et al. 2010; Saxena et al. 2011) demonstrating resemblance to the oxidative stress condition in the human sAD patients post-mortem (Götz et al. 1992; Gsell et al. 1995).

Morphological changes in the brain of the STZ-icv rat model have been investigated in much lesser extent than the behavioral and neurochemical ones. The earliest study published on that suggested that a single STZ-icv dose (1.5 mg/kg) might cause neuronal damage indicated by a reduction of 30–50 % in the weight of the septum (Terwel et al. 1995). The first more direct evidence of specific neuronal damage after the STZ-icv injection has been provided by the group of Weinstock and co-workers. Using silver impregnation staining, they found that three consecutive STZ-icv injections (in total 3 mg/kg dose) caused degeneration in the fornix, a loss of white matter, and occasional clusters of myelinated axons with distorted morphology, 40 days after the first injection (Weinstock and Shoham 2004). At that posttreatment time point, selective injury to myelin and axons in the fornix and hippocampus was found in association with the activation of microglia, as demonstrated by the increased expression of the glial fibrillary acidic protein (GFAP), a marker of astrogliosis (Prieckaerts et al. 2000). The 3rd ventricle was found enlarged by 100–150 % because of a loss of ependymal cells and damage to hypothalamic periventricular myelin (Shoham et al. 2003). Their work published afterwards indicated that activated microglia and astrocytes found in the cortex, around the cannula penetration area, in the hippocampal CA1 region, corpus callosum, and medial and lateral septum, could be found already 1 week after a single STZ-icv injection (1.5 mg/kg), while 8 weeks after the injection, the whole area of the cannula penetration site contained scar tissue that was filled with tightly packed nonreactive astrocytes and no reactive microglia around (Shoham et al. 2007). Inflammatory processes, reduced neuronal density, and axonal neurotoxicity following the STZ-icv treatment have also been confirmed by recent reports (Dhull et al. 2012; Diwu et al. 2011) in addition to the report of decreased hippocampal neurogenesis found 1 month following the treatment (Qu et al. 2012). Reduced expression of neuronal- and oligodendroglia-specific genes and increased expression of genes encoding GFAP and microglia-specific proteins were also found in rat pups administered with STZ intracortically (STZ-ic) (Lester-Coll et al. 2006).

With regard to the ultrastructural changes induced by the STZ-icv treatment, a significant enlargement of the Golgi apparatus in the frontoparietal cortical neuronal cells caused by 2x-fold expansion of the trans-Golgi segment of the

cellular protein secretory pathway was found 3 weeks after STZ-icv injection (Grieb et al. 2004). Considering that proamyloidogenic processing of β-amyloid precursor protein may occur preferentially in the trans-Golgi segment, the observed early response of neuronal ultrastructure might be of importance for predisposing cells to form β-amyloid deposits.

Demonstration of structural changes in the brain of the STZ-icv-treated rats manifested as Aβ pathology has been a great step in the process of the characterization and validation of this non-transgenic sAD model. Pathological Aβ aggregation in the form of cerebral amyloid angiopathy was visualized by Congo red staining in meningeal capillaries but not earlier than 3 months after the STZ-icv injection (Salkovic-Petrisic et al. 2006) and was further supported by positive Aβ1–42 immunolabelling found in the blood vessel wall at that time point (Salkovic-Petrisic et al. 2011). In the recently published paper, thioflavine-S and Congo red staining revealed diffuse congophilic deposits in the wall of meningeal and cortical blood vessels both 6 and 9 months after the STZ-icv treatment (Salkovic-Petrisic et al. 2011), suggesting a process of progression of the cerebral amyloid angiopathy. However, no diffuse or compact amyloid plaques and neurofibrillary tangles could be detected in the rat brain up to 3 months after icv STZ treatment (Salkovic-Petrisic et al. 2006), while some preliminary work of the same authors suggests a possibility of development of the primitive plaque-like formations appearing from 6 month post-STZ-icv treatment onward (unpublished data). A positive Aβ42 immunosignal has been found in the brain of the STZ-intracerebrocortically treated rat pups (Lester-Coll et al. 2006). These results provide further evidence of the resemblance of the STZ-icv rat model to the human sAD patient pathophysiology and strongly suggest that there is a need to characterize the post-STZ-icv time dependency of the development of amyloid pathology in the brain of this non-transgenic model in which clear differences in comparison to the APP transgenic mice models become obvious.

None of the previously explored alterations were able to provide a missing link to the primary event which could initiate the pathological hallmarks of sAD, hyperphosphorylated tau protein in neurofibrillary tangles, and aggregated Aβ peptide in amyloid plaques. Evidence has gathered indicating that central STZ administration induces region-specific and, for some parameters, time-progressive dysfunction in the rat brain insulin signalling, i.e., an insulin-resistant brain state (IRBS) which highly resembles those found in sAD patients post-mortem (Hoyer 2004; Cole and Frautschy 2007; Qiu and Folstein 2006; Watson and Craft 2006; Wada et al. 2005), suggesting the IRBS might be the missing link. Insulin in the brain binds to IRs which have been found functionally linked to improved cognition, in particular general and spatial memory (Zhao et al. 1999; for review Park 2001; Zhao et al. 2004). Activated IR tyrosine kinase, in addition to the protein kinase (MAPK) mitogen pathway, activates also the phosphatidylinositol-3 kinase (PI3K) pathway and its downstream elements (Johnston et al. 2003), protein kinase B (Akt/PKB) which phosphorylates and consequently inactivates both α and β cytosolic forms of glycogen synthase kinase-3 (GSK-3) (Cross et al. 1995), involved in regulation of APP metabolism and Aß peptides production

(Phiel et al. 2003) and in tau protein phosphorylation (Ishiguro et al. 1993), respectively. IRBS in the STZ-icv rat model is manifested as decreased expression of the insulin genes 1 and 2, as well as the IR gene mostly pronounced in hippocampus but present also in the cerebral cortex, 2 and 12 weeks following the drug administration (Agrawal et al. 2011; Grünblatt et al. 2007). This is followed by decrement in IR-β protein level and unchanged Akt/PKB protein expression at 4 weeks but decreased by 12 weeks posttreatment (Salkovic-Petrisic et al. 2006). Recent data demonstrates significant decrease in IR protein expression, phosphorylation of its downstream substrate (IRS-1), and Akt/PKB in hippocampal CA3 region of STZ-icv-treated rats already 2 weeks following the treatment compared to the control rats subjected to cognitive training (Agrawal et al. 2011). Decreased phosphorylation of PI3K has been also demonstrated in cerebral cortex 3 weeks following the STZ-icv treatment in rats (Deng et al. 2009). Downstream the IR–PI3K pathway, IRBS is manifested by unchanged total GSK-3β levels and decreased p-GSK-3β levels with consequently decreased p-GSK-3/GSK-3 ratio in the hippocampus and cerebral cortex, found 2, 3, and 12 weeks following STZ-icv treatments, indirectly suggesting increased GSK-3β activity (Salkovic-Petrisic et al. 2006; Deng et al. 2009; Ponce-Lopez et al. 2011). In line with dysfunction in the upper part of IR–PI3K signalling pathway manifested already 2 weeks after the STZ-icv treatment, IRBS seems to eventually progress to its lower parts with increased hippocampal expression of tau protein and the p-tau/tau ratio being manifested 4 and 12 weeks following STZ-icv treatment (Salkovic-Petrisic et al. 2006; Grünblatt et al. 2007). Similar IRBS condition with altered IR–PI3K signalling pathway has been also found in STZ-intracortically treated rat pups (Lester-Coll et al. 2006). Recent data demonstrated that the STZ-icv treatment effects on the IR–MAPK pathway might be also time and region dependent as decreased levels of phospho-ERK were found in the cerebrum 3 weeks after the treatment (Deng et al. 2009) while phospho-ERK and its upstream element, phospho-Shc, were found unchanged 2 weeks after the treatment in the hippocampus (Agrawal et al. 2011). Disturbances in insulin action, IR function, and downstream signalling pathways have been found post-mortem in the brain of sAD patients (Hoyer 2004; Cole and Frautschy 2007; Qiu and Folstein 2006; Watson and Craft 2006; Wada et al. 2005; Frölich et al. 1998), but it was only the STZ-icv rat model which has provided evidence that IRBS forms the core of the sAD pathophysiology by preceding and eventually triggering the sAD-like pathology. This hypothesis seems to gain more and more attention in the sAD research field, in particular now when the "amyloid cascade" hypothesis has been argued and the idea of causally different sAD forms is starting to be considered (Correia et al. 2011).

To conclude, a growing body of evidence indicates similarities in cognitive deficits, brain neurochemical and structural pathology between the sAD patients and STZ-icv rats which have been proposed as a non-transgenic model of sAD. Considering the recognition of the IRBS condition in the sAD patients post-mortem, the development of IRBS following the STZ-icv treatment suggests that the STZ-icv rat model might be a promising experimental tool in both the sAD etiopathogenesis research and novel drug testing. Preliminary data on IRBS being

the primary event in this non-transgenic model which could eventually progress and initiate the pathological hallmarks of sAD might provide a new perspective in the research of early diagnosis of this disease in humans. Considering the literature data on environmental and food contaminant exposures to compounds structurally related to STZ, the hypothesis that environmental toxins might play a significant role in the sAD etiopathogenesis should not be neglected (Landrigan et al. 2005; Tong et al. 2009; de la Monte and Tong 2009; de la Monte et al. 2009). In line with that, the STZ-icv rat model provides a wide range of application in the translational neuroscience, and hopefully its further characterization and validation, in particular regarding the long-term follow-up, will bring about the change in elucidating of the sAD enigma.

2.3 Streptozotocin-icv + Transgenic Mice

Considering the STZ-icv rat as a model for the sporadic form and the transgenic (Tg) mice overexpressing beta-amyloid protein precursor (APP Tg2576) as a model for the familial (hereditary) form of AD (Hsiao et al. 1996), the question has arisen on how the STZ-icv treatment would affect the Alzheimer-like pathology in the latter model. Generally, in APP Tg2576 mice, a significant increase in soluble Aβ1–40 and Aβ1–42/43 peptides is found from 3 to 9–10 months of age, and the senile amyloid plaques are formed at the age of 1 year and older (Hsiao et al. 1996), while the impaired spatial memory is found at the age of 9–10 months and later (Ashe 2001). STZ-icv treatment of 3-month-old APP Tg2576 mice induced increased mortality, and measurements done at the age of 9 months (6 months after the STZ-icv treatment) demonstrated reduced spatial cognition and increased cerebral aggregated Aβ fragments, total tau protein, and congophilic amyloid deposits in comparison to the untreated APP Tg2576 mice (Plaschke et al. 2010b). These changes were associated with decreased GSK-3α/GSK-3β ratio (phosphorylated/total) and additionally with a linear negative correlation between Aβ1–42 and cognition and between GSK-3α/GSK-3β ratio and aggregated Aβ1–40 + 1–42, indicating that the STZ-icv treatment induced significant aggravation of the Alzheimer-like pathology in this transgenic mice model of familial AD.

2.4 Cholinotoxin Model

One of the oldest theories of the AD etiopathogenesis proposed the cholinergic deficits, demonstrated as a dramatic decrease of ChAT in the hippocampus and frontal cortex and the marked reduction in cholinergic neuron counts in the nucleus basalis in AD patient brains post-mortem, to be the core in the AD pathophysiology (Greenwald and Davis 1983). Importantly, ChAT deficit has been shown to correlate with histopathologic changes in the brain and psychologic test scores in AD patients (Greenwald and Davis 1983). In line with that, the putative cholinergic

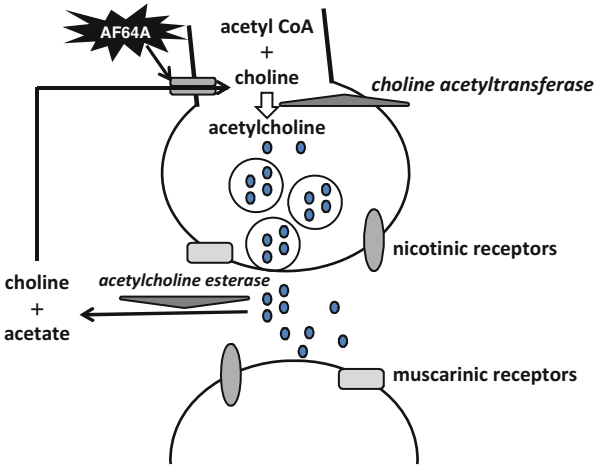

Fig. 3 *Mechanism of action of cholinotoxin AF64A in the cholinergic synapse*. Acetylcholine is synthesized in the presynaptic cholinergic neuron from acetyl coenzyme A (acetyl-CoA) and the precursor choline by means of the choline acetyltransferase. Degradation of acetylcholine in the synaptic cleft is mediated by the acetylcholine esterase, generating acetate and choline. The uptake of choline back into the presynaptic nerve ending is mediated by the high-affinity choline membrane transporter, and this process provides the only source of choline necessary for the acetylcholine synthesis. AF64A is an ethylcholine mustard aziridinium ion, a neurotoxic choline analogue, which is taken up by the high-affinity choline transport system into cholinergic neurons (Fisher et al. 1982)

neurotoxin AF64A was injected intracerebroventricularly in order to determine whether it would produce specific damage to the cholinergic cell bodies and thus be useful in developing animal models of AD (Fig. 3). AF64A is an ethylcholine mustard aziridinium ion, a neurotoxic choline analogue, which is taken up by the high-affinity choline transport system into cholinergic neurons (Fisher et al. 1982). Intracerebroventricular administration of 65 nmol of AF64A to mice was not acutely lethal, but produced delayed behavioral effects similar to those found after parenteral administration of AF64A (ataxia, hypokinesia, weight loss) (Fisher et al. 1982). Seven days following the icv treatment, there was a significant decrease in acetylcholine content in the cortex, striatum, and hippocampus, but no change in choline levels. Acetylcholine content was still significantly reduced in the hippocampus at 3 weeks after this treatment (Fisher et al. 1982). The reduction in activity of choline acetyltransferase and high-affinity choline transport paralleled the reduction in acetylcholine measured at 7 days post AF64A treatment, whereas muscarinic receptors in all three brain areas were unchanged (Fisher et al. 1982). Data has indicated that AF64A is a presynaptic chemical neurotoxin, capable of inducing a persistent deficiency in central cholinergic transmission. The work that followed has demonstrated that AF64A-icv injections in addition to causing cholinergic damage in the septum cause also glial lesions in the lateral septal nucleus and in the lateral zones of the hippocampus (Dudas et al. 2004). Small amounts of

AF64A (0.01 nmol/μL) injected in each ventricle had little effect on the appearance of the nucleus basalis of Meynert (nbM) or on the ChAT levels in the cortex (Kozlowski and Arbogast 1986). Larger amounts of AF64A (0.02 and 0.05 nmol/μL and 0.02 nmol/10 μL, respectively) produced a loss of diffuse acetylcholinesterase (AChE) staining in the nbM and a loss of large positively staining neurons as well as a significant reduction of ChAT activity in the central portion of the cortex without affecting non-cholinergic neurons (Kozlowski and Arbogast 1986). ChAT activity in the AF64A rat model was preferentially reduced in tissue samples of the dorsal compared to the ventral hippocampus (Ayala-Grosso and Urbina-Paez 1999). At the gene level, AF64A-icv injection (2 nmol) was accompanied by a tenfold increase in the AChE mRNA and a twofold decrease in the butyrylcholinesterase (BChE) and AChE mRNA expression in the hippocampus by day 7 with the latter remaining decreased until day 60 posttreatment (Lev-Lehman et al. 1994). AF64A treatment affects also monoaminergic transmission in the brain, as demonstrated by a dose-dependent decrease in noradrenaline level and increased level of its metabolite in the hippocampus 2 days after the treatment (Hörtnagl et al. 1989) and increased serotoninergic neuronal activity and decreased densities of 5-HT uptake sites in different brain regions (Park and Lim 2010).

AF64A-treated rats exhibit learning and memory deficits which have been recorded in different cognitive tests (Nakahara et al. 1988; Lim et al. 2001; Mukhina et al. 2004). In the Morris water maze, these rats showed the delayed latencies to find the platform from the 6th day after the infusion, while in pretrained rats, AF64A treatment caused the significant delay of latency at the 7th day, but not the 8th day (Lim et al. 2001). In the passive avoidance test, AF64A-treated rats shortened the latency 1.5 h but not 24 h after the electroshock, suggesting that AF64A impairs the learning and memory but also that the disturbed memory might rapidly recover after the first retrain (Lim et al. 2001). No literature data are available regarding the amyloid and tau protein pathologies as well as the IRBS in the cholinotoxin AF64A animal model of AD which has not been frequently used in the contemporary AD research.

References

Agrawal, R., Tyagi, E., Shukla, R., & Nath, C. (2011). Insulin receptor signaling in rat hippocampus: A study in STZ (ICV) induced memory deficit model. *European Neuropsychopharmacology, 21*, 261–273.

Arluison, M., Quignon, M., Thorens, B., Leloup, C., & Penicaud, L. (2004a). Immunocytochemical localization of the glucose transporter 2 (GLUT2) in the adult rat brain. II. Electron microscopic study. *Journal of Chemical Neuroanatomy, 28*, 137–146.

Arluison, M., Quignon, M., Nguyen, P., Thorens, B., Leloup, C., & Penicaud, L. (2004b). Distribution and anatomical localization of the glucose transporter 2 (GLUT2) in the adult rat brain–an immunohistochemical study. *Journal of Chemical Neuroanatomy, 28*, 117–136.

Ashe, K. H. (2001). Learning and memory in transgenic mice modeling Alzheimer's disease. *Learning and Memory, 8*, 301–308.

Ayala-Grosso, C. A., & Urbina-Paez, R. (1999). Septohippocampal adaptive GABAergic responses by AF64A treatment. *Journal of Neuroscience Research, 55*, 178–186.

Bennett, D. A., Cochran, E. J., Saper, C. B., Leverenz, J. B., Gilley, D. W., & Wilson, R. S. (1993). Pathological changes in frontal cortex from biopsy to autopsy in Alzheimer's disease. *Neurobiology of Aging, 14*, 589–596.

Blennow, K., de Leon, M., & Zetterberg, H. (2006). Alzheimer's disease. *The Lancet, 368*, 387–403.

Blokland, A., & Jolles, J. (1993). Spatial learning deficit and reduced hippocampal ChAT activity in rats after an ICV injection of streptozotocin. *Pharmacology Biochemistry and Behavior, 44*, 491–494.

Blokland, A., & Jolles, J. (1994). Behavioral and biochemical effects of an ICV injection of streptozotocin in old Lewis rats. *Pharmacology Biochemistry and Behavior, 47*, 833–837.

Blondel, O., & Portha, B. (1989). Early appearance of in vivo insulin resistance in adult streptozotocin-injected rats. *Diabète & Métabolisme, 15*, 382–387.

Brant, A. M., Jess, T. J., Milligan, G., Brown, C. M., & Gould, G. W. (1993). Immunological analysis of glucose transporters expressed in different regions of the rat brain and central nervous system. *Biochemical and Biophysical Research Communications, 192*, 1297–1302.

Cole, G. M., & Frautschy, S. A. (2007). The role of insulin and neurotrophic factor signaling in brain aging and Alzheimer's disease. *Experimental Gerontology, 42*, 10–21.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., & Pericak-Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science, 261*, 921–923.

Correia, S. C., Santos, R. X., Perry, G., Zhu, X., Moreira, P. I., & Smith, M. A. (2011). Insulin-resistant brain state: The culprit in sporadic Alzheimer's disease? *Ageing Research Reviews, 10*, 264–273.

Cross, D. A. E., Alessi, D. R., Cohen, P., Andjelkovich, M., & Hemmings, B. A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated protein kinase. *Nature, 378*, 785–789.

Crystal, H., Dickson, D., Fuld, P., Masur, D., Scott, R., Mehler, M., Masdeu, J., Kawas, C., Aronson, M., & Wolfson, L. (1988). Clinico-pathologic studies in dementia: Nondemented subjects with pathologically confirmed Alzheimer's disease. *Neurology, 38*, 1682–1687.

Davies, C. A., Mann, D. M., Sumpter, P. Q., & Yates, P. O. (1987). A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. *Journal of the Neurological Sciences, 78*, 151–164.

Davis, D. G., Schmitt, F. A., Wekstein, D. R., & Markesbery, W. R. (1999). Alzheimer neuropathologic alterations in aged cognitively normal subjects. *Journal of Neuropathology and Experimental Neurology, 58*, 376–388.

de la Monte, S. M., & Tong, M. (2009). Mechanisms of nitrosamine-mediated neurodegeneration: Potential relevance to sporadic Alzheimer's disease. *Journal of Alzheimer's Disease, 17*, 817–825.

de la Monte, S.M., Tong, M., Lawton, M., & Longato, L. (2009). Nitrosamine exposure exacerbates high fat diet-mediated type 2 diabetes mellitus, non-alcoholic steatohepatitis, and neurodegeneration with cognitive impairment. *Molecular Neurodegeneration, 4*, 54. http://www.ncbi.nlm.nih.gov/pubmed/20034403. doi: 10.1186/1750-1326-4-54.

de la Monte, S. M., Tong, M., Lester-Coll, N., Plater, M., Jr., & Wands, J. R. (2006). Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: Relevance to Alzheimer's disease. *Journal of Alzheimer's Disease, 10*, 89–109.

de Quervain, D. J., Poirier, R., Wollmer, M. A., Grimaldi, L. M., Tsolaki, M., Streffer, J. R., Hock, C., Nitsch, R. M., Mohajeri, M. H., & Papassotiropoulos, A. (2004). Glucocorticoid-related genetic susceptibility for Alzheimer's disease. *Human Molecular Genetics, 13*, 47–52.

Deng, Y., Li, B., Liu, Y., Iqbal, K., Grundke-Iqbal, I., & Gong, C. X. (2009). Dysregulation of insulin signaling, glucose transporters, O-GlcNAcylation, and phosphorylation of tau and neurofilaments in the brain: Implication for Alzheimer's disease. *The American Journal of Pathology, 175*, 2089–2098.

Dhull, D. K., Bhateja, D., Dhull, R. K., Padi, S. S. (2012). Differential role of cyclooxygenase isozymes on neuronal density in hippocampus CA1 region of intracerebroventricular streptozotocin treated rat brain. *Journal of Chemical Neuroanatomy, 43*, 58–51.

Diwu, Y. C., Tian, J. Z., & Shi, J. (2011). Effects of Chinese herbal medicine Yinsiwei compound on spatial learning and memory ability and the ultrastructure of hippocampal neurons in a rat model of sporadic Alzheimer disease. *Zhong Xi Yi Jie He Xue Bao, 9*, 209–215.

Dudas, B., Hanin, I., Rose, M., & Wülfert, E. (2004). Protection against inflammatory neurodegeneration and glial cell death by 7beta-hydroxy epiandrosterone, a novel neurosteroid. *Neurobiology of Disease, 15*, 262–268.

Duelli, R., Schrock, H., Kuschinsky, W., & Hoyer, S. (1994). Intracerebroventricular injection of streptozotocin induces discrete local changes in cerebral glucose utilization in rats. *International Journal of Developmental Neuroscience, 12*, 737–743.

Evans, D. A., Funkenstein, H. H., Albert, M. S., Scherr, P. A., Cook, N. R., Chown, M. J., Hebert, L. E., Hennekens, C. H., & Taylor, J. O. (1989). Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. *JAMA : The Journal of the American Medical Association, 262*, 2551–2556.

Feldmann, R. E., Jr., Maurer, M. H., Hunzinger, C., Lewicka, S., Buergers, H. F., Kalenka, A., Hinkelbein, J., Broemme, J. O., Seidler, G. H., Martin, E., & Plaschke, K. (2008). Reduction in rat phosphatidylethanolamine binding protein-1 (PEBP1) after chronic corticosterone treatment may be paralleled by cognitive impairment: A first study. *Stress, 11*, 134–147.

Fisher, A., Mantione, C. R., Abraham, D. J., & Hanin, I. (1982). Long-term central cholinergic hypofunction induced in mice by ethylcholine aziridinium ion (AF64A) in vivo. *The Journal of Pharmacology and Experimental Therapeutics, 222*, 140–145.

Foster, N. L., Chase, T. N., Mansi, L., Brooks, R., Fedio, P., Patronas, N. J., & Di Chiro, G. (1984). Cortical abnormalities in Alzheimer's disease. *Annals of Neurology, 16*, 649–654.

Frölich, L., Blum-Degen, D., Bernstein, H. G., Engelsberger, S., Humrich, J., Laufer, S., Muschner, D., Thalheimer, A., Türk, A., Hoyer, S., Zöchling, R., Boissl, K. W., Jellinger, K., & Riederer, P. (1998). Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *Journal of Neural Transmission, 105*, 423–438.

Giorgino, F., Chen, J. H., & Smith, R. J. (1992). Changes in tyrosine phosphorylation of insulin receptors and a 170,000 molecular weight nonreceptor protein in vivo in skeletal muscle of streptozotocin-induced diabetic rats: Effects of insulin and glucose. *Endocrinology, 130*, 1433–1444.

Gómez-Isla, T., Hollister, R., West, H., Mui, S., Growdon, J. H., Petersen, R. C., Parisi, J. E., & Hyman, B. T. (1997). Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Annals of Neurology, 41*, 17–24.

Götz, M., Freyberger, A., Hauer, E., Heckers, S., Sofic, E., Jellinger, K., Hebenstreit, G., Beckmann, H., & Riederer, P. (1992). Susceptibility to brains from patients with Alzheimer's disease to oxygen-stimulated lipid peroxidation and differential scanning calorimetry. *Dementia, 3*, 213–222.

Greenwald, B. S., & Davis, K. L. (1983). Experimental pharmacology of Alzheimer disease. *Advances in Neurology, 38*, 87–102.

Grieb, P., Kryczka, T., Fiedorowicz, M., Frontczak-Baniewicz, M., & Walski, M. (2004). Expansion of the Golgi apparatus in rat cerebral cortex following intracerebroventricular injections of streptozotocin. *Acta Neurobiologiae Experimentalis (Wars), 64*, 481–489.

Grünblatt, E., Salkovic-Petrisic, M., Osmanovic, J., Riederer, P., & Hoyer, S. (2007). Brain insulin system dysfunction in streptozotocin intracerebroventricularly treated rats generates hyperphosphorylated tau protein. *Journal of Neurochemistry, 101*, 757–770.

Grunfeld, C., Baird, K., Van Obberghen, E., & Kahn, C. R. (1981). Glucocorticoid-induced insulin resistance in vitro: Evidence for both receptor and postreceptor defects. *Endocrinology, 109*(5), 1723–1730.

Gsell, W., Conrad, R., Hickethier, M., Sofic, E., Froelich, L., Wichart, I., Jellinger, K., Moll, G., Ransmayr, G., & Beckmann, H. (1995). Decreased catalase activity but unchanged superoxide

dismutase activity in brains of patients with dementia of Alzheimer type. *Journal of Neurochemistry, 64*, 1216–1223.

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science, 297*, 353–356.

Hellweg, R., Nitsch, R., Hock, C., Jaksch, M., & Hoyer, S. (1992). Nerve growth factor and choline acetyltransferase activity levels in the rat brain following experimental impairment of cerebral glucose and energy metabolism. *Journal of Neuroscience Research, 31*, 479–486.

Heo, J. H., Lee, S. R., Lee, S. T., Lee, K. M., Oh, J. H., Jang, D. P., Chang, K. T., & Cho, Z. H. (2011). Spatial distribution of glucose hypometabolism induced by intracerebroventricular streptozotocin in monkeys. *Journal of Alzheimer's Disease, 25*, 517–523.

Holmes, C., Boche, D., Wilkinson, D., Yadegarfar, G., Hopkins, V., Bayer, A., Jones, R. W., Bullock, R., Love, S., Neal, J. W., Zotova, E., & Nicoll, J. A. (2008). Long-term effects of Abeta42 immunisation in Alzheimer's disease: Follow-up of a randomised, placebo-controlled phase I trial. *The Lancet, 372*, 216–223.

Hörtnagl, H., Potter, P. E., Singer, E. A., Kindel, G., & Hanin, I. (1989). Clonidine prevents transient loss of noradrenaline in response to cholinergic hypofunction induced by ethylcholine aziridinium (AF64A). *Journal of Neurochemistry, 52*, 853–858.

Hoyer, S. (1993). Editor's Note for debate. Sporadic dementia of Alzheimer type: Role of amyloid in etiology is challenged. *Journal of Neural Transmission, 6*, 159–165 [P-D Sect].

Hoyer, S. (2004a). Glucose metabolism and insulin receptor signal transduction in Alzheimer disease. *European Journal of Pharmacology, 490*, 115–125.

Hoyer, S. (2004b). Causes and consequences of disturbances of cerebral glucose metabolism in sporadic Alzheimer disease: Therapeutic implications. *Advances in Experimental Medicine and Biology, 541*, 135–152. Review.

Hoyer, S., & Lannert, H. (2008). Long-term effects of corticosterone on behavior, oxidative and energy metabolism of parietotemporal cerebral cortex and hippocampus of rats: Comparison to intracerebroventricular streptozotocin. *Journal of Neural Transmission, 115*, 1241–1249.

Hoyer, S., Nitsch, R., & Oesterreich, K. (1991). Predominant abnormality in cerebral glucose utilization in late-onset dementia of the Alzheimer type: A cross-sectional comparison against advanced late-onset and incipient early-onset cases. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 3*, 1–14.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., & Cole, G. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science, 274*, 99–102.

Ishiguro, K., Shiratsuchi, A., Sato, S., Omori, A., Arioka, M., Kobayashi, S., & Uchida, T. (1993). Glycogen synthase kinase 3-beta is identical to tau protein kinase I generating several epitopes of paired helical filaments. *FEBS Letters, 325*, 167–172.

Ishrat, T., Khan, M. B., Hoda, M. N., Yousuf, S., Ahmad, M., Ansari, M. A., Ahmad, A. S., & Islam, F. (2006). Coenzyme Q10 modulates cognitive impairment against intracerebroventricular injection of streptozotocin in rats. *Behavioural Brain Research, 171*, 9–16.

Johnston, A. M., Pirola, L., & Van Obberghen, E. (2003). Molecular mechanisms of insulin receptor substrate protein-mediated modulation of insulin signalling. *FEBS Letters, 546*, 32–36.

Joseph, J., Shukitt-Hale, B., Denisova, N. A., Martin, A., Perry, G., & Smith, M. A. (2001). Copernicus revisited: Amyloid beta in Alzheimer's disease. *Neurobiology of Aging, 22*(1), 131–146.

Kadowaki, T., Kasuga, M., Akanuma, Y., Ezaki, O., & Takaku, F. (1984). Decreased autophosphorylation of the insulin receptor-kinase in streptozotocin-diabetic rats. *The Journal of Biological Chemistry, 259*, 14208–14216.

Kozlowski, M. R., & Arbogast, R. E. (1986). Specific toxic effects of ethylcholine nitrogen mustard on cholinergic neurons of the nucleus basalis of Meynert. *Brain Research, 372*, 45–54.

Kulstad, J. J., McMillan, P. J., Leverenz, J. B., Cook, D. G., Green, P. S., Peskind, E. R., Wilkinson, C. W., Farris, W., Mehta, P. D., & Craft, S. (2005). Effects of chronic glucocorticoid administration on insulin-degrading enzyme and amyloid-beta peptide in the aged macaque. *Journal of Neuropathology and Experimental Neurology, 64*, 139–146.

Kumar, R., Jaggi, A. S., & Singh, N. (2010). Effects of erythropoietin on memory deficits and brain oxidative stress in the mouse models of dementia. *The Korean Journal of Physiology and Pharmacology, 14*, 345–352.

Landrigan, P. J., Sonawane, B., Butler, R. N., Trasande, L., Callan, R., & Droller, D. (2005). Early environmental origins of neurodegenerative disease in later life. *Environmental Health Perspectives, 113*, 1230–1233.

Lannert, H., & Hoyer, S. (1998). Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. *Behavioral Neuroscience, 112*, 1199–1208.

Leloup, C., Arluison, M., Lepetit, N., Cartier, N., Marfaing-Jallat, P., Ferre, P., & Penicaud, L. (1994). Glucose transporter 2 (GLUT2): Expression in specific brain nuclei. *Brain Research, 638*, 221–226.

Lester-Coll, N., Rivera, E. J., Soscia, S. J., Doiron, K., Wands, J. R., & de la Monte, S. M. (2006). Intracerebral streptozotocin model of type 3 diabetes: Relevance to sporadic Alzheimer's disease. *Journal of Alzheimer's Disease, 9*, 13–33.

Lev-Lehman, E., el-Tamer, A., Yaron, A., Grifman, M., Ginzberg, D., Hanin, I., & Soreq, H. (1994). Cholinotoxic effects on acetylcholinesterase gene expression are associated with brain-region specific alterations in G, C-rich transcripts. *Brain Research, 661*, 75–82.

Lim, D. K., Oh, Y. H., & Kim, H. S. (2001). Impairments of learning and memory following intracerebroventricular administration of AF64A in rats. *Archives of Pharmacal Research, 24*, 234–239.

Mahley, R. W., & Rall, S. C., Jr. (2000). Apolipoprotein E: Far more than a lipid transport protein. *Annual Review of Genomics and Human Genetics, 1*, 507–537.

Mann, D. M., Yates, P. O., & Marcyniuk, B. (1985). Some morphometric observations on the cerebral cortex and hippocampus in presenile Alzheimer's disease, senile dementia of Alzheimer type and Down's syndrome in middle age. *Journal of the Neurological Sciences, 69*, 139–159.

Maurer, K., & Hoyer, S. (2006). Alois Alzheimer revisited: Differences in origin of the disease carrying his name. *Journal of Neural Transmission, 113*, 1645–1658.

Mayer, G., Nitsch, R., & Hoyer, S. (1990). Effects of changes in peripheral and cerebral glucose metabolism on locomotor activity, learning and memory in adult male rats. *Brain Research, 532*, 95–100.

Mielke, R., Herholz, K., Grond, M., Kessler, J., & Heiss, W. D. (1994). Clinical deterioration in probable Alzheimer's disease correlates with progressive metabolic impairment of association areas. *Dementia, 5*, 36–41.

Minoshima, S., Giordani, B., Berent, S., Frey, K. A., Foster, N. L., & Kuhl, D. E. (1997). Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Annals of Neurology, 42*, 85–94.

Moloney, A. M., Griffin, R. J., Timmons, S., O'Connor, R., Ravid, R., & O'Neill, C. (2010). Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiology of Aging, 31*, 224–243.

Mosconi, L. (2005). Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. *European Journal of Nuclear Medicine and Molecular Imaging, 32*, 486–510. Review.

Mukhina, T. V., Lermontova, N. N., Van'kin, G. I., Oettel, M., P'chev, V. K., & Bachurin, S. O. (2004). The effects of estrogens on learning in rats with chronic brain cholinergic deficiency in a Morris water test. Identification of the "passive swimming" component. *Neuroscience and Behavioral Physiology, 34*, 213–219.

Nakahara, N., Iga, Y., Mizobe, F., & Kawanishi, G. (1988). Effects of intracerebroventricular injection of AF64A on learning behaviors in rats. *Japanese Journal of Pharmacology, 48*, 121–130.

Ngarmukos, C., Baur, E. L., & Kumagai, A. K. (2001). Co-localization of GLUT1 and GLUT4 in the blood–brain barrier of the rat ventromedial hypothalamus. *Brain Research, 900*, 1–8.

Nitsch, R., & Hoyer, S. (1991). Local action of the diabetogenic drug, streptozotocin, on glucose and energy metabolism in rat brain cortex. *Neuroscience Letters, 128*, 199–202.

Osmanovic, J., Plaschke, K., Salkovic-Petrisic, M., Grünblatt, E., Riederer, P., & Hoyer, S. (2010). Chronic exogenous corticosterone administration generates an insulin-resistant brain state in rats. *Stress, 13*, 123–131.

Ott, A., Breteler, M. M., van Harskamp, F., Claus, J. J., van der Cammen, T. J., Grobbee, D. E., & Hofman, A. (1995). Prevalence of Alzheimer's disease and vascular dementia: Association with education. The Rotterdam study. *BMJ, 310*, 970–973.

Park, C. R. (2001). Cognitive effects of insulin in the central nervous system. *Neuroscience and Biobehavioral Reviews, 25*, 311–323.

Park, S. H., & Lim, D. K. (2010). Increases in serotonergic neuronal activity following intracerebroventricular administration of AF64A in rats. *Archives of Pharmacal Research, 33*, 301–308.

Pascualy, M., Petrie, E. C., Brodkin, K., Peskind, E. R., Wilkinson, C. W., & Raskind, M. A. (2000). Hypothalamic pituitary adrenocortical and sympathetic nervous system responses to the cold pressor test in Alzheimer's disease. *Biological Psychiatry, 48*, 247–254.

Pathan, A. R., Viswanad, B., Sonkusare, S. K., & Ramarao, P. (2006). Chronic administration of pioglitazone attenuates intracerebroventricular streptozotocin induced-memory impairment in rats. *Life Sciences, 79*, 2209–22016.

Phiel, C. J., Wilson, C. A., Lee, V. M. Y., & Klein, P. S. (2003). GSK-3α regulates production of Alzheimer's disease amyloid-β peptides. *Nature, 423*, 435–439.

Pinton, S., da Rocha, J. T., Gai, B. M., & Nogueira, C. W. (2011). Sporadic dementia of Alzheimer's type induced by streptozotocin promotes anxiogenic behavior in mice. *Behavioural Brain Research, 223*, 1–6.

Plaschke, K., & Hoyer, S. (1993). Action of the diabetogenic drug streptozotocin on glycolytic and glycogenolytic metabolism in adult rat brain cortex and hippocampus. *International Journal of Developmental Neuroscience, 11*, 477–483.

Plaschke, K., Müller, D., & Hoyer, S. (1996). Effect of adrenalectomy and corticosterone substitution on glucose and glycogen metabolism in rat brain. *Journal of Neural Transmission, 103*, 89–100.

Plaschke, K., Müller, D., & Hoyer, S. (2010a). Insulin-resistant brain state (IRBS) changes membrane composition of fatty acids in temporal and entorhinal brain cortices of rats: Relevance to sporadic Alzheimer's disease? *Journal of Neural Transmission, 117*, 1419–1422.

Plaschke, K., Kopitz, J., Siegelin, M., Schliebs, R., Salkovic-Petrisic, M., Riederer, P., & Hoyer, S. (2010b). Insulin-resistant brain state after intracerebroventricular streptozotocin injection exacerbates Alzheimer-like changes in Tg2576 AbetaPP-overexpressing mice. *Journal of Alzheimer's Disease, 19*, 691–704.

Ponce-Lopez, T., Liy-Salmeron, G., Hong, E., & Meneses, A. (2011). Lithium, phenserine, memantine and pioglitazone reverse memory deficit and restore phospho-GSK3β decreased in hippocampus in intracerebroventricular streptozotocin induced memory deficit model. *Brain Research, 1426*, 73–85.

Prickaerts, J., Fahrig, T., & Blokland, A. (1999). Cognitive performance and biochemical markers in septum, hippocampus and striatum of rats after an i.c.v. Injection of streptozotocin: A correlation analysis. *Behavioural Brain Research, 102*, 73–88.

Prickaerts, J., De Vente, J., Honig, W., Steinbusch, H., Ittersum, M. M. V., Blokland, A., & Steinbusch, H. W. (2000). Nitric oxide synthase does not mediate neurotoxicity after an i.c.v. Injection of streptozotocin in the rat. *Journal of Neural Transmission, 107*, 745–766.

Qiu, Q. W., & Folstein, M. F. (2006). Insulin, insulin-degrading enzyme and amyloid-β peptide in Alzheimer's disease: Review and hypothesis. *Neurobiology of Aging, 27*, 190–198.

Qu, Z. Q., Zhou, Y., Zeng, Y. S., Lin, Y. K., Li, Y., Zhong, Z. Q., & Chan, W. Y. (2012). Protective effects of a rhodiola crenulata extract and salidroside on hippocampal neurogenesis against streptozotocin-induced neural injury in the rat. *PloS One, 7*, e29641.

Rivera, E. J., Goldin, A., Fulmer, N., Tavares, R., Wands, J. R., & de la Monte, S. M. (2005). Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: Link to brain reductions in acetylcholine. *Journal of Alzheimer's Disease, 8*, 247–268.

Salkovic, M., Sabolic, I., & Lackovic, Z. (1995). Striatal dopaminergic D1 and D2 receptors after intracerebroventricular application of alloxan and streptozotocin in rat. *Journal of Neural Transmission. General Section, 100*, 137–145.

Salkovic-Petrisic, M., & Hoyer, S. (2007). Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: An experimental approach. *Journal of Neural Transmission. Supplementum, 72*, 217–233.

Salkovic-Petrisic, M., Tribl, F., Schmidt, M., Hoyer, S., & Riederer, P. (2006). Alzheimer-like changes in protein kinase B and glycogen synthase kinase-3 in rat frontal cortex and hippocampus after damage to the insulin signalling pathway. *Journal of Neurochemistry, 96*, 1005–1015.

Salkovic-Petrisic, M., Osmanovic, J., Grünblatt, E., Riederer, P., & Hoyer, S. (2009). Modeling sporadic Alzheimer's disease: The insulin resistant brain state generates multiple long-term morphobiological abnormalities including hyperphosphorylated tau protein and amyloid-beta. *Journal of Alzheimer's Disease, 18*, 729–750.

Salkovic-Petrisic, M., Osmanovic-Barilar, J., Brückner, M. K., Hoyer, S., Arendt, T., & Riederer, P. (2011). Cerebral amyloid angiopathy in streptozotocin rat model of sporadic Alzheimer's disease: A long-term follow up study. *Journal of Neural Transmission, 118*, 765–772.

Sandbrink, R., Hartmann, T., Masters, C. L., & Beyreuther, K. (1996). Genes contributing to Alzheimer's disease. *Molecular Psychiatry, 1*, 27–40.

Saxena, G., Patro, I. K., & Nath, C. (2011). ICV STZ induced impairment in memory and neuronal mitochondrial function: A protective role of nicotinic receptor. *Behavioural Brain Research, 224*, 50–57.

Selkoe, D. J. (2003). Aging, amyloid, and Alzheimer's disease: A perspective in honor of Carl Cotman. *Neurochemical Research, 28*, 1705–1713.

Sharma, M., & Gupta, Y. K. (2001). Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. *Life Sciences, 68*, 1021–1029.

Sharma, M., & Gupta, Y. K. (2002). Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. *Life Sciences, 71*, 2489–2498.

Shoham, S., Bejar, C., Kovalev, E., & Weinstock, M. (2003). Intracerebroventricular injection of streptozotocin causes neurotoxicity to myelin that contributes to spatial memory deficits in rats. *Experimental Neurology, 184*, 1043–1052.

Shoham, S., Bejar, C., Kovalev, E., Schorer-Apelbaum, D., & Weinstock, M. (2007). Ladostigil prevents gliosis, oxidative-nitrative stress and memory deficits induced by intracerebroventricular injection of streptozotocin in rats. *Neuropharmacology, 52*, 836–843.

Snowdon, D. A. (1997). Aging and Alzheimer's disease: Lessons from the Nun study. *The Gerontologist, 37*, 150–156.

Sonkusare, S., Srinivasan, K., Kaul, C., & Ramarao, P. (2005). Effect of donepezil and lercanidipine on memory impairment induced by intracerebroventricular streptozotocin in rats. *Life Sciences, 77*, 1–14.

Steen, E., Terry, B. M., Rivera, E. J., Cannon, J. L., Neely, T. R., Tavares, R., Xu, X. J., Wands, J. R., & de la Monte, S. M. (2005). Impaired insulin and insulin-like growth factor expression

and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? *Journal of Alzheimer's Disease, 7*, 63–80.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research, 50*, 336–346.

Tanzi, R. E., & Bertram, L. (2001). New frontiers in Alzheimer's disease genetics. *Neuron, 32*, 181–184.

Terwel, D., Prickaerts, J., Meng, F., & Jolles, J. (1995). Brain enzyme activities after intracerebroventricular injection of streptozotocin in rats receiving acetyl-L-carnitine. *European Journal of Pharmacology, 287*, 65–71.

Tong, M., Neusner, A., Longato, L., Lawton, M., Wands, J. R., & de la Monte, S. M. (2009). Nitrosamine exposure causes insulin resistance diseases: Relevance to type 2 diabetes mellitus, non-alcoholic steatohepatitis, and Alzheimer's disease. *Journal of Alzheimer's Disease, 17*, 827–844.

Tota, S., Kamat, P. K., Saxena, G., Hanif, K., Najmi, A. K., & Nath, C. (2011). Central angiotensin converting enzyme facilitates memory impairment in intracerebroventricular streptozotocin treated rats. *Behavioural Brain Research, 226*, 317–330.

Vallée, M., MacCari, S., Dellu, F., Simon, H., Le Moal, M., & Mayo, W. (1999). Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: A longitudinal study in the rat. *The European Journal of Neuroscience, 11*, 2906–2916.

Wada, A., Yokoo, H., Yanagita, T., & Kobayashi, H. (2005). New twist on neuronal insulin receptor signaling in health, disease and therapeutics. *Journal of Pharmacological Sciences, 99*, 128–143.

Watson, G. S., & Craft, S. (2006). Insulin resistance, inflammation, and cognition in Alzheimer's disease: Lessons for multiple sclerosis. *Journal of the Neurological Sciences, 245*, 21–33.

Weinstock, M., & Shoham, S. (2004). Rat models of dementia based on reductions in regional glucose metabolism, cerebral blood flow and cytochrome oxidase activity. *Journal of Neural Transmission, 111*, 347–366.

Wüppen, K., Oesterle, D., Lewicka, S., Kopitz, J., & Plaschke, K. (2010). A subchronic application period of glucocorticoids leads to rat cognitive dysfunction whereas physostigmine induces a mild neuroprotection. *Journal of Neural Transmission, 117*, 1055–1065.

Zhao, W., Chen, H., Xu, H., Moore, E., Meiri, N., Quon, M. J., & Alkon, D. L. (1999). Brain insulin receptors and spatial memory. *The Journal of Biological Chemistry, 274*, 34893–34902.

Zhao, W. Q., Chen, H., Quon, M. H., & Alkon, D. L. (2004). Insulin and the insulin receptor in experimental models of learning and memory. *European Journal of Pharmacology, 490*, 71–81.

Fusion Models and "Fusioning" in Parkinsonism: Protection and Restoration by Exercise

Trevor Archer and Anders Fredriksson

Contents

Abstract

Fusion models, or the "fusioning" of models, of Parkinson's disease (PD) to attain the sufficiency of disorder etiopathogenesis have spurred the development of laboratory models incorporating neurotoxin treatments in combination with genetic manipulations of animals studied. The present review describes fusion from two directions: (i) through the fusioning of neurotoxin and genetic models, and, It was observed that wheel-running exercise (four 30-min sessions/week) attenuated the motor deficits, both assessed as distance run in the running wheels and as locomotor, rearing and total activity counts in the motor activity test chambers, and the dopamine (DA) loss induced by the DA neurotoxin, MPTP compared with the no exercise MPTP group (one 30-min session/week).

Keywords

DA • Exercise • Mice • Motor activity • MPTP • PD

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1 Introduction

Parkinson's disease (PD) is second only to Alzheimer's disease as a neurodegenerative disorder afflicting humankind with four to five million individuals, diagnosed or undiagnosed in the USA alone (Korell and Tanner 2005). PD most commonly affects individuals in their sixth decade or later and involves several risk factors including pesticides, head trauma, and aging (Allam et al. 2005). Pathways mediated through mitochondrial actions are implicated heavily in the neurodegenerative cascades that result in dopaminergic cell death (Jordan et al. 2003, 2011), with mitochondrial outer membrane permeabilization intimately associated with the apoptotic process (Galluzzi et al. 2009; Gomez-Lazaro et al. 2008). PD is a relatively common, idiopathic neurodegenerative movement disorder with the cardinal features of impaired motor function, resting tremor, rigidity, akinesia/bradykinesia, and postural instability (Gaggelli et al. 2006; Jankovic 2008; Lees et al. 2009). It is a progressive neurodegenerative disorder with late onset and, compared with familial forms, is associated most often with advanced age (>55 years of age). PD symptoms are manifested following 50–75 % loss of striatal dopamine (DA, Riederer and Wuketich 1976; Scherman et al. 1989) and 30–60 % destruction of nigrostriatal DA neurons (Cheng et al. 2010; Fearnley and Lees 1991). Axonal pathology in PD is supported by various sources, not least genetic mutational models (Li et al. 2009; Mortiboys et al. 2008; Saha et al. 2004). For example, Mortiboys et al. (2010) obtained impaired mitochondrial function and morphology in LRRK2 (G2019S)-mutant PD patient tissue, i.e., shared features in early-onset and late-onset PD. The consensus of these and other studies indicating alterations of axonal transport (Meissner et al. 2011; Weihofen et al. 2009) reinforces the notion that neurons in the substantia nigra degenerate through "retrogradely perishing" axonopathy whereby deterioration originates in the distal axon and proceeds to the cell body temporally (Antenor-Dorsey and O'Malley 2012). Converging pathogenic mechanisms imply a restricted number of mechanisms that include oxidative stress, mitochondrial defects and dysfunctionality, proteolytic stress, and neuroinflammation (Braak et al. 2006).

The pathophysiology of PD involves dopaminergic neuron death and accumulation of Lewy bodies associated with mutations in α-synuclein, a 14-kDa protein predominantly expressed in the brain and CNS (Rasia et al. 2005). PD patients show decreased levels of presynaptic DA neuron terminal markers in the basal ganglia (Felicio et al. 2009), consistent with loss of dopaminergic terminals due to degeneration of neuronal cell bodies in the substantia nigra pars compacta (Hattori et al. 2006). They also exhibit decreased levels of DA transporters (DATs) and vesicular monoamine transporter type 2 (VMAT2) as well as reduced activity of dopa decarboxylase, assessed by striatal conversion of L-Dopa to DA, according to PET and SPECT analyses. Wu et al. (2012) using MRI showed that the substantia nigra pars compacta expressed a decreased connectivity with several regions, including the striatum, globus pallidus, subthalamic nucleus, thalamus, supplementary motor area, dorsolateral prefrontal cortex, insula, default mode network, temporal lobe, cerebellum, and pons in patients compared

to controls. They found that L-Dopa administration partially normalized the pattern of connectivity to a similarity such as that expressed by the healthy volunteers involving causal connectivity of basal ganglia networks from the substantia nigra pars compacta. Postsynaptic D_2 DA receptors (D_2Rs) are either unaffected or increased in the striatum of untreated PD patients (Antonini et al. 1994). Oxidative injury appears to be one effect of α-synuclein (α-Syn) aggregates and could ultimately produce neuronal cell death. α-Syn, a 140 residue, intrinsically disordered protein is localized in presynaptic terminals of DA neurons (Yang et al. 2010). Autonomic nervous system involvement occurs at early stages in both PD and incidental Lewy body disease and affects the sympathetic, parasympathetic, and enteric nervous systems. It has been proposed that α-Syn pathology in PD has a distal to proximal progression along autonomic pathways. According to Braakian notions, the enteric nervous systems is affected before the dorsal motor nucleus of the vagus, and distal axons of cardiac sympathetic nerves degenerate before there is loss of paravertebral sympathetic ganglion neurons. Cersosimo and Benarroch (2012a) have shown that consistent with neuropathological findings, some autonomic manifestations such as constipation or impaired cardiac uptake of norepinephrine precursors occur at early stages of the disease even before the onset of motor symptoms (cf. Braak et al. 2006; Cersosimo and Benarroch 2012b; Hawkes et al. 2007).

2 Fusion in PD

The notion concerning the "fusion of models" of PD, the current and latest phase of Parkinsonian model development, has provided a major basis for studying the etiopathogenesis of the disorder through the presentation of a whole new set of animal models and clinical scenarios whereby gene x environment interactions may be analyzed (Manning-Bog and Langston 2007). Several models of PD induced by systemic treatment with neurotoxins included 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the pesticide rotenone, the herbicide paraquat, and liposaccharide (LPS) occurring in outer membrane of gram-negative bacteria, which combine to provide dopaminergic vulnerability for PD that endows the "multiple-hit" aspect to establish PD symptom profiles, if not a complete pathophysiology. Other models of PD are based upon some form of genetic mutation of α-synuclein or Parkin deficiency, DJ-1 deficiency, and *PINK-1* and *LRRK2* genes that are employed in transgenic models (Manning-Bog and Langston 2007). Remarkably, Westerlund et al. (2010) advocated genetic factors as the major causative factor in PD due to the multiplicity of genes now implicated in the etiopathogenesis that involve several molecular pathways and downstream effectors affecting the trophic support and/or the survival of DA neurons. Nevertheless, much evidence suggests that multiple factors combining environmental agents and genetic predispositions orchestrate the fate of DA neurons (de Lau and Breteler 2006; Gupta et al. 2008; Schapiro et al. 2009). Manning-Bog and Langston (2007), as well as others (Nieto et al. 2006; Song et al. 2004), have presented the notion of

"fusion" between transgenic mouse lines, which indicate subtle alterations with risk factor relevance but generally fail to show overt damage to the nigrostriatal DA system, and laboratory models of neurotoxin-induced DA depletion and altered neurotransmission. Several instances of genetic and neurotoxin model have been reported: Liu et al. (2012) have found that SIRT2 (a sirtuin) deacetylates Foxo3a, increases RNA and protein levels of Bim, and, as a result, enhances apoptosis following MPTP treatment and that chronic MPTP- induced neurodegeneration was prevented by the genetic deletion of SIRT2 in mice. The deletion of SIRT2 led to the reduction of apoptosis due to an increase in acetylation of Foxo3a and a decrease in Bim levels. Peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), a transcriptional coactivator regulating cell metabolism, mitochondrial biogenesis, oxidative stress, and gene expression, modulates the sensitivity of neurons in the brain to insults; PGC-1α knockout mice were shown to be supersensitive to MPTP (St-Pierre et al. 2006). Several recent studies have shown that PGC-1α is necessary for the integrity of DA neurons through its actions against oxidative stress and enhancing neuronal viability (Breidert et al. 2002; Zheng et al. 2010). Mudo et al. (2012) applied resveratrol to modulate/activate PGC-1α in dopaminergic SN4741 cells in vitro via the deacetylase SIRT1, thereby enhancing PGC-1α gene transcription with elevated SOD2 and Trx2.

The necessity for a model fusion approach has been spurred by several avenues of discontent stemming from what has been described as a "myopic" predilection for neurotoxin-based assaults of the dopaminergic nigrostriatal system (Langston 2006); all viable notions pertaining to the etiopathogenesis of the disorder must accommodate the circumstance that PD presents not a single entity that is the cumulative effect of a dopaminergic structural and functional defect accompanied by several related deficits but expresses rather a concatenation of destructive regional, cellular, and molecular events brought together by a combination of genetic and environmental factors (Litvan et al. 2007a, b). The existence of familial (i.e., genetic) forms of the disorder that appears to account for about 10–18 % of PD diagnoses has been considered (Wirdefeldt et al. 2011). Gao et al. (2012) showed that a family history of Parkinsonism was associated with PD risk (odds ratio = 2.71, 95 % confidence interval, 1.97–3.74), but at the same time was affected marginally, or not at all, by further adjustment for these SNPs and environmental exposures; they obtained a significant interaction with rs2896905 at SLC2A13, near LRRK2, with 32–35 % higher risk levels. Blandini and Armentero (2012) have classified animal models of PD on the basis of systemic/intracerebral administration of neurotoxins that replicated pathological and phenotypic characteristics of the disorder yet describe the proliferation of mammalian and nonmammalian transgenic models presenting disease-inducing mutations identified for monogenic forms of familial PD. Nevertheless, the current trend towards "fusioning" of animal models in this respect holds both specific characteristics that may not be commensurate with essential aspects of the disorders, such as "staging" (Archer et al. 2011c), or limitations requiring much careful consideration prior to selection and application. Soreq et al. (2012) carried out a meta-analysis on 131 brain region transcriptomes from mice overexpressing

native or mutated α-synuclein (SNCA) with or without the protective HSP70 chaperone or exposed MPTP, with or without the protective acetylcholinesterase variant, AChE-R, all of which presented risk-inducible and protection-suppressible transcript modifications. Their conclusions imply that metal ions mediated the "cross talk" between nuclear and mitochondrial pathways by both environmental and genetic risk and those protective factors which eventually culminate in neuroinflammation in the PD cascade. In nonhuman primate studies of PD, both the motor and cognitive capacities of nonhuman primates (NHPs) provide levels of structural and functional complexity that permits laboratory observations to attain insights for applications in the clinical realities (Capitanio and Emborg 2007).

Applying a different point of departure to the investigation of Parkinsonism in the laboratory, fusion dynamics (mitochondrial biogenesis, bioenergetics, dynamics, transport, quality control, and the genes involved) are critical for the life cycle and maintenance of dopaminergic neuron mitochondria. Mitochondrial integrity, dependent upon specific morphological, biochemical, and physiological features, is of critical importance for the maintenance and survival of neurons (Exner et al. 2012) with dysfunctions linked to severe cellular consequences that result in neurodegenerative diseases and aging. Thus, Arduino et al. (2011) have proposed that the cellular demise and neurodegenerative propensity in PD pathophysiology may be modulated by an interaction between mitochondrial dysfunction, mitochondrial trafficking disruption, and impaired autophagic clearance. Sporadic PD case studies, neurotoxin-mediated animal models, and genetically based (familial PD) PD etiology imply that mitochondrial dysfunction/dysregulation constitutes an earl feature of the disorder (Burman et al. 2012; Cooper et al. 2012; Moran et al. 2012). Increased mitochondrial damage due to complex I inhibition, derangement of Ca^{++}, oxidant stress, or impaired clearance of mitochondrial debris leads to the accumulation of nonfunctional organelles contributing to neuronal disrepair (McCoy and Cookson 2012). Several gene mutations regulate the responses to cellular stress with enhanced sensitivity to mitochondrial neurotoxins and oxidative stress (Alberio et al. 2012; Heo et al. 2012; Rochet et al. 2012). Macroautophagy is associated with prevention and limitation of mitochondrial damage and debris through degradation of superfluous or severely damaged mitochondria (Lynch-Day et al. 2012; Wang and Klionsky 2011). Mitochondrial fusion processes, distinguished from membrane fusion mechanisms in secretory pathway or membrane-bound organelles, are divided into three events: "docking," outer membrane fusion, and inner membrane fusion. Injuries to mitochondria impair fusion activate fission-dependent fragmentation and trigger mitophagy (Kim et al. 2007). For instance, mitochondria-derived reactive oxygen species (ROS) at low concentrations may act as signaling molecules and induce mitophagy (Scherz-Shouval et al. 2007). Mitochondrial quantity and quality are regulated to prevent excess energy and harmful ROS. Mitophagy limits the mitochondria to a basal level to fulfill cellular energy requirements and preventing excess ROS production (Kurihara et al. 2012). Arduino et al. (2011) have proposed that cellular demise and neurodegeneration in PD stem from the interactions between mitochondrial

dysfunction and trafficking disruption and autophagic clearance. In this context, fusion-fission models of the disorder pertain to conditions wherein mitochondrial structural-functional integrity through the effective working of cellular autophagy and mitophagy has been compromised (Santos and Cardoso 2012; Youle and van der Bliek 2012).

From a PD perspective, axonal integrity and function are disrupted also by a variety of environmental and laboratory neurotoxins, including 6-hydroxydopamine (Segura Aguilar and Kostrzewa 2004; Kostrzewa et al. 2006), MPTP (see below, Annese et al. 2012; Villalba and Smith 2011), rotenone (Norazit et al. 2010; Press and Milbrandt 2008), and paraquat (Brooks et al. 1999; Peng et al. 2004). Both MPP^+ and rotenone disrupt axonal integrity and function by inhibiting mitochondrial complex I activity (Sterky et al. 2012) and depolymerizing microtubules leading to axon fragmentation and decreased synaptic function (Cabeza-Arvelaiz and Schiestl 2012; Cartelli et al. 2010; Hongo et al. 2012; Qureshi and Paudel 2011); the neurotoxic insults of these agents linked to inhibition of axon transport directly (Behrouz et al. 2007; Kim-Han et al. 2011; Morfini et al. 2007). Axon degeneration, characteristic to neurodegeneration, shares many morphological features with events expressed in acute injuries; the expression of the Wallerian degeneration slow (Wld^S) transgene delays nerve degeneration in both types of insult (Wang et al. 2012). Wallerian degeneration occurs through crushing/cutting nerve fibers whereby the section of the axon separated from the cell body degenerates distal to the injury; Wallerian-type degeneration occurs in PD through compromise of axonal transport. The Wallerian degeneration slow fusion protein, Wld^S, delays axonal degeneration about 10-fold (Avery et al. 2012; Chitnis et al. 2007; Coleman and Freeman 2010; Samsam et al. 2003; Takada et al. 2011) and blocks axon degeneration in several models, such as animal models of PD (Mi et al. 2005; Sajadi et al. 2004) that include the MPTP model (Antenor-Dorsey and O'Malley 2012; Hasbani and O'Malley 2006). Using mutant mice and lentiviral transduction of DA neurons, Antenor-Dorsey and O'Malley (2012) showed that Wld^S, but not Nmnat1, Nmnat3, or cytoplasmically targeted Nmnat1, protected DA axons from MPTP treatment that resulted in MPP^+. Lentiviral transduction refers to a form of "gene therapy" used to designate a process through which viruses are exploited to carry specific genetic information into a cell, thereby transducing the cells' function (cf. Tolu et al. 2010). NAD^+ synthesis was not required but was, by itself, sufficiently axonally protective and was additive in effect together with Wld^S. It appears that Wld^S and NAD^+ act through separate pathways to protect DA neurons through preserving mitochondrial health and/or maintaining cellular metabolism.

The regulation of processes incorporating mitochondrial dynamics encompasses essential mechanisms that control the eventual fate of cells, such as dopaminergic neurons (Liesa et al. 2009); these processes include fission, fusion, and mitophagy, with fusion and fission acting antagonistically, and imbalances in mitochondrial dynamics contributing to PD (Cho et al. 2010; Lees et al. 2009; Su et al. 2010). Mitophagy eliminates damaged or aged mitochondria, thereby maintaining mitochondrial quality, an autophagy-dependent degradation of mitochondria that

contributes to the removal of dysfunctional, aged, or excess mitochondria (Hirota et al. 2012). Galindo et al. (2012) have shown that the regulation of dynamic mitochondrial processes such as fusion, fission, and mitophagy has been unfolded as cascades contributing to important mechanism controlling neuronal fate.

3 MPTP Model of PD

Repeated administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to C57/BL6 mice produces selective lesions of dopaminergic neurons in substantia nigra pars compacta and long-lasting depletion of dopamine (DA) in basal ganglia (Jackson-Lewis et al. 1995; Jones-Humble et al. 1994; Langston 1985; Langston et al. 1984). The susceptibility of mice to the neurotoxic actions of MPTP can be quite variable, depending on gender and strain differences, expressed in functional, neurochemical, and histochemical analyses (Schwarting et al. 1999; Sedelis et al. 2000a, b, 2001, 2003). C57/BL6 and Swiss Webster strains were shown to differ in c-Jun N-terminal kinases (JNKs) and c-JUN activation in response to MPTP. JNKs, of the mitogen-activated protein kinase family, are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock; c-Jun is the name of a gene and protein that, in combination with c-Fos, forms the AP-1 early-response transcription factor. MPTP induced COX-2, an enzyme responsible for inflammation and pain, responding exclusively in C57/BL6 mice (Boyd et al. 2007). MPTP, administered systemically, induces Parkinsonism in human and nonhuman primates (Langston 1985) that results in the loss of *substantia nigra* cells in the *pars compacta* of adult animals (Chiueh et al. 1985). MPTP selectively destroys nigrostriatal neurons, thereby inducing acute, subacute, long-lasting, and even permanent effects that resemble certain features of PD, particularly the hypokinesia effects (Schultz et al. 1989). Systemic administration of MPTP (2×40 mg/kg, s.c.) caused L-Dopa reversible hypoactivity (Fredriksson et al. 1990; Sundström et al. 1990). A less rigorous dose regime, e.g., 2×20 or 25 or 30 mg/kg, of MPTP has been found not to reduce motility in the C57 black mice, although DA brain concentrations may indicate up to 50–80 % reductions (Heikkila et al. 1989; Sonsalla and Heikkila 1986), unless given much more repeatedly (cf. Kurz et al. 2007). The parameters of MPTP treatment neurotoxicity in mice are long-lasting (up to and beyond 52 weeks after treatment), with strong correlations between the functional deficits, particularly hypokinesia, the main biomarker, severe DA depletions, and a dose- and time-dependent recovery of several parameters of motor behavior following treatment with the DA precursor, L-Dopa (Archer and Fredriksson 2003; Fredriksson and Archer 1994; Fredriksson et al. 1999).

4 Exercise Intervention in PD

In the unilateral 6-hydroxydopamine rat model of PD, Tillerson et al. (2001) abolished the lesion-induced motor asymmetry by forcing rats to use the affected (contralateral)

limb, whereas forced nonuse exacerbated the injury (Tillerson et al. 2002). Both DA and 3,4-dihydroxyphenylacetic acid (Dopac) were elevated markedly in "casted" 6-OHDA-treated rats (forced to use the contralateral limb) compared with "non-casted" rats (Cohen et al. 2003). More recently, Choe et al. (2012) showed that exercise recovers PD-induced dopaminergic neuron loss and contralateral soleus muscle atrophy. Using the DA neurotoxin, MPTP as their model of PD, Sung et al. (2012) observed that treadmill exercise prevented dopaminergic neuronal loss by inhibiting brain inflammation through suppression of microglial activation in the PD mice. Archer and Fredriksson (2010) found that daily running-wheel activity attenuated the hypokinesia effects of MPTP in both a concentrated (2 × 40 mg/kg, 24-h interval) and progressive (1 × 40 mg/kg, weekly doses over 4 weeks) schedule with regard to spontaneous motor behavior and activity following a subthreshold dose of L-Dopa. The loss of DA in each case was attenuated by exercise also (experiment I, 61 % of control vs. 17 %; experiment II, 24 % vs. 11 %). Using the progressive schedule of MPTP treatment and extending the exercise intervention from 7 to 14 weeks, it was shown that spontaneous motor activity after MPTP was close to restoration, whereas activity after subthreshold L-Dopa was completely recovered (Fredriksson et al. 2011); DA levels were restored from 17 % (non-exercised) to 64 % in the 14-week exercise intervention, and levels of brain-derived neurotrophic factor were increased significantly. Also, both the functional and DA deficits by MPTP were attenuated even by delayed introduction of exercise (Archer and Fredriksson 2012).

Zigmond et al. (e.g., Cohen et al. 2003; Tillerson et al. 2001, 2002) have utilized the unilateral 6-hydroxydopamine (6-OHDA) rat model of PD whereby they increased the use of the normally denervated contralateral by constraining the other forelimb with a cast, thereby reducing the function and neurochemical effects of the DA neurotoxin evidenced by marked elevations of DA and metabolites. Using MPTP to deplete DA, Klaissle et al. (2012) analyzed newborn cells in the substantia nigra at different stages of maturation and time points as a function of voluntary physical activity, environmental enrichment, and L-Dopa treatment. They observed a motor activity-induced, running-wheel increase in neuroglial antigen-2-positive cells and mature oligodendrocytes in the substantia nigra of healthy mice. In MPTP-treated mice, the stimulation of neuroglial antigen-2-positive cell generation and oligodendrogenesis was dependent upon L-Dopa administration. The authors have suggested that DA is the key regulator for generation of neuroglial antigen-2-positive cells and oligodendrocytes in the substantia nigra that ought to be taken into account as an important contributor to endogenous nigral cellular plasticity. The integrity of the nigrostriatal system under MPTP insult is linked further with dynamics of glial cell activation: CD11b expression, a surface marker on the plasma membrane of microglia and the α-component of several integrins, is increased in the striatum and substantia nigra following MPTP (Kang et al. 2008; Singh et al. 2011). Integrins, transmembrane receptors that mediate the attachment between neurons and surrounding tissues, pass information about the chemical composition and mechanical status of the extracellular matrix. Sung et al. (2012) have shown that treadmill

running exercise prevented the loss of dopaminergic neurons and ameliorated coordination dysfunction in MPTP mice by inhibiting brain inflammation through microglial activation. The effects of physical exercise upon functional and biomaker integrity have been amply shown (Archer 2011, 2012; Archer et al. 2011a, b, 2012; Archer and Kostrzewa 2012; Archer and Fredriksson 2013).

5 Conclusion

The fusion modeling of PD, whether viewed as neurotoxin-genetic model fusions or fusioning within mitochondria, offers an abundance of structural, epigenetic, bio-, and even neurophysiologic markers (Baziyan 2012) as evidence for essential neurodegenerative processes involved in the disorder, if not a complete etiopathogenesis. There is an emerging consensus that genetic- and toxin-induced animal together with cellular models and postmortem human brain tissue provide evidence that mitochondrial dysfunction/dysregulation forms the basis disorder pathophysiology (Chaturvedi and Beal 2012; Van Laar and Berman 2012). From the distinguishing perspective of neuroprotection and/or neurorestoration, Ohshima-Hosoyama et al. (2012) have combined the human insulin receptor (HIR) with glial-derived neurotrophic factor (GDNF) in the fusion protein, HIRMAb-GDNF, in order to induce neuroprotection in Parkinsonian monkeys. However, their results demonstrated that HIRmAb-GDNF dosing in a monkey model of PD was not an effective neuroprotective strategy. Physical exercise has proven effective even using other laboratory models of PD: using the chronic haloperidol administration model of PD, Baptista et al. (2013) showed that exercise alleviated haloperidol-induced akinesia (see also Abrantes et al. 2012). Finally, taking into account noninvasive, non-pharmacological strategies to prevent/restore against brain degeneration in PD, physical exercise has been shown to offer a remarkably effective strategy, alleviating and even restituting brain tissue and mitochondrial dysfunction. Marquis-Aleixo et al. (2012) have described the role of physical exercise in the modulation of the mechanisms involved in neuroprotection including the activation of signaling pathways underlying brain protection.

References

Abrantes, A. M., Friedman, J. H., Brown, R. A., Strong, D. R., Desaulniers, J., Ing, E., Saritelli, J., & Riebe, D. (2012). Physical activity and neuropsychiatric symptoms of Parkinson disease. *Journal of Geriatric Psychiatry and Neurology, 25*, 138–145. doi:10.1177/0891988712455237.

Alberio, T., Lopiano, L., & Fasano, M. (2012). Cellular models to investigate biochemical pathways in Parkinson's disease. *The FEBS Journal, 279*, 1146–1155. doi:10.1111/j.1742-4658.2012.08516.x.

Allam, M. F., Del Castillo, A. S., & Navajas, R. F. (2005). Parkinson's disease risk factors: Genetic, environmental, or both? *Neurological Research, 7*, 206–208.

Annese, V., Barcia, C., Ros-Bernal, F., Gómez, A., Ros, C. M., De Pablos, V., Fernández-Villalba, E., De Stefano, M. E., & Herrero, M. T. (2012). Evidence of oligodendrogliosis in MPTP-induced Parkinsonism. *Neuropathology and Applied Neurobiology*. doi:10.1111/j.1365-2990.2012.01271.x.

Antenor-Dorsey, J. A., & O'Malley, K. L. (2012). WldS but not Nmnat1 protects dopaminergic neurites from MPP^+ neurotoxicity. *Molecular Neurodegeneration, 7*, 5–19.

Archer, T. (2011). Physical exercise alleviates debilities of normal aging and Alzheimer's disease. *Acta Neurologica Scandinavica, 123*, 221–238.

Archer, T. (2012). Influence of physical exercise on traumatic brain injury deficits: Scaffolding effect. *Neurotoxicity Research, 21*, 418–434. doi:10.1007/s12640-011-9297-0.

Archer, T., & Fredriksson, A. (2003). An antihypokinesic action of α2-adrenoceptors upon MPTP-induced behavior deficits in mice. *Journal of Neural Transmission, 110*, 183–200.

Archer, T., & Fredriksson, A. (2010). Physical exercise attenuates MPTP-induced deficits in mice. *Neurotoxicity Research, 18*, 313–327.

Archer, T., & Fredriksson, A. (2012). Delayed exercise-induced functional and neurochemical partial restoration following MPTP. *Neurotoxicity Research, 21*, 210–221. doi:10.1007/s12640-011-9261-z.

Archer, T., Fredriksson, A. (2013). Pharmacogenomics and personalized medicine in Parkinson's disease. In D. Barh (Ed.), *Pharmacogenomics*. New York: Springer.

Archer, T., & Kostrzewa, R. M. (2012). Physical exercise alleviates ADHD symptoms: Regional deficits and development trajectory. *Neurotoxicity Research, 21*, 195–209. doi:10.1007/s12640-011-9260-0.

Archer, T., Fredriksson, A., & Johansson, B. (2011a). Exercise alleviates Parkinsonism: Clinical and laboratory evidence. *Acta Neurologica Scandinavica, 123*, 73–84.

Archer, T., Fredriksson, A., Schütz, E., & Kostrzewa, R. M. (2011b). Influence of physical exercise on neuroimmunological functioning and health: Aging and stress. *Neurotoxicity Research, 20*, 69–83.

Archer, T., Kostrzewa, R. M., Beninger, R. J., & Palomo, T. (2011c). Staging neurodegenerative disorders: Structural, regional, biomarker, and functional progressions. *Neurotoxicity Research, 19*, 211–234. doi:10.1007/s12640-010-9190-2.

Archer, T., Svensson, K., & Alricsson, M. (2012). Physical exercise ameliorates deficits induced by traumatic brain injury. *Acta Scand Neurol, 125*(5), 293–302.

Arduino, D. M., Esteves, A. R., & Cardoso, S. M. (2011). Mitochondrial fusion/fission, transport and autophagy in Parkinson's disease: When the mitochondria get nasty. *Parkinson's Disease*. doi:10.4061/2011/767230.

Avery, M. A., Rooney, T. M., Pandya, J. D., Wishart, T. M., Gillingwater, T. H., Geddes, J. W., Sullivan, P. G., & Freeman, M. R. (2012). WldS prevents axon degeneration through increased mitochondrial flux and enhanced mitochondrial Ca^{2+} buffering. *Current Biology, 22*, 596–600.

Baptista, P. P., de Senna, P. N., Paim, M. F., Saur, L., Blank, M., do Nascimento, P., Ilha, J., Vianna, M. R., Mestriner, R. G., Achaval, M., & Xavier, L. L. (2013). Physical exercise down-regulated locomotor side effects induced by haloperidol treatment in Wistar rats. *Pharmacology Biochemistry and Behavior, 104*, 113–118. doi:10.1016/j.pbb.2012.12.020.

Baziyan, B. K. (2012). Possible neurophysiological markers of early neurodegenerative process: Parkinson's disease. *Bulletin of Experimental Biology and Medicine, 154*, 186–188.

Behrouz, B., Drolet, R. E., Sayed, Z. A., Lookingland, K. J., & Goudreau, J. L. (2007). Unique responses to mitochondrial complex I inhibition in tuberoinfundibular dopamine neurons may impart resistance to toxic insult. *Neuroscience, 147*, 592–598.

Blandini, F., & Armentero, M. T. (2012). Animal models of Parkinson's disease. *The FEBS Journal, 279*, 1156–1166.

Boyd, J. D., Jang, H., Shepherd, K. R., Faherty, C., Slack, S., Jiao, Y., & Smeyne, R. J. (2007). Brain response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) differs in mouse strains and reveals a divergence in JNK signaling and COX-2 induction prior to loss of neurons in the substantia nigra pars compacta. *Brain Research, 1175*, 107–116.

Braak, H., Müller, C. M., Rüb, U., Ackermann, H., Bratzke, H., de Vos, R. A., & Del Tredici, K. (2006). Pathology associated with sporadic Parkinson's disease–where does it end? *Journal of Neural Transmission, 70*, 89–97.

Breidert, T., Callebert, J., Heneka, M. T., Landreth, G., Launay, J. M., & Hirsch, E. C. (2002). Protective action of the peroxisome proliferator-activated receptor-gamma agonist pioglitazone in a mouse model of Parkinson's disease. *Journal of Neurochemistry, 82*, 615–624.

Brooks, A. I., Chadwick, C. A., Gelbard, H. A., Cory-Slechta, D. A., & Federoff, H. J. (1999). Paraquat elicited neurobehavioural syndrome caused by dopaminergic neuron loss. *Brain Research, 823*, 1–10.

Burman, J. L., Yu, S., Poole, A. C., Decal, R. B., & Pallanck, L. (2012). Analysis of neural subtypes reveals selective mitochondrial dysfunction in dopaminergic neurons from parkin mutants. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 10438–10443.

Cabeza-Arvelaiz, Y., & Schiestl, R. H. (2012). Transcriptome analysis of a rotenone model of parkinsonism reveals complex I-tied and -untied toxicity mechanisms common to neurodegenerative diseases. *PLoS One, 7*(9), e44700. doi:10.1371/journal.pone.0044700.

Capitanio, J. P., & Emborg, M. E. (2007). Contributions of nonhuman primates to neuroscience research. *Lancet, 371*, 1126–1135. doi:10.1016/S0140-6736(08)60489-4.

Cartelli, D., Ronchi, C., Maggioni, M. G., Rodighiero, S., Giavini, E., & Cappelletti, G. (2010). Microtubule dysfunction precedes transport impairment and mitochondria damage in MPP^+−induced neurodegeneration. *Journal of Neurochemistry, 115*, 247–258.

Cersosimo, M. G., & Benarroch, E. E. (2012a). Pathological correlates of gastrointestinal dysfunction in Parkinson's disease. *Neurobiology of Disease, 46*, 559–564. doi:10.1016/j.nbd.2011.10.014.

Cersosimo, M. G., & Benarroch, E. E. (2012b). Autonomic involvement in Parkinson's disease: Pathology, pathophysiology, clinical features and possible peripheral biomarkers. *Journal of Neurological Sciences, 313*, 57–63. doi:10.1016/j.jns.2011.09.030.

Chaturvedi, R. K., & Beal, M. F. (2012). Mitochondria targeted therapeutic approaches in Parkinson's and Huntington's diseases. *Molecular and Cellular Neuroscience*. doi:S1044-7431(12)00210-2.%2010.1016/j.mcn.2012.11.011.

Cheng, H. C., Ulane, C. M., & Burke, R. E. (2010). Clinical progression in Parkinson's disease and the neurobiology of axons. *Annals of Neurology, 67*, 715–725.

Chitnis, T., Imitola, J., Wang, Y., Elyaman, W., Chawla, P., Sharuk, M., Raddassi, K., Bronson, R. T., & Khoury, S. J. (2007). Elevated neuronal expression of CD200 protects Wlds mice from inflammation-mediated neurodegeneration. *The American Journal of Pathology, 170*, 1695–1712.

Chiueh, C. C., Burns, R. S., Markey, S. P., Jacobowitz, D. M., & Kopin, I. J. (1985). Primate model of parkinsonism: Selective lesion of nigrostriatal neurons by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine produces an extrapyramidal syndrome in rhesus monkeys. *Life Sciences, 36*, 213–218.

Cho, D. H., Nakamura, T., & Lipton, S. A. (2010). Mitochondrial dynamics in cell death and neurodegeneration. *Cellular and Molecular Life Sciences, 67*, 3435–3447. doi:10.1007/s00018-010-0435-2.

Choe, M. A., Koo, B. S., An, G. J., & Jeon, S. (2012). Effects of treadmill exercise on the recovery of dopaminergic neuron loss and muscle atrophy in the 6-OHDA lesioned parkinson's disease rat model. *The Korean Journal of Physiology & Pharmacology, 16*, 305–312. doi:10.4196/kjpp.2012.16.5.305.

Cohen, A. D., Tillerson, J. L., Smith, A. D., Schallert, T., & Zigmond, M. J. (2003). Neuroprotective effects of prior limb use in 6-hydroxydopamine-treated rats: Possible role of GDNF. *Journal of Neurochemistry, 85*, 299–305.

Coleman, M. P., & Freeman, M. R. (2010). Wallerian degeneration, wld(s), and nmnat. *Annual Review of Neuroscience, 33*, 245–267.

Cooper, O., Seo, H., Andrabi, S., Guardia-Laguarta, C., Graziotto, J., Sundberg, M., McLean, J. R., Carrillo-Reid, L., Xie, Z., Osborn, T., Hargus, G., Deleidi, M., Lawson, T., Bogetofte, H., Perez-Torres, E., Clark, L., Moskowitz, C., Mazzulli, J., Chen, L., Volpicelli-Daley, L., Romero, N., Jiang, H., Uitti, R. J., Huang, Z., Opala, G., Scarffe, L. A., Dawson, V. L., Klein, C., Feng, J., Ross, O. A., Trojanowski, J. Q., Lee, V. M., Marder, K., Surmeier, D. J., Wszolek, Z. K., Przedborski, S., Krainc, D., Dawson, T. M., & Isacson, O. (2012). Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. *Science Translational Medicine, 4*, 141ra90. doi:10.1126/scitranslmed.3003985.

De Lau, L. M., & Breteler, M. M. (2006). Epidemiology of Parkinson's disease. *Lancet Neurology, 5*, 525–535.

Exner, N., Lutz, A. K., Haass, C., & Winklhofer, K. F. (2012). Mitochondrial dysfunction in Parkinson's disease: Molecular mechanisms and pathophysiological consequences. *The EMBO Journal, 31*, 3038–3062. doi:10.1038/emboj.2012.170.

Fearnley, J. M., & Lees, A. J. (1991). Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain, 114*, 2283–2301.

Felicio, A. C., Shih, M. C., Godeiro-Junior, C., Andrade, L. A., Bressan, R. A., & Ferraz, H. B. (2009). Molecular imaging studies in Parkinson disease: Reducing diagnostic uncertainty. *The Neurologist, 15*, 6–16. doi:10.1097/NRL.0b013e318183fdd8.

Fredriksson, A., & Archer, T. (1994). MPTP-induced behavioral and biochemical deficits: A parametric analysis. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 7*, 123–132.

Fredriksson, A., Plaznik, A., Sundström, E., Jonsson, G., & Archer, T. (1990). MPTP-induced hypoactivity in mice: Reversal by L-Dopa. *Pharmacology and Toxicology, 67*, 295–301.

Fredriksson, A., Palomo, T., Chase, T. N., & Archer, T. (1999). Tolerance to a suprathreshold dose of L-Dopa in MPTP mice: Effects of glutamate antagonists. *Journal of Neural Transmission, 106*, 283–300.

Fredriksson, A., Stigsdotter, I. M., Hurtig, A., Ewalds-Kvist, B., & Archer, T. (2011). Running wheel activity restores MPTP-induced functional deficits. *Journal of Neural Transmission, 18*, 407–420.

Gaggelli, E., Kozlowski, H., Valensin, D., & Valensin, G. (2006). Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). *Chemical Review, 106*, 1995–2044.

Galindo, M. F., Solesio, M. E., Atienzar-Aroca, S., Zamora, M. J., & Bueso, J. J. (2012). Mitochondrial dynamics and mitophagy in the 6-hydroxydopamine preclinical model of Parkinson's disease. *Parkinsons Disease, 2012*, 131058. doi:10.1155/2012/131058.

Galluzzi, L., Blomgren, K., & Kroemer, G. (2009). Mitochondrial membrane permeabilization in neuronal injury. *Nature Reviews Neuroscience, 10*, 481–494. doi:10.1038/nrn2665.

Gao, J., Nalls, M. A., Shi, M., Joubert, B. R., Hernandez, D. G., Huang, X., Hollenbeck, A., Singleton, A. B., & Chen, H. (2012). An exploratory analysis on gene-environment interactions for Parkinson disease. *Neurobiology of Aging, 33*, 2528.e1–2528.e6. doi:10.1016/j.neurobiolaging.2012.06.007.

Gomez-Lazaro, M., Galindo, M. F., Concannon, C. G., Segura, M. F., Fernandez-Gomez, F. J., Llecha, N., Comella, J. X., Prehn, J. H., & Jordan, J. (2008). 6-Hydroxydopamine activates the mitochondrial apoptosis pathway through p38 MAPK-mediated, p53-independent activation of Bax and PUMA. *Journal of Neurochemistry, 104*, 1599–1612.

Gupta, A., Dawson, V. L., & Dawson, T. M. (2008). What causes cell death in Parkinson's disease? *Annals of Neurology, 64*(Suppl. 2), S3–S15.

Hasbani, D. M., & O'Malley, K. L. (2006). Wld(S) mice are protected against the Parkinsonian mimetic MPTP. *Experimental Neurology, 202*, 93–99.

Hattori, N., Machida, Y., Sato, S., Noda, K., Iijima-Kitami, M., Kubo, S., & Mizuno, Y. (2006). Molecular mechanisms of nigral neurodegeneration in Park2 and regulation of parkin protein by other proteins. *Journal of Neural Transmission. Supplementum, 70*, 205–208.

Hawkes, C. H., Del Tredici, K., & Braak, H. (2007). Parkinson's disease: A dual-hit hypothesis. *Neuropathology and Applied Neurobiology, 33*, 599–614.

Heikkila, R. E., Sieber, B.-A., Manzino, L., & Sonsalla, P. K. (1989). Some features of the nigrostriatal dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the mouse. *Molecular and Chemical Neuropathology, 10*, 171–183.

Heo, J. Y., Park, J. H., Kim, S. J., Seo, K. S., Han, J. S., Lee, S. H., Kim, J. M., Park, J. I., Park, S. K., Lim, K., Hwang, B. D., Shong, M., & Kweon, G. R. (2012). DJ-1 null dopaminergic neuronal cells exhibit defects in mitochondrial function and structure: Involvement of mitochondrial complex I assembly. *PLoS One, 7*, e32629.

Hirota, Y., Kang, D., & Kanki, T. (2012). The physiological role of mitophagy: New insights into phosphorylation events. *International Journal of Cell Biology*. doi:10.1155/2012/354914.

Hongo, H., Kihara, T., Kume, T., Izumi, Y., Niidome, T., Sugimoto, H., & Akaike, A. (2012). Glycogen synthase kinase-3β activation mediates rotenone-induced cytotoxicity with the involvement of microtubule destabilization. *Biochemical and Biophysical Research Communications, 426*(1), 94–99. doi:10.1016/j.bbrc.2012.08.042.

Jackson-Lewis, V., Jakowec, M., Burke, R. E., & Przedborski, S. (1995). Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurodegeneration, 4*, 257–269.

Jankovic, J. (2008). Parkinson's disease: Clinical features and diagnosis. *Journal of Neurology, Neurosurgery, and Psychiatry, 79*, 368–376. doi:10.1136/jnnp.2007.131045.

Jones-Humble, S. A., Morgan, P. F., & Cooper, B. R. (1994). The novel anticonvulsant lamotrigine prevents dopamine depletion in C57 black mice in the MPTP animal model of Parkinson's disease. *Life Sciences, 54*, 245–252.

Jordan, J., Cena, V., & Prehn, J. H. M. (2003). Mitochondrial control of neuron death and its role in neurodegenerative disorders. *Journal of Physiology and Biochemistry, 59*, 129–141.

Jordan, J., de Groot, P. W. J., & Galindo, M. F. (2011). Mitochondria: The headquarters in ischemia-induced neuronal death. *Central Nervous System Agents in Medicinal Chemistry, 11*, 98–106.

Kang, J. M., Jung, J. C., Kim, H., Lim, H., Jang, S., & Oh, S. (2008). Neuroprotective effect of benzylideneacetophenone derivative on the MPTP model of neurodegeneration in mice. *Archives of Pharmacal Research, 31*, 1098–1107.

Kim, I., Rodriguez-Enriquez, S., & Lemasters, J. J. (2007). Selective degradation of mitochondria by mitophagy. *Archives of Biochemistry and Biophysics, 462*, 245–253.

Kim-Han, J. S., Antenor-Dorsey, J. A., & O'Malley, K. L. (2011). The Parkinson mimetic, MPP^+, specifically impairs mitochondrial transport in dopamine axons. *The Journal of Neuroscience, 31*, 7212–7221.

Klaisse, P., Lesemann, A., Huehnchen, P., Hermann, A., Storch, A., & Steiner, B. (2012). Physical activity and environmental enrichment regulate the generation of neural precursors in the adult mouse substantia nigra in a dopamine-dependent manner. *BMC Neuroscience, 13*, 132–146.

Korell, M., & Tanner, C. M. (2005). Epidemiology of Parkinson's disease: an overview. In M. Ebadi & R. F. Pfeiffer (Eds.), *Parkinson's disease* (pp. 39–50). New York: CRC Press.

Kostrzewa, R. M., Kostrzewa, J. P., Brus, R., Kostrzewa, R. A., & Nowak, P. (2006). Proposed animal model of severe Parkinson's disease, neonatal 6-hydroxydopamine lesion of dopaminergic innervation of striatum. *J Neural Transm, 70*, 277–279.

Kurihara, Y., Kanki, T., Aoki, Y., Hirota, Y., Saigusa, T., Uchiumi, T., & Kang, D. (2012). Mitophagy plays an essential role in reducing mitochondrial production of reactive oxygen species and mutation of mitochondrial DNA by maintaining mitochondrial quantity and quality in yeast. *The Journal of Biological Chemistry, 287*, 3265–3272. doi:10.1074/jbc.M111.280156.

Kurz, M. J., Pothakos, K., Jamaluddon, S., Scott-Pandorf, M., Arellano, C., & Lau, Y.-S. (2007). A chronic mouse model of Parkinson's disease has a reduced gait pattern certainty. *Neuroscience Letters, 429*, 39–42.

Langston, J. W. (1985). MPTP neurotoxicity: An overview and characterization of phases of toxicity. *Life Sciences, 36*, 201–206.

Langston, J. W. (2006). The Parkinson's complex, parkinsonism is just the tip of the iceberg. *Annals of Neurology, 59*, 591–596.

Langston, J. W., Forno, L. S., Rebert, C. S., & Irwin, I. (1984). Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. *Brain Research, 292*, 390–394.

Lees, A. J., Hardy, J., & Revesz, T. (2009). Parkinson's disease. *The Lancet, 373*, 2055–2066. doi:10.1016/S0140-6736(09)60492-X.

Li, Y., Liu, W., Oo, T. F., Wang, L., Tang, Y., Jackson-Lewis, V., Zhou, C., Geghman, K., Bogdanov, M., Przedborski, S., Beal, M. F., Burke, R. E., & Li, C. (2009). Mutant LRRK2 (R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. *Nature Neuroscience, 12*, 826–828.

Liesa, M., Palacín, M., & Zorzano, A. (2009). Mitochondrial dynamics in mammalian health and disease. *Physiological Reviews, 89*, 799–845. doi:10.1152/physrev.00030.2008.

Litvan, I., Chesselet, M. F., Gasser, T., Di Monte, D. A., Parker, D., Jr., Hagg, T., Hardy, J., Jenner, P., Myers, R. H., Price, D., Hallett, M., Langston, W. J., Lang, A. E., Halliday, G., Rocca, W., Duyckaerts, C., Dickson, D. W., Ben-Shlomo, Y., Goetz, C. G., & Melamed, E. (2007a). The etiopathogenesis of Parkinson disease and suggestions for future research. *Part II Journal of Neuropathology and Experimental Neurology, 66*, 329–336.

Litvan, I., Halliday, G., Hallett, M., Goetz, C. G., Rocca, W., Duyckaerts, C., Ben-Shlomo, Y., Dickson, D. W., Lang, A. E., Chesselet, M. F., Langston, W. J., Di Monte, D. A., Gasser, T., Hagg, T., Hardy, J., Jenner, P., Melamed, E., Myers, R. H., Parker, D., Jr., & Price, D. L. (2007b). The etiopathogenesis of Parkinson disease and suggestions for future research. *Part I Journal of Neuropathology and Experimental Neurology, 66*, 251–257.

Liu, L., Arun, A., Ellis, L., Peritore, C., & Donmez, G. (2012). Sirtuin 2 (SIRT2) enhances 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced nigrostriatal damage via deacetylating forkhead box O3a (Foxo3a) and activating Bim protein. *The Journal of Biological Chemistry, 287*, 32307–32311.

Lynch-Day, M. A., Mao, K., Wang, K., Zhao, M., & Klionsky, D. J. (2012). The role of autophagy in Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine, 2*, a009357.

Manning-Bog, A. B., & Langston, J. W. (2007). Model fusion, the next phase in developing animal models for Parkinson's disease. *Neurotoxicity Research, 11*, 219–240.

Marques-Aleixo, I., Oliveira, P. J., Moreira, P. I., Magalhães, J., & Ascensão, A. (2012). Physical exercise as a possible strategy for brain protection: Evidence from mitochondrial-mediated mechanisms. *Progress in Neurobiology, 99*, 149–162. doi:10.1016/j.pneurobio.2012.08.002.

McCoy, M. K., & Cookson, M. R. (2012). Mitochondrial quality control and dynamics in Parkinson's disease. *Antioxidants & Redox Signaling, 16*, 869–882. doi:10.1089/ars.2011.4074.

Meissner, C., Lorenz, H., Weihofen, A., Selkoe, D. J., & Lemberg, M. K. (2011). The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. *Journal of Neurochemistry, 117*, 856–867. doi:10.1111/j.1471-4159.2011.07253.x.

Mi, W., Beirowski, B., Gillingwater, T. H., Adalbert, R., Wagner, D., Grumme, D., Osaka, H., Conforti, L., Arnhold, S., Addicks, K., Wada, K., Ribchester, R. R., & Coleman, M. P. (2005). The slow Wallerian degeneration gene, WldS, inhibits axonal spheroid pathology in gracile axonal dystrophy mice. *Brain, 128*, 405–416.

Moran, M., Moreno-Lastres, D., Marín-Buera, L., Arenas, J., Martín, M. A., & Ugalde, C. (2012). Mitochondrial respiratory chain dysfunction: Implications in neurodegeneration. *Free Radical Biology & Medicine, 53*, 595–609.

Morfini, G., Pigino, G., Opalach, K., Serulle, Y., Moreira, J. E., Sugimori, M., Llinas, R. R., & Brady, S. T. (2007). 1-Methyl-4-phenylpyridinium affects fast axonal by activation of caspase and protein kinase C. *Proceedings of the National Academy of Sciences, 104*, 2442–2447.

Mortiboys, H., Thomas, K. J., Koopman, W. J., Klaffke, S., Abou-Sleiman, P., Olpin, S., Wood, N. W., Willems, P. H., Smeitink, J. A., Cookson, M. R., & Bandmann, O. (2008). Mitochondrial function and morphology are impaired in Parkin-mutant fibroblasts. *Annals of Neurology, 64*, 555–565.

Mortiboys, H., Johansen, K. K., Aasly, J. O., & Bandmann, O. (2010). Mitochondrial impairment in patients with Parkinson disease with the G2019S mutation in LRRK2. *Neurology, 75*, 2017–2020.

Mudò, G., Mäkelä, J., Di Liberto, V., Tselykh, T. V., Olivieri, M., Piepponen, P., Eriksson, O., Mälkiä, A., Bonomo, A., Kairisalo, M., Aguirre, J. A., Korhonen, L., Belluardo, N., & Lindholm, D. (2012). Transgenic expression and activation of PCG-1α protect dopaminergic neurons in the MPTP mouse model of Parkinson's disease. *Cellular and Molecular Life Sciences, 69*, 1153–1165.

Nieto, M., Gil-Bea, F. J., Dalfo, E., Guadrado, M., Cabodevilla, F., Sanchez, B., Catena, S., Sesma, T., Ribe, E., Ferrer, I., Ramirez, M. J., & Gomez-Isla, T. (2006). Increased sensitivity to MPTP in human alpha-synuclein A30P transgenic mice. *Neurobiology of Aging, 27*, 848–856.

Norazit, A., Meedeniya, A. C., Nguyen, M. N., & Mackay-Sim, A. (2010). Progressive loss of dopaminergic neurons induced by unilateral rotenone infusion into the medial forebrain bundle. *Brain Research, 1360*, 119–129.

Ohshima-Hosoyama, S., Simmons, H. A., Goecks, N., Joers, V., Swanson, C. R., Bondarenko, V., Velotta, R., Brunner, K., Wood, L. D., Hruban, R. H., & Emborg, M. E. (2012). A monoclonal antibody-GDNF fusion protein is not neuroprotective and is associated with proliferative pancreatic lesions in parkinsonian monkeys. *PLoS One, 7*(6), e39036. doi:10.1371/journal.pone.0039036.

Peng, J., Mao, X. O., Stevenson, F. F., Hsu, M., & Andersen, J. K. (2004). The herbicide paraquat induces dopaminergic nigral apoptosis through sustained activation of the JNK pathway. *The Journal of Biological Chemistry, 279*, 32626–32632.

Press, C., & Milbrandt, J. (2008). Nmnat delays axonal degeneration caused by mitochondrial and oxidative stress. *The Journal of Neuroscience, 28*, 4861–4871.

Qureshi, H. Y., & Paudel, H. K. (2011). Parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and alpha-synuclein mutations promote Tau protein phosphorylation at Ser262 and destabilize microtubule cytoskeleton in vitro. *The Journal of Biological Chemistry, 286*, 5055–5068.

Rasia, R. M., Bertoncini, C. W., Marsh, D., Hoyer, W., Cherny, D., Zweckstetter, M., Griesinger, C., Jovin, T. M., & Fernández, C. O. (2005). Structural characterization of copper(II) binding to alpha-synuclein: Insights into the bioinorganic chemistry of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 4294–4299.

Riederer, P., & Wuketich, S. (1976). Time course of nigrostriatal degeneration in Parkinson's disease. A detailed study of influential factors in human brain amine analysis. *Journal of Neural Transmission, 38*, 277–301.

Rochet, J. C., Hay, B. A., & Guo, M. (2012). Molecular insights into Parkinson's disease. *Progress in Molecular Biology and Translational Science, 107*, 125–188.

Saha, A. R., Hill, J., Utton, M. A., Asuni, A. A., Ackerley, S., Grierson, A. J., Miller, C. C., Davies, A. M., Buchman, V. L., Anderton, B. H., & Hanger, D. P. (2004). Parkinson's disease alpha-synuclein mutations exhibit defective axonal transport in cultured neurons. *Journal of Cell Science, 117*, 1017–1024.

Sajadi, A., Schneider, B. L., & Aebischer, P. (2004). Wlds-mediated protection of dopaminergic fibers in an animal model of Parkinson's disease. *Current Biology, 14*, 326–330.

Samsam, M., Mi, W., Wessig, C., Zielasek, J., Toyka, K. V., Coleman, M. P., & Martini, R. (2003). The Wlds mutation delays robust loss of motor and sensory axons in a genetic model for myelin-related axonopathy. *The Journal of Neuroscience, 23*, 2833–2839.

Santos, D., & Cardoso, S. M. (2012). Mitochondrial dynamics and neuronal fate in Parkinson's disease. *Mitochondrion, 12*, 428–437. doi:10.1016/j.mito.2012.05.002.

Schapiro, A. H., Agid, Y., Barone, P., Jenner, P., Lemke, M. R., Poewe, W., Rascol, O., Reichmann, H., & Tolosa, E. (2009). Perspectives on recent advances in the understanding and treatment of Parkinson's disease. *European Journal of Neurology, 16*, 1090–1099.

Scherman, D., Desnos, C., Darchen, F., Pollak, P., Javoy-Agid, F., & Agid, Y. (1989). Striatal dopamine deficiency in Parkinson's disease: Role of aging. *Annals of Neurology, 26*, 551–557.

Scherz-Shouval, R., Shvets, E., Fass, E., Shorer, H., Gil, L., & Elazar, Z. (2007). Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. *The EMBO Journal, 26*, 1749–1760.

Schultz, W., Scarnati, E., Sundström, E., & Romo, R. (1989). Protection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism by the catecholamine uptake inhibitor nomifensine: Behavioral analysis in monkeys with partial striatal dopamine depletions. *Neuroscience, 31*, 219–230.

Schwarting, R. K., Sedelis, M., Hofele, K., Auburger, G. W., & Huston, J. P. (1999). Strain-dependent recovery of open-field behavior and striatal dopamine deficiency in the mouse MPTP model of Parkinson's disease. *Neurotoxicity Research, 1*, 41–56.

Sedelis, M., Hofele, K., Auburger, G. W., Morgan, S., Huston, J. P., & Schwarting, R. K. (2000a). Evidence for resistance to MPTP in C57BL/6 mice x BALB/c F1 hybrids as compared to their progenitor strains. *Neuroreport, 11*, 1093–1096.

Sedelis, M., Hofele, K., Auburger, G. W., Morgan, S., Huston, J. P., & Schwarting, R. K. (2000b). MPTP susceptibility in the mouse: Behavioural, neurochemical and histological analysis of gender and strain differences. *Behavior Genetics, 30*, 171–182.

Sedelis, M., Schwarting, R. K., & Huston, J. P. (2001). Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. *Behavioural Brain Research, 125*, 109–125.

Sedelis, M., Hofele, K., Schwarting, R. K., Huston, J. P., & Belknap, J. K. (2003). Chromosomal loci influencing the susceptibility to the parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *The Journal of Neuroscience, 23*, 8247–8253.

Segura-Aguilar, J., & Kostrzewa, R. M. (2004). Neurotoxins and neurotoxic species implicated in neurodegeneration. *Neurotoxicity Research, 6*, 615–630.

Singh, A. K., Tiwari, M. N., Dixit, A., Upadhyay, G., Patel, D. K., Singh, D., Prakash, O., & Singh, M. P. (2011). Nigrostriatal proteomics of cypermethrin-induced dopaminergic neurodegeneration: Microglial activation-dependent and -independent regulations. *Toxicological Sciences, 122*, 526–538. doi:10.1093/toxsci/kfr115.

Song, D. D., Shults, C. W., Sisk, A., Rockenstein, E., & Masliah, E. (2004). Enhanced substantia nigra mitochondrial pathology in human α-synuclein transgenic mice after treatment with MPTP. *Experimental Neurology, 186*, 158–172.

Sonsalla, P. K., & Heikkila, R. E. (1986). The influence of dose and dosing interval on MPTP-induced dopaminergic neurotoxicity in mice. *European Journal of Pharmacology, 129*, 339–345.

Soreq, L., Ben-Shaul, Y., Israel, Z., Bergman, H., & Soreq, H. (2012). Meta-analysis of genetic and environmental Parkinson's disease models reveals a common role of mitochondrial protection pathways. *Neurobiology of Disease, 45*, 1018–1030. doi:10.1016/j.nbd.2011.12.021.

Sterky, F. H., Hoffman, A. F., Milenkovic, D., Bao, B., Paganelli, A., Edgar, D., Wibom, R., Lupica, C. R., Olson, L., & Larsson, N. G. (2012). Altered dopamine metabolism and increased vulnerability to MPTP in mice with partial deficiency of mitochondrial complex I in dopamine neurons. *Human Molecular Genetics, 21*, 1078–1089.

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jäger, S., Handschin, C., Zheng, K., Lin, J., Yang, W., Simon, D. K., Bachoo, R., & Spiegelman, B. M. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell, 127*, 397–408.

Su, B., Wang, X., Zheng, L., Perry, G., Smith, M. A., & Zhu, X. (2010). Abnormal mitochondrial dynamics and neurodegenerative diseases. *Biochimica et Biophysica Acta, 1802*, 135–142. doi:10.1016/j.bbadis.2009.09.013.

Sundström, E., Fredriksson, A., & Archer, T. (1990). Chronic neurochemical and behavioral changes in MPTP-lesioned C57BL/6 mice: A model for Parkinson's disease. *Brain Research, 528*, 181–188.

Sung, Y. H., Kim, S. C., Hong, H. P., Park, C. Y., Shin, M. S., Kim, C. J., Seo, J. H., Kim, D. Y., Kim, D. J., & Cho, H. J. (2012). Treadmill exercise ameliorates dopaminergic neuronal loss

through suppressing microglial activation in Parkinson's disease mice. *Life Sciences, 91*, 1309–1316. doi:10.1016/j.lfs.2012.10.003.

Takada, H., Yuasa, S., & Araki, T. (2011). Demyelination can proceed independently of axonal degradation during Wallerian degeneration in wlds mice. *The European Journal of Neuroscience, 34*, 531–537. doi:10.1111/j.1460-9568.2011.07783.x.

Tillerson, J. L., Cohen, A. D., Philhower, J., Miller, G. W., & Zigmond, M. J. (2001). Forced limb-use effects on the behavioral and neurochemical effects of 6-hydroxydopamine. *The Journal of Neuroscience, 21*, 4427–4435.

Tillerson, J. L., Cohen, A. D., Philhower, J., Miller, G. W., & Zigmond, M. J. (2002). Forced nonuse in unilateral Parkinsonian rats exacerbates injury. *The Journal of Neuroscience, 22*, 6790–6799.

Tolu, S., Avale, M. E., Nakatani, H., Pons, S., Parnaudeau, S., Tronche, F., Vogt, A., Monyer, H., Vogel, R., de Chaumont, F., Olivo-Marin, J. C., Changeux, J. P., & Maskos, U. (2010). A versatile system for the neuronal subtype specific expression of lentiviral vectors. *The FASEB Journal, 24*, 723–730.

Van Laar, V. S., & Berman, S. B. (2012). The interplay of neuronal mitochondrial dynamics and bioenergetics: Implications for Parkinson's disease. *Neurobiology of Disease, 51*, 43–55. PMID: 22668779.

Villalba, R. M., & Smith, Y. (2011). Differential structural plasticity of corticostriatal and thalamostriatal axo-spinous synapses in MPTP-treated Parkinsonian monkeys. *The Journal of Comparative Neurology, 519*, 989–1005. doi:10.1002/cne.22563.

Wang, K., & Klionsky, D. J. (2011). Mitochondria removal by autophagy. *Autophagy, 7*, 297–300.

Wang, J. T., Medress, Z. A., & Barres, B. A. (2012). Axon degeneration: Molecular mechanisms of a self-destruction pathway. *The Journal of Cell Biology, 196*, 7–18.

Weihofen, A., Thomas, K. J., Ostaszewski, B. L., Cookson, M. R., & Selkoe, D. J. (2009). Pink1 forms a multiprotein complex with Miro and Milton, Pink1 function to mitochondrial trafficking. *Biochemistry, 48*, 2045–2052.

Westerlund, M., Hoffer, B., & Olson, L. (2010). Parkinson's disease: Exit toxins, enter genetics. *Progress in Neurobiology, 90*, 146–156. doi:10.1016/j.pneurobio.2009.11.001.

Wirdefeldt, K., Adami, H. O., Cole, P., Trichopoulos, D., & Mandel, J. (2011). Epidemiology and etiology of Parkinson's disease: A review of the evidence. *European Journal of Epidemiology, 1*, S1–S58. doi:10.1007/s10654-011-9581-6.

Wu, T., Wang, J., Wang, C., Hallett, M., Zang, Y., Wu, X., & Chan, F. (2012). Basal ganglia circuits changes in Parkinson's disease patients. *Neuroscience Letters, 524*, 55–59. doi:10.1016/j.neulet.2012.07.012.

Yang, M. L., Hasadsri, L., Woods, W. S., & George, J. M. (2010). Dynamic transport and localization of alpha-synuclein in primary hippocampal neurons. *Molecular Neurodegeneration, 5*, 9. doi:10.1186/1750-1326-5-9.

Youle, R. J., & van der Bliek, A. M. (2012). Mitochondrial fission, fusion, and stress. *Science, 337*, 1062–1065. doi:10.1126/science.1219855.

Zheng, B., Liao, Z., Locascio, J. J., Lesniak, K. A., Roderick, S. S., Watt, M. L., Eklund, A. C., Zhang-James, Y., Kim, P. D., Hauser, M. A., Grünblatt, E., Moran, L. B., Mandel, S. A., Riederer, P., Miller, R. M., Federoff, H. J., Wüllner, U., Papapetropoulos, S., Youdim, M. B., Cantuti-Castelvetri, I., Young, A. B., Vance, J. M., Davis, R. L., Hedreen, J. C., Adler, C. H., Beach, T. G., Graeber, M. B., Middleton, F. A., Rochet, J. C., Scherzer, C. R., & Global PD Gene Expression (GPEX) Consortium. (2010). PGC-1α, a potential therapeutic target for early intervention in Parkinson's disease. *Science Translational Medicine, 2*, 52ra73.

Iron-Induced Dopaminergic Cell Death In Vivo as a Model of Parkinson's Disease

Manfred Gerlach, Kay L. Double, and Peter Riederer

Contents

Abstract

Histopathological, biochemical, and in vivo brain imaging techniques have revealed a consistent increase of total iron in the substantia nigra pars compacta (SNc) of Parkinson's disease (PD) patients. Interestingly, the increased iron was shown to be restricted to the SNc and occurs in the ferric (Fe^{3+}), rather than ferrous (Fe^{2+}), form of the metal.

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The objective of this chapter is to discuss the evidence for dopaminergic cell death in the rat following unilateral ferric iron injections into the SN. In addition, this chapter will briefly review findings showing that NM-bound ferric iron represents a pool of iron that, under certain circumstances, can be released to interact with free radical-producing pathways and the ubiquitin proteasome system, ultimately leading to dopaminergic nerve cell death in the rat. Special attention will be paid to summarize evidence that iron-induced dopaminergic cell death reproduces key features of PD and mimics molecular mechanisms underlying dopaminergic cell death in PD.

Keywords
Animal models • Iron • Neuromelanin • Oxidative stress • Parkinson's disease • Progression

1 Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder. The most striking neuropathological characteristic of PD is the relatively specific loss of the pigmented dopaminergic neurons of the substantia nigra (SN) pars compacta (SNc) and the resulting pallor of the midbrain, as well as the development of abnormal α-synuclein-immunopositive inclusion bodies within the boundaries of the pigment (Jellinger 2012). While the full clinical syndrome of PD is a complex multi-transmitter disorder, the characteristic motor symptoms associated with the sporadic, and all genetic, forms of this disorder result from the degeneration of the dopamine-producing neurons of the SNc. While the trigger for this relatively selective neuronal vulnerability remains unknown, the cascade of degenerative events leading to cell death is beginning to be understood. The major hypotheses believed to contribute to the eventual demise of nigral dopamine-producing cells include altered protein handling, disturbed iron homeostasis, oxidative stress, mitochondrial dysfunction, and neuroinflammation (Gerlach et al. 1994, 2006a; Sian-Hulsmann et al. 2011; Schapira and Jenner 2011). Age is the single most important risk factor for PD, and the biochemical changes that are consequences of aging amplify these abnormalities in the PD brain (Schapira and Jenner 2011).

Histopathological, biochemical, and in vivo brain imaging techniques have revealed a consistent increase in total SNc iron in PD (Gerlach et al. 1994, 2006a; Sian-Hulsmann et al. 2011). Interestingly, the increased iron was shown to be restricted to the SNc and occurs in the ferric (Fe^{3+}), rather than ferrous (Fe^{2+}), form of the metal (Sofic et al. 1988).

Iron increases have also been observed in the brain of nonhuman primates and rodents after exposure to dopaminergic neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Mochizuki et al. 1994), 6-hydroxydopamine (6-OHDA; Oestreicher et al. 1994), methamphetamine (Melega et al. 2007), and lipopolysaccharide (Zhang et al. 2012). For a detailed treatment of MPTP,

6-OHDA, and methamphetamine, the interested reader is referred to corresponding chapters in this handbook.

Increased iron deposits in the SNc in PD may result from several sources, including a damaged or "leaky" blood-brain barrier in the SN and disturbances of iron uptake, storage, and transport as neurodegeneration progresses (Gerlach et al. 2006b; Sian-Hulsmann et al. 2011). Major iron stores are ferritin and hemosiderin in glial cells, and neuromelanin (NM) in neurons. Age- and disease-dependent overload of iron storage molecules may result in iron release upon reduction. Consequently, the low molecular weight chelatable iron complexes may trigger redox reactions (e.g., oxidative stress), leading to damage of biomolecules, ultimately leading to dopaminergic nerve cell death. This damage includes a defect in complex I in mitochondria, DNA fragmentation, lipid peroxidation of cell membranes, protein misfolding, and disturbance of calcium homeostasis. Additionally, upon neurodegeneration, there is strong microglial activation that can be another source of high iron concentrations in the brain. Although the current evidence suggests that increased brain iron may be a secondary result of neuronal degeneration and reactive gliosis in several neurological disorders affecting the basal ganglia including PD, progressive supranuclear palsy, and multisystem atrophy, the question of whether iron-associated degenerative pathways are a significant factor driving progressive neuronal death in these disorders is yet to be definitively answered.

The objective of this chapter is to discuss the evidence for dopaminergic cell death in the rat following unilateral ferric iron injections into the SN. In addition, this chapter will briefly review findings showing that NM-bound ferric iron represents a pool of iron that, under certain circumstances, can be released to interact with free radical-producing pathways and the ubiquitin proteasome system, ultimately leading to dopaminergic nerve cell death in the rat. Special attention will be paid to summarize evidence that iron-induced dopaminergic cell death reproduces key features of PD and mimics molecular mechanisms underlying dopaminergic cell death in PD.

2 Effects of Unilateral Intranigral Injections of Ferric Iron

The unilateral injection of 50 μg of ferric iron into the right SN of the rat produced a strongly altered motor behavior in the animals, which even 3 weeks after application is expressed by a reduction in spontaneous locomotor activity in a strange environment, by a lower frequency of getting up onto the hind-legs, by a transient appearance of "freezing" phenomena and by spontaneous ipsilateral rotations that are accentuated by amphetamine (Ben-Shachar et al. 1991). This altered motor behavior was accompanied by an average 95 % reduction of the dopamine concentration in the ipsilateral striatum, as well as smaller reductions in the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (85 % or 45 % respectively) measured *post mortem*. Similar changes were also observed at lower iron concentrations (70–350 ng; Sengstock et al. 1992, 1993). In contrast, other neurochemical striatal markers,

such as noradrenaline, serotonin, and 5-hydroxyindoleacetic acid, were unchanged (Ben-Shachar et al. 1991; Arendash et al. 1993).

Histopathological investigations predominantly showed damage of the SNc (Sengstock et al. 1992; Arendash et al. 1993). As early as 24 h after intranigral application of 70 ng of ferric iron, iron-stained astrocytes and microglia were demonstrated. Immediately adjacent to these iron-stained cells, there was a severe loss of neurons and a reactive gliosis, still demonstrable after at least 6 months, as well as a decreased number of neurons stained immunohistochemically of tyrosine hydroxylase (TH).

This damage to the dopaminergic system produced by intranigral ferric iron injection was also demonstrated in vivo by voltammetric procedures measuring dopamine release (Junxia et al. 2003) and extracellular DOPAC concentrations (Wesemann et al. 1993, 1994). In the striatum of rats that have been lesioned with 50-μg ferric iron, the DOPAC signal was diminished. It is interesting to note the time course of this signal: During the 1st week following the lesion, there has been a 79 % reduction on the side of the injection compared to the opposite side, while 3–6 weeks later, the signals were diminished by 86 % or 97 % (Wesemann et al. 1994). This was the first time that a toxic insult has been shown to result in a chronic and progressive course in an animal model of PD. This chronic and progressive course provoked by the nigral iron injection was confirmed by other parameters (Sengstock et al. 1994): First, there was a similarly progressive course on dopamine and HVA changes in the striatum, determined at *post mortem*; second, a progressive atrophy of the SN has been found; and third, there was progressive alteration in apomorphine-elicited rotational behavior.

Interestingly, the unilateral injection of low amounts of ferric iron (1.5 μg) into the left ventral striatum of the rat induced also a long-lasting disturbance of the dopaminergic function (Kolasiewicz et al. 1995). Ten weeks following injection, there was a decrease of the spontaneous night locomotor activity; 14 weeks post-injection, there was a decrease of spontaneous locomotor activity during the adaptation phase in a new environment and an induction of contralateral rotation behavior following subcutaneous administration of 2 mg/kg apomorphine.

The mechanism of the neurotoxic effect of injected ferric iron has not been extensively investigated and is only partly understood. It is assumed that ferric iron is taken up into the neurons and glia by transferrin-receptors (Arendash et al. 1993). Indeed, it was demonstrated in SH-SY5Y neuroblastoma cells that exposure to ferric iron (1–80 μM) leads to a concentration-dependent accumulation of intracellular ferric iron (Nunez et al. 2004). Ferric iron is probably reduced to ferrous iron in the cytosol of these cells. Ferrous iron showed a cytotoxic action in primary cultures of neurons derived from the mesencephalon of rat embryos (Michel et al. 1992). In a reaction similar to the Fenton reaction, excess ferrous iron could catalyze the formation of hydroxyl free radicals from hydrogen peroxide which is an endogenous product of enzymatic dopamine metabolism. This proposed mechanism for the neurotoxic effect was indirectly confirmed by the demonstration of increased amounts of thiobarbituric acid-reactive compounds (a measure of lipid peroxidation) in the SN of ferric iron-infused rats (Arendash et al. 1993;

Wesemann et al. 1993; Sengstock et al. 1997) and the inhibitory effect of desferrioxamine, an iron chelator (Wesemann et al. 1993; Lan and Jiang, 1997). In addition, cell culture studies have demonstrated that exposure of ferric iron (40–150 μM) leads to oxidative stress, assessed by reduction of reduced glutathione (GSH) and total glutathione (GSH and GSSG) concentrations (Nunez et al. 2004) as well as activation of redox-sensitive signals (Salvador and Oteiza 2011), and apoptotic cell death (Salvador and Oteiza 2011).

It has also been shown that ferrous iron led to the formation of large perinuclear inclusion bodies in SH-SY5Y cells that overexpress α-synuclein (Hasegawa et al. 2004). However, the presence of Lewy body-like nigral inclusions has not been established in animals injected with ferric iron into the SN. This is probably the result of the acute administration of iron. It has been observed that inclusions form only in MPTP-treated mice when the neurotoxin is administered continuously at low doses (Fornai et al. 2005).

Extracellular iron has also been shown to increase activated microglia, the principal immune cells in the CNS, and subsequent neurodegeneration in animal models of PD (Shoham and Youdim 2000). Interestingly, an increased SN iron content and increased amounts of activated microglia are associated with SN hyperechogenicity in postmortem brains, whereas increased SN neuromelanin content is related to reduced SN echogenicity (Berg et al. 2010).

3 Effects of Unilateral Injections of Human Neuromelanin-Bound Ferric Iron into the Substantia Nigra

NM is considered as an endogenous iron-binding molecule in pigmented neurons as ferritin is primarily located in glia, rather than neurons, and is believed to play a physiological role in intraneuronal iron homeostasis (Double et al. 2000; Gerlach et al. 2006a). Isolated human NM consists of 2.8 % iron as estimated by Mössbauer spectroscopy (Gerlach et al. 1995), while the concentration of ferric iron in the SN has been determined using electron paramagnetic resonance at 11.3-μg iron/mg isolated NM (Shima et al. 1997). Our Mössbauer spectroscopy data demonstrated also that the iron bound to NM is purely ferric iron that is bound to ferritin-like oxyhydroxide clusters (Gerlach et al. 1995). In PD, where nigral iron levels are increased, saturation of high affinity iron-binding sites on NM may overwhelm the protective capacity of this molecule, leading instead to an increase in redox-active iron, and subsequent cellular damage (Gerlach et al. 2008). Support for this theory comes from changes in NM in the PD brain where significantly less iron is bound to NM than that seen in the normal brain (Lopiano et al. 1999). This suggests that changes in iron-binding to NM may result in increased levels of intraneuronal free iron and subsequent cell damage.

Indeed, it has been demonstrated in adult male Sprague-Dawley rats that injection of 1 μl of a NM/ferric iron (2.5 nmol or 0.139 μg iron) suspension into the ventrolateral region of the left SNc leads to a 50 % reduction of the dopamine neuron number (counted as TH-immunopositive neurons) 8 weeks after injection

compared to that in vehicle-injected animals (Double et al. 2003). The absence of motoric disturbances or a striatal dopamine deficit in these animals suggests a subclinical dopaminergic lesion. This is in contrast to many of the commonly used animal models of PD including the unilateral injection of 50 μg of ferric iron, where nigral TH-immunopositive neurons' cell number is dramatically reduced (up to 95 % cell loss; Gerlach and Riederer 1996).

In humans, at least 70 % of dopaminergic neurons in the SN and 70 % of striatal dopamine must be lost before the symptoms of PD develop (Riederer and Wuketich 1976; Halliday et al. 1996). Progressive neurodegeneration thus occurs several years prior to the onset of the characteristic motor symptoms. The current lesion mimics that which occurs in the parkinsonian brain, where nigral cell loss exhibits a distinct pattern, beginning with the loss of the ventrolateral neurons and progressing to loss of dorsolateral and medial nigral cell groups (Halliday et al. 1996). A localized loss of 50 % of ventrolateral dopaminergic neurons thus represents the pattern of cell loss occurring in preclinical PD. The success of this approach is demonstrated by the absence of behavioral changes following apomorphine challenge or a striatal dopamine deficit in these animals (Double et al. 2003).

A nearly 50 % reduction of TH-positive neuronal cells was also found in the SN of adult male Wistar rats 1 week following unilateral intranigral injection of 2-μl human NM (0.5 mg/ml; Zecca et al. 2008). This injection also induced a strong inflammatory response assessed by immunostaining with glial fibrillary acidic protein and Iba-1 in close vicinity to the NM granules. It has been recently shown that human NM in vitro induces microglial proliferation, chemotaxis and activates proinflammatory microglial properties by stimulating the release of tumor necrosis factor, interleukine-6, and nitric oxide (Wilms et al. 2003).

4 Conclusion

In summary, studies using unilateral intranigral administration of ferric iron and NM-bound ferric iron have been shown to result in dopaminergic neurodegeneration with characteristic features of the human disease in the rat (Table 1). Further, these studies provided insights into the pathophysiological mechanisms of redox-active iron underlying dopaminergic neuronal cell death in PD.

The ideal model of PD (Duty and Jenner 2011) would exhibit a high degree of construct validity (that is similar pathogenesis to the disease), of face validity (that is, similarity in symptoms, neurochemistry, and neuropathology to the human condition) as well as predictive validity (that is, the ability to positively identify agents that are clinically effective). Unilateral administration of ferric iron and NM-bound ferric iron into the SN is therefore a valid experimental model of mechanisms, leading to dopaminergic neurodegeneration in PD, and for the development of neuroprotective agents for PD that stop or slow disease progression (Table 1).

Table 1 Similarities of ferric iron-induced characteristic features to the human condition

	Unilateral intranigral ferric iron injection	Unilateral intranigral human NM-bound ferric iron injection
Construct validity	High (increased SNc ferric iron, oxidative stress, inflammation)	High (increased SNc ferric iron, oxidative stress, inflammation, proteasome inhibition)
Face validity		
Symptoms	Reduction of spontaneous locomotor activity	Absence of motoric disturbances: subclinical model
Neurochemistry	Severe striatal dopamine depletion (up to 95 %)	Moderate striatal dopamine depletion (50 %)
Neuropathology	Nearly complete dopaminergic SNc cell loss	Dopaminergic SNc cell loss
		Moderate dopaminergic SNc cell loss (50 %)
Progression	Intracellular proteinaceous aggregates: No	Not investigated
	Yes	Not investigated
Predictive validity	Yes (development of iron chelators for protection of dopaminergic neurons)	Not investigated

NM neuromelanin, *SNc* substantia nigra pars compacta

References

Arendash, G. W., Olanow, C. W., & Sengstock, G. J. (1993). Intranigral iron infusion in rats: a progressive model for excess nigral iron levels in Parkinson's disease? In P. Riederer & M. B. H. Youdim (Eds.), *Key topics in brain research. Iron in central nervous system disorders* (pp. 87–101). Wien/New York: Springer.

Ben-Shachar, D., Riederer, P., & Youdim, M. B. H. (1991). Iron-melanin interaction and lipid peroxidation: Implications for Parkinson's disease. *Journal of Neurochemistry, 57*, 1609–1614.

Berg, D., Godau, J., Riederer, P., Gerlach, M., & Arzberger, T. (2010). Microglia activation is related to substantia nigra echogenicity. *Journal of Neural Transmission, 117*, 1287–1292.

Double, K. L., Zecca, L., Ben-Shachar, D., Youdim, M. B. H., Riederer, P., & Gerlach, M. (2000). Neuromelanin may mediate neurotoxicity via its interaction with redox active iron. In A. Storch & M. A. Collins (Eds.), *Neurotoxic factors in Parkinson's disease and related disorders* (pp. 211–218). New York: Kluwer Academic/Plenum.

Double, K. L., Halliday, G. M., Henderson, J., Griffiths, F. M., Heinemann, T., Riederer, P., et al. (2003). The dopamine receptor agonist lisuride attenuates iron-mediated dopaminergic neurodegeneration. *Experimental Neurology, 184*, 530–535.

Duty, S., & Jenner, P. (2011). Animal models of Parkinson's disease: A source of novel treatments and clues to the cause of the disease. *British Journal of Pharmacology, 164*, 1357–1391.

Fornai, F., Schlueter, O. M., Lenzi, P., Gesi, M., Ruffoli, R., Ferrucci, M., et al. (2005). Parkinson-like syndrome induced by continuous MPTP infusion: Convergent roles of the ubiquitin-proteasome system and {alpha}-synuclein. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 3413–3418.

Gerlach, M., & Riederer, P. (1996). Animal models of Parkinson's disease: An empirical comparison with the phenomenology of the disease in man. *Journal of Neural Transmission, 103*, 987–1041.

Gerlach, M., Ben-Shachar, D., Riederer, P., & Youdim, M. B. H. (1994). Altered brain metabolism of iron as a cause of neurodegenerative diseases? *Journal of Neurochemistry, 63*, 793–807.

Gerlach, M., Trautwein, A. X., Zecca, L., Youdim, M. B. H., & Riederer, P. (1995). Mössbauer spectroscopic studies of purified human neuromelanin isolated from the substantia nigra. *Journal of Neurochemistry, 65*, 923–926.

Gerlach, M., Double, K., Götz, M. E., Youdim, M. B. H., & Riederer, P. (2006a). The role of iron in the pathogenesis of Parkinson's disease. In A. Sigel, H. Sigel, & R. K. O. Sigel (Eds.), *Neurodegenerative diseases and metal ions* (Metal ions in life sciences, Vol. 1, pp. 125–149). Chichester: Wiley.

Gerlach, M., Double, K. L., Youdim, M. B. H., & Riederer, P. (2006b). Potential sources of increased iron in the substantia nigra of Parkinsonian patients. *Journal of Neural Transmission, Supplement, 70*, 133–142.

Gerlach, M., Riederer, P., & Double, K. L. (2008). Neuromelanin-bound ferric iron as an experimental model of dopaminergic neurodegeneration in Parkinson's disease. *Parkinsonism& Related Disorders, 14*(Suppl 2), S185–S188.

Halliday, G., McRitchie, D., Cartwright, H., Pamphlett, R., Hely, M., & Morris, J. (1996). Midbrain neuropathology in idiopathic Parkinson's disease and diffuse Lewy body disease. *Journal of Clinical Neurosciences, 3*, 52–60.

Hasegawa, T., Matsuzaki, M., Takeda, A., Kikuchi, A., Akita, H., Perry, G., et al. (2004). Accelerated alpha-synuclein aggregation after differentiation of SH-SY5Y neuroblastoma cells. *Brain Research, 1013*, 51–59.

Jellinger, K. A. (2012). Neuropathology of sporadic Parkinson's disease: Evaluation and changes of concepts. *Movement Disorders, 27*, 8–30.

Junxia, X., Hong, J., Wenfang, C., & Ming, Q. Z. (2003). Dopamine release rather than content in the caudate putamen is associated with behavioral changes in the iron rat model of Parkinson's disease. *Experimental Neurology, 182*, 483–489.

Kolasiewicz, W., Jaros, T., Heim, C., Melzacka, M., Sieklucka, M., Weiner, N., et al. (1995). Injection of minuscule dose of $FeCl_3$ within the ventrolateral striatum causes a chronic disturbance of the integrative function within the limbic part of the ventral striatum. *Journal of Neural Transmission, P-D Section, 9*, 15–29.

Lan, J., & Jiang, D. H. (1997). Excessive iron accumulation in the brain: A possible potential risk of neurodegeneration in Parkinson's disease. *Journal of Neural Transmission, 104*, 649–660.

Lopiano, L., Digilio, G., Fasano, M., Giraudo, S., Rizzone, M., Torre, E., et al. (1999). Iron and neuromelanin in Parkinson's disease. *Journal of Neural Transmission, 106*, XXIV.

Melega, W. P., Lacan, G., Harvey, D. C., & Way, B. M. (2007). Methamphetamine increases basal ganglia iron to levels observed in aging. *Neuroreport, 18*, 1741–1745.

Michel, P. P., Vyas, S., & Agid, Y. (1992). Toxic effects of iron for cultured mesencephalic dopaminergic neurons derived from rat embryonic brains. *Journal of Neurochemistry, 59*, 118–127.

Mochizuki, H., Imai, H., Endo, K., Yokomizo, K., Murata, Y., Hatorri, N., et al. (1994). Iron accumulation in the substantia nigra of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrdine (MPTP)-induced hemiparkinsonian monkeys. *Neuroscience Letters, 168*, 251–253.

Nunez, M. T., Gallardo, V., Munoz, P., Tapia, V., Esparza, A., Salazar, J., et al. (2004). Progressive iron accumulation induces a biphasic change in the glutathione content of neuroblastoma cells. *Free Radical Biology and Medicine, 37*, 953–960.

Oestreicher, E., Sengstock, G. J., Riederer, P., Olanow, C. W., Dunn, A. J., & Arendash, G. W. (1994). Degeneration of nigrostriatal dopaminergic neurons increases iron within the substantia nigra: a histochemical and neurochemical study. *Brain Research, 660*, 8–18.

Riederer, P., & Wuketich, S. (1976). Time course of nigrostriatal degeneration in Parkinson's disease. A detailed study of influential factors in human brain amine analysis. *Journal of Neural Transmission, 38*, 277–301.

Salvador, G. A., & Oteiza, P. I. (2011). Iron overload triggers redox-sensitive signals in human IMR-32 neuroblastoma cells. *Neurotoxicology, 32*, 75–82.

Schapira, A. H., & Jenner, P. (2011). Etiology and pathogenesis of Parkinson's disease. *Movement Disorders, 26*, 1049–1055.

Sengstock, G. J., Olanow, C. W., Dunn, A. J., & Arendash, G. W. (1992). Iron induces degeneration of nigrostriatal neurons. *Brain Research Bulletin, 28*, 645–649.

Sengstock, G. J., Olanow, C. W., Menzies, R. A., Dunn, A. J., & Arendash, G. W. (1993). Infusion of iron into the rat substantia nigra: Nigral pathology and dose-dependent loss of striatal dopaminergic markers. *Journal of Neuroscience Research, 35*, 67–82.

Sengstock, G. J., Olanow, C. W., Dunn, A. J., Barone, S., Jr., & Arendash, G. W. (1994). Progressive changes in striatal dopaminergic markers, nigral volume, and rotational behavior following iron infusion into the rat substantia nigra. *Experimental Neurology, 130*, 82–94.

Sengstock, G. J., Zawia, N. H., Olanow, C. W., Dunn, A. J., & Arendash, G. W. (1997). Intranigral iron infusion in the rat – Acute elevations in nigral lipid peroxidation and striatal dopaminergic markers with ensuing nigral degeneration. *Biological Trace Element Research, 58*, 177–195.

Shima, T., Sarna, T., Swartz, H. M., Stroppolo, A., Gerbasi, R., & Zecca, L. (1997). Binding of iron to neuromelanin of human substantia nigra and synthetic melanin: An electron paramagnetic resonance spectroscopy study. *Free Radical Biology and Medicine, 23*, 110–119.

Shoham, S., & Youdim, M. B. H. (2000). Iron involvement in neural damage and microgliosis in models of neurodegenerative diseases. *Cellular and Molecular Biology, 46*, 743–760.

Sian-Hulsmann, J., Mandel, S., Youdim, M. B. H., & Riederer, P. (2011). The relevance of iron in the pathogenesis of Parkinson's disease. *Journal of Neurochemistry, 118*, 939–957.

Sofic, E., Riederer, P., Heinsen, H., Beckmann, H., Reynolds, G. P., Hebenstreit, G., et al. (1988). Increased iron (III) and total iron content in post mortem substantia nigra of Parkinsonian brain. *Journal of Neural Transmission, 74*, 199–205.

Wesemann, W., Blaschke, St., Clement, H. W., Grote, Ch., Weiner, N., Kolasiewicz, W., et al. (1993). Iron and neurotoxin intoxication: Comparative in vitro and in vivo studies. In P. Riederer P, M. B. H. Youdim (Eds.), *Key topics in brain research. Iron in central nervous system disorders* (pp. 79–86). Wien/New York: Springer.

Wesemann, W., Blaschke, S., Solbach, M., Grote, C., Clement, H. W., & Riederer, P. (1994). Intranigral injected iron progressively reduces striatal dopamine metabolism. *Journal of Neural Transmission [P.D.-Dem. Sect.], 8*, 209–214.

Wilms, H., Rosenstiel, P., Sievers, J., Deuschl, G., Zecca, L., & Lucius, R. (2003). Activation of microglia by human neuromelanin is NF-kappa B-dependent and involves p38 mitogen-activated protein kinase: Implications for Parkinson's disease. *Faseb Journal, 17*, 500–502.

Zecca, L., Wilms, H., Geick, S., Claasen, J. H., Brandenburg, L. O., Holzknecht, C., et al. (2008). Human neuromelanin induces neuroinflammation and neurodegeneration in the rat substantia nigra: Implications for Parkinson's disease. *Acta Neuropathologica, 116*, 47–55.

Zhang, Z., Zhang, K., Du, X., & Li, Y. (2012). Neuroprotection of desferrioxamine in lipopolysaccharide-induced nigrostriatal neuron degeneration. *Molecular Medicine Reports, 5*, 562–566.

Molecular, Cellular, and Behavioural Effects Produced by Perinatal Asphyxia: Protection by Poly (ADP-Ribose) Polymerase 1 (PARP-1) Inhibition

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Contents

Abstract

Perinatal asphyxia implies oxygen interruption at birth, leading to death whenever reoxygenation is not promptly reestablished. Reoxygenation triggers a cascade of biochemical events for restoring function at the cost of improper

The contribution of TN-P and PE-M has been equally relevant.

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homeostasis. The effects observed long after perinatal asphyxia have been explained by overexpression of sentinel proteins, such as poly(ADP-ribose) polymerase 1 (PARP-1), competing for NAD^+ during reoxygenation, leading to the idea that sentinel protein inhibition constitutes a suitable therapeutic strategy. Asphyxia also induces transcriptional activation of proinflammatory factors, including NFκB, and its subunit p65, whose translocation to the nucleus was found here, is significantly increased in brain tissue from asphyxia-exposed animals, in tandem with PARP-1 overactivation, suggesting that PARP-1 inhibition downregulates the expression of proinflammatory cytokines. Indeed, TNF-α and IL-1β were found to be increased 8 and 24 h after perinatal asphyxia in mesencephalon and hippocampus of rat neonates.

The possible neuroprotection effect of nicotinamide has been studied in an experimental model of global perinatal asphyxia in rats, inducing the insult by immersing rat fetuses into a water bath for various periods of time. Following asphyxia, the pups are delivered, immediately treated, or given to surrogate dams for nursing, pending further experiments. Systemic administration of nicotinamide was found to rapidly distribute into the brain reaching a steady-state concentration sufficient to inhibit PARP-1 activity for several hours. Nicotinamide prevented several of the long-term consequences elicited by perinatal asphyxia, supporting the idea that it constitutes a lead for exploring compounds with similar or better pharmacological profiles.

Keywords

Behavior • Development • Hypoxia • Neonatal • Obstetric complications • Plasticity • Poly(ADP-ribose) polymerase • Rats • Sentinel proteins

List of Abbreviations

AIF	Apoptosis-inducing factor
AN	Nicotinamide-treated, asphyxia-exposed rats
AS	Asphyxia exposed, saline treated
ATP	Adenosine triphosphate
BAD	Bcl-2-associated death factor
BAX	Bcl-2-associated X factor
BBB	Blood–brain barrier
bFGF	Basic fibroblast growth factor
CNS	Central nervous system
COX-2	Cyclooxygenase-2
CREB	cAMP-response element-binding protein
CS	Caesarean delivered, saline treated
DG	Dentate gyros
DNMT1	DNA(cytosine-5-)-methyl transferase 1

DPQ	3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone
DR2313	2-methyl-3,5,7,8-tetrahydrothiopyranol[4,3-d]pyrimidine-4-one
E2F	Family of DNA-binding transcription factors
EPO	Erythropoietin
ERCC2	Excision repair cross-complementing rodent repair group 2
ERK	Extracellular signal-regulated kinases
FOXO	Subclass of forkhead O family of transcription factors
FR247304	5-Chloro-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl) propyl]-4(3H)-quinazoline
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HDAC	Histone deacetylases
HIF	Hypoxia-inducible factor
HRE	Hypoxia-responsive elements
ICAM-1	Intercellular adhesion molecule-1
IGF-1	Insulin-like growth factor-1
IL-1β	Interleukin-1β
iNOS	Inducible nitric oxide synthase
IQ	Intelligence quotient
IκB	Inhibitor of kappa B protein
Ku70	Protein encoded by the XRCC1 gene, required for the nonhomologous end joining pathway of DNA repair
LIG3	DNA ligase 3
LPS	Lipopolysaccharides
NAD^+	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NFκB	Nuclear factor-κB
ONO-1924H	*N*-3-(4-oxo-3,4-dihydrophthalazin-1-yl)phenyl-4-(morpholin-4-yl) butanamide methane sulfonate monohydrate
P	Postnatal day
p300	Histone acetyltransferase p300
p65	Transcription factor p65
PAR	Poly(ADP-ribose) polymers
PARG	Poly(ADP-ribose) glycohydrolase
PARPs	Poly(ADP-ribose) polymerases
PARylated PARP	Poly-ADP-ribosylated PARP
PBS	Phosphate-buffered saline
PCAF	P300/CBP-associated factor
PCr	Phosphocreatine
PHD	Prolyl hydroxylase domain
PJ34	[*N*-(6-oxo-5,6-dihydrophenanthridin-2-yL)-*N*,*N*-dimethylacetamide.HCl]

POLB, DNA	Polymerase-β
ROS	Reactive oxygen species
SIRT	Sirtuin
SRY	Sex-determining region Y
Strep-HRP	Streptavidin-horseradish peroxidase
SVZ	Subventricular zone
TH	Tyrosine hydroxylase
TNF	Tumor necrosis factor
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labelling
VEGF	Vascular endothelial factor
XRCC1	X-ray cross-complementing factor 1

1 Introduction

Obstetric complications are important risk factors for several psychiatric and neurological disorders characterized by a delayed clinical onset. Hypoxia is a common factor of obstetric complications, priming brain development by mechanisms not yet established (Basovich 2010).

Delay in starting pulmonary ventilation at birth implies decreased oxygen saturation in blood and decreased oxygen supply to the brain, which depends upon aerobic metabolism for generating energy and for maintaining the respiratory chain and mitochondrial ATPase activity. Hypoxia implies a switch to glycolysis, which for neurons is a poor metabolic alternative, because of low stores of glucose in brain tissue and deficient ATP output by the glycolysis pathway. Furthermore, glycolysis implies production of lactate, which is accumulated in extracellular compartments, causing acidosis, although there is evidence indicating that lactate can provide a significant source of energy to neurons (Wyss et al. 2011). Prolonged hypoxia further involves suppression of gene expression and translation, favoring the activation of genes for sustained survival, such as hypoxia-inducible factor (HIF) and its target genes (Iyer et al. 1998).

The incidence of perinatal asphyxia is still high, despite improvements of perinatal care (2–6/1,000 term births; De Hann et al. 2006), occurring with higher prevalence in developing countries (Lawn et al. 2010). After asphyxia, infants can develop long-term neurological sequelae, their severity depending upon the extent of the insult. Severe asphyxia has been linked to cerebral palsy, mental retardation, and epilepsy, while mild to severe asphyxia has been associated with attention deficits, hyperactivity, and schizophrenia. No strict correlation has yet been demonstrated between the clinical outcome and the severity and/or the extent of the insult, but the outcome and how the infants recover from the insult are related. In a cohort study monitoring approximately 6,000 children for 8 years, it was demonstrated that resuscitated infants, even if asymptomatic for encephalopathy,

showed an increased risk for low IQ score (Odd et al. 2009). At present, there is no therapeutic strategy to significantly prevent the long-term effects produced by perinatal asphyxia, apart from hypothermia, which still is a controversial issue (Robertson et al. 2012).

2 Reoxygenation

While the average pO_2 in tissue is approximately 65 mmHg, it can decline below 5 mmHg following severe hypoxia. Reoxygenation is mandatory for restoration of function and survival. However, functional constraints of the cells affected by reduced oxygen may be exacerbated during reoxygenation, implying oxidative stress and production of proinflammatory cytokines, delaying the onset of proper homeostasis and complete recovery (Hagberg et al. 1996; Shalak et al. 2002; Girard et al. 2009). Reoxygenation can even enhance protein synthesis over normal levels (see Fähling 2009), including ribosomal biogenesis, leading to defects in protein synthesis fidelity, a mechanism associated to several neurodegenerative diseases (Lee et al. 2006). A number of proteins has to be increased upon reoxygenation, triggering (i) cell death, (ii) cell repairing (e.g., basic fibroblast growth factor, bFGF; VEGF, CREB-binding protein; ribosomal protein S_{19}; casein kinase 1), and (iii) *neuritogenesis* and *synaptogenesis*, required for maturation of neurocircuitries, also implying neurogenesis. Increased postnatal neurogenesis has been observed in subventricular zones (SVZ) and dentate gyrus (DG) of hippocampus in response to hypoxia/ischemia (Scheepens et al. 2003; Bartley et al. 2005).

The leading role in these early events following hypoxia is taken over by HIF-1α that is both an oxygen sensor and a transcription factor. After heterodimerization with HIF-1β, it binds to hypoxia-responsive elements (HRE) on promoters of genes like VEGF, iNOS, hemoxigenase-1, and EPO or to the oxygen sensors prolyl hydroxylase domain (PHD) proteins 2 and 3. HIF-1 also binds to other transcription factors, such as X-ray cross-complementing factor 1 (XRCC1) (Green et al. 1992), excision repair cross-complementing rodent repair group 2 (ERCC2) (Sung et al. 1993; Chiappe-Gutierrez et al. 1998; Lubec et al. 2002), and poly(ADP-ribose) polymerases (PARPs), mainly PARP-1 (Amé et al. 2004). The coordinated action of the products of these genes is believed to minimize and compensate for the damage induced by hypoxic conditions, acting on genes involved in glycolysis, oxygen transport, cell survival, and apoptosis (see Correia and Moreira 2010).

3 Sentinel Proteins

Most of the adaptive changes elicited by hypoxia begin at the level of the DNA. Indeed, within minutes of cerebral hypoxia/ischemia, oxidative damage of DNA is observed in brain parenchyma, including oxidation of guanosine to 8-oxo-guanine and deamination of cytosine to uracil. A major repair mechanism to reconstitute

damaged DNA is base excision repair, shown to be elevated following hypoxia/ischemia, implying activation of PARP-1, XRCC1, ERCC2, DNA polymerase-β (POLB), and DNA ligase (LIG3) (see Ellenberger and Tomkinson 2008; Herrera-Marschitz et al. 2011).

PARPs, XRCC1, ERCC2, POLB, and LIG3 are rapidly activated whenever there is a risk of genome damage. These proteins play a pivotal role not only in repairing mechanisms but also in other environmentally induced DNA modifications. They can act as both cell survival- and cell death-inducing factors by regulation of DNA repair, chromatin remodelling, and regulation of transcription. PARP-1 overactivation may be elicited by P300/CBP-associated factor (PCAF), resulting in caspase-independent translocation of apoptosis-inducing factor (AIF) from mitochondria to the nucleus and subsequent cell death by *chromatinolysis*. Indeed, PARP-1 activation is essential for AIF truncation and release from mitochondria (Kolthur-Seetharam et al. 2006). PARP-1 can be acetylated for enhancing nuclear factor-κB (NFκB)-, FOXO- and Ku70-mediated transcription and for that of tumor suppressor protein p53 (Zampieri et al. 2009). Otherwise, PARP-1 can be deacetylated by proteins of the sirtuin (SIRT) family, histone deacetylases (HDAC) type III, resulting in its inactivation. Activated SIRT also blocks release of truncated AIF from mitochondria (Kolthur-Seetharam et al. 2006; Rajamohan et al. 2009).

PARP-1 activity is strictly controlled to maintain the balance between cell survival and cell death. The equilibrium between the antagonistic actions of SIRT and PARP-1 is maintained through their strict dependency from and competition for NAD^+ (Berger 1985). Increased demand for NAD^+ by PARP-1 inhibits SIRT and vice versa. If both are overactive simultaneously, NAD^+ depletion generates large quantities of nicotinamide that has been shown to inhibit both SIRT and PARP activities (Rajamohan et al. 2009), probably by an end-product inhibitory mechanism. Thus, PARP and SIRT are at the core of epigenetic actions encompassing DNA and histone modifications.

4 Apoptosis

Apoptosis is the prevalent type of delayed cell death in the perinatal brain, mediated by caspase-dependent and caspase-independent mechanisms (Northington et al. 2001; see Morales et al. 2008). Indeed, pro-apoptotic proteins have been observed to be increased following perinatal asphyxia, including Bcl-2-associated X (BAX), and Bcl-2-associated death (BAD) factors, and also anti-apoptotic proteins, including Bcl-2, ERK2, and bFGF, suggesting the activation of neuroprotective and repair pathways (Morales et al. 2008). Extensive and regionally selective nuclear fragmentation has been observed in control and asphyctic rat pups, depending upon the stage of development and the analyzed brain region (Dell'Anna et al. 1997). Signs of apoptosis were observed in para- and presubiculum of both control and asphyxia-exposed animals, independently upon the severity of the insult, with the highest number of cells showing nuclear fragmentation observed

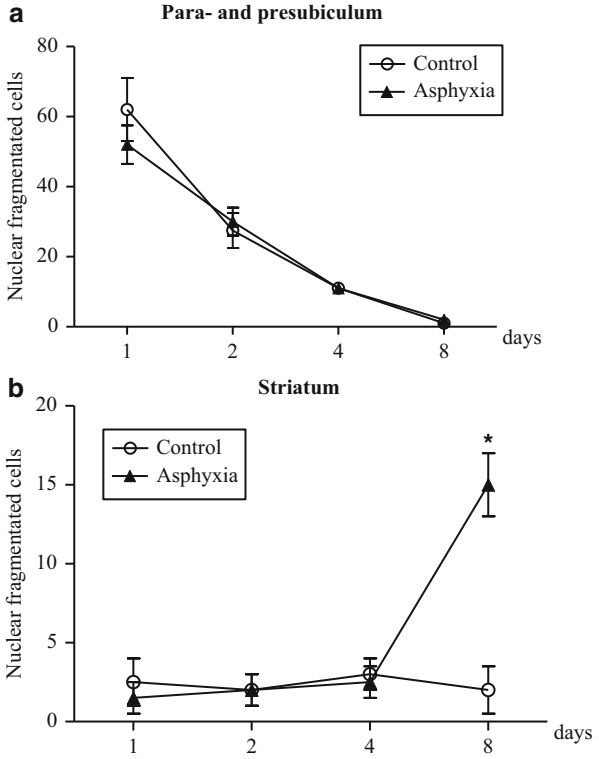

Fig. 1 *Number of neurons showing nuclear fragmentation in para- and presubiculum* (**a**) *and neostriatum* (**b**) *of asphyxia-exposed and control rat pups 1–8 days after birth***.** Brain sections (sagittal 20 μm serial formalin-fixed sections) were obtained from asphyxia-exposed (20–21 min of asphyxia) ($n = 5$–7 animals per group) (*filled triangles*) and the corresponding control ($n = 9$–16) (*open circles*) animals, 1–8 days after delivery. The sections were stained for hematoxylin-eosin and in situ DNA double-strand breaks and analyzed under light microscopy at 40× magnification to detect cellular alterations, using Foster atlas (1998) for proper brain region identification (see Dell'Anna et al. 1997). Values are expressed as means ± SEM (*$p < 0.05$; Student's *t*-test)

at P1-2, decreasing thereafter, with no cells showing nuclear fragmentation at P8 (Fig. 1a). In contrast, no significant nuclear fragmentation was observed in neostriatum of control pups, but a progressive increase in the number of cells showing chromatin fragmentation was observed in asphyxia-exposed animals, with a maximum observed at P8 (Fig. 1b). Similar results were also observed in frontal cortex and cerebellum, increasing with the length of perinatal asphyxia (Dell'Anna et al. 1997).

The effect of perinatal asphyxia on apoptosis is heterogeneous even on a particular region. Indeed, when assayed with an *Apop Tag*^{R} TUNEL kit (Millipore, Temecula, CA), the number of apoptotic cells was particularly

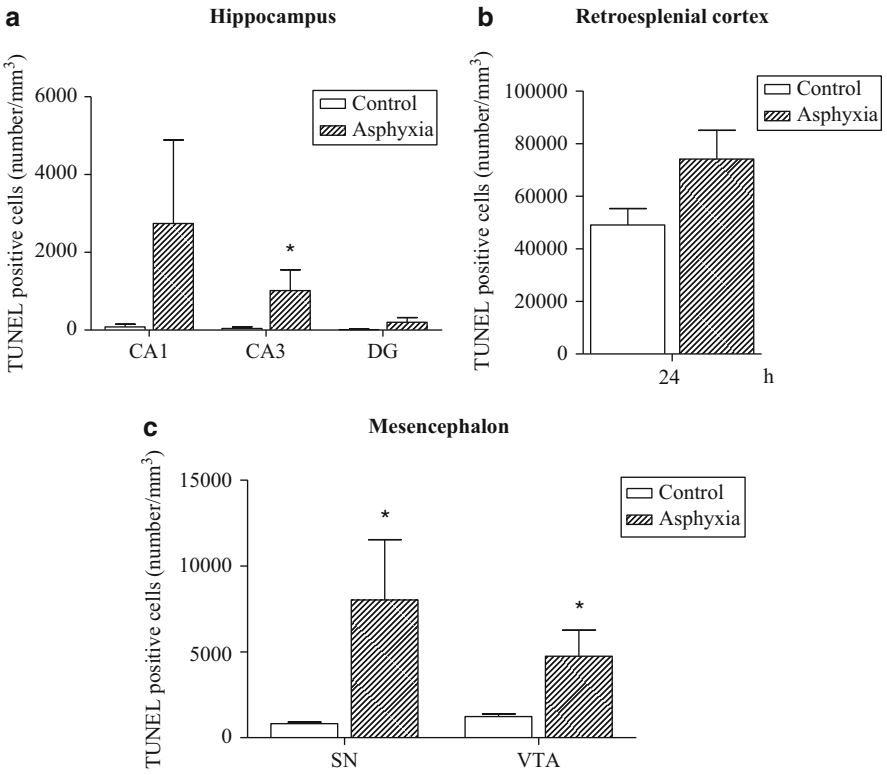

Fig. 2 *Delayed cell death following perinatal asphyxia*. Twenty-four hours after delivery, asphyxia-exposed and control animals were sacrificed, the brains rapidly removed and fixed in 0.1 M pH 7.4 phosphate-buffered saline (PBS) containing 4 % paraformaldehyde, kept in 20 % sucrose, embedded in cryomatrix (Thermo Electron Corp, Pittsburgh, PA, USA), and stored at −80° C. DNA fragmentation was evaluated in coronal brain sections (20 μm thick) with the TUNEL assay (*ApopTag*®; Millipore, Temecula, CA). TUNEL-positive cells (brown) were manually counted in a Nikon TS100 microscope (magnification 100×). The number of TUNEL-positive cells per mm^3 was determined in sections showing hippocampus (**a**), retrosplenial granular zone (**b**), and mesencephalon (**c**) (Foster atlas 1998) from the asphyxia exposed (*dashed bars*) and the corresponding controls (*open bars*). Comparisons were tested with a Student's *t*-test (*$p < 0.05$)

increased in CA1 and CA3 regions of the hippocampus of asphyxia-exposed pups, compared to that from controls (Fig. 2a). A large increase was also observed in the retrosplenial granular cortex (Fig. 2b) (Neira-Peña et al. unpublished), a region connecting the hippocampus with the entorhinal cortex (Wyss and Van Groen 1992). This is an exciting observation, because entorhinal cortex is a brain area consistently observed to be abnormal in psychiatric disorders associated to metabolic insults occurring at birth (Bachus et al. 1997). Indeed, entorhinal cortex (Akil et al. 2000) receives dopamine nerve terminals from mesencephalon

(Hökfelt et al. 1974). In agreement, as shown in Fig. 2c, apoptosis was also increased in mesencephalon following perinatal asphyxia, both in substantia nigra and VTA (Neira-Peña et al. unpublished).

5 Neuroinflammation

Asphyxia can induce transcriptional activation of proinflammatory factors, including NFκB that is normally located in the cytoplasm as a heterodimer, composed of p65 and p50 subunits, coupled to IκB. IκB dissociates from the complex when phosphorylated, liberating p65/p50, which are translocated to the nucleus for inducing the transcription of TNF-α, IL-1β intercellular adhesion molecule-1 (ICAM-1), cyclooxygenase-2 (COX-2), iNOS, and IL-6, all genes involved in inflammation (see Girard et al. 2009).

The immature CNS is capable of mounting innate and adaptive immune responses, through microglial and astrocytes, although it has been argued that the immune responses of the developing CNS differ from that of the adult, in part due to immaturity of blood–brain barrier (BBB). Tight junctions are, however, present early during embryonic development (Kniesel et al. 1996), controlling the interchange of proteins, including leukocytes (Engelhardt 2003; Vexler and Yenari 2009). The developing brain is, however, vulnerable to increases of inflammatory cytokines, leading to a substantial cross talk between peripheral and local brain immune components (see Ransohoff et al. 2003; Vexler and Yenari 2009). Indeed, Qiao et al. (2001) have demonstrated that in neonatal brain, disruption of the BBB to proteins occurs earlier after hypoxic-ischemic insult than in the mature brain.

Microglia provide immunosurveillance to the brain by stimulus-dependent activation (see Vexler and Yenari 2009). Microglia populate the developing brain at birth, and activated microglia can release a number of cytokines, including IL-1β, IGF-1, and TNF-α. It is not clear yet whether microglia activation contributes or protects against the long-term deficits induced by perinatal asphyxia, but it has been reported that minocycline, a tetracycline derivative with anti-inflammatory properties, protects the neonatal brain against ischemia, partly by inhibiting microglia activation and monocyte infiltration (Arvin et al. 2002; Dommergues et al. 2003; Denker et al. 2007). Nevertheless, to investigate the involvement of different cytokines is complex, depending upon the type of the insult, upon the timing along the development axis, and also upon specific brain regions.

The translocation of NFκB was investigated in tissue samples from mesencephalon, hippocampus, and telencephalon from asphyxia-exposed and control rat pups, 1–24 h after birth, with an antibody specific to p65. It was found (Neira-Peña et al. unpublished) that translocation of p65 to the nucleus was significantly increased ($>10\times$) in mesencephalon of both control and asphyxia-exposed pups 24 h after birth, but that increase was already remarkable 8 h after birth in asphyxia-exposed animals (Fig. 3b). In hippocampus, translocation of p65 was enhanced 1 h after birth in control animals, decreasing thereafter at 8–24 h (Fig. 3a). In contrast, in asphyxia-exposed animals, translocation of p65 was remarkably enhanced in

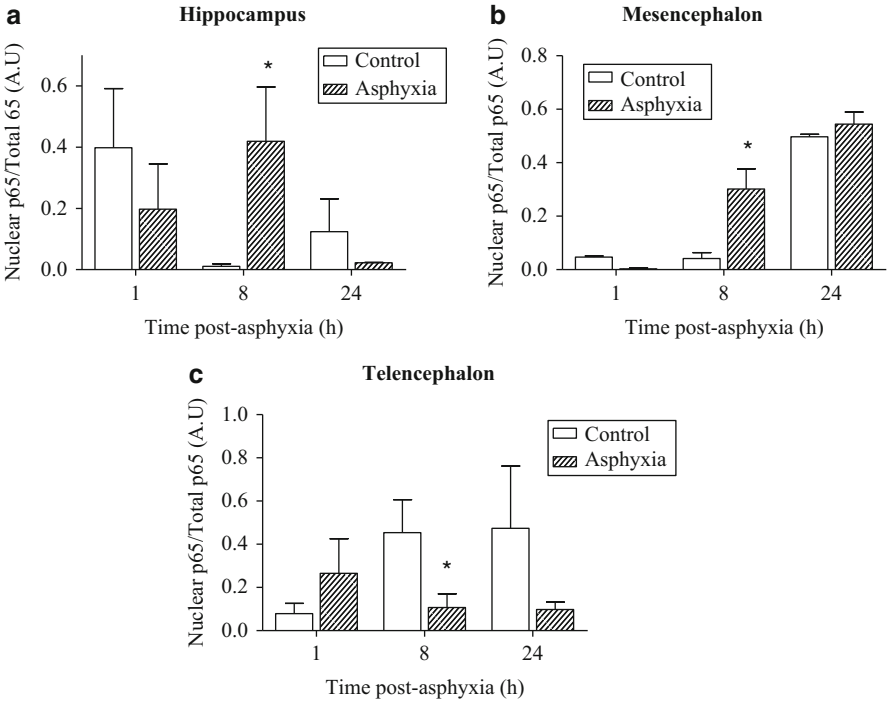

Fig. 3 *Effect of reoxygenation on p65 activation.* Asphyxia and control rats were sacrificed 1, 8, and 24 h post-birth; the brain was rapidly removed, taking up tissue samples from hippocampus (**a**), mesencephalon (**b**), and telencephalon (**c**), placed in Eppendorf tubes, frozen in liquid nitrogen, and stored at −80 °C, pending further analysis. Cytosolic and nuclear protein was extracted with a ProteoJet™ kit (Fermentas). P65 levels were determined in cytosolic and nuclear protein fractions of tissue samples from asphyxia-exposed (*dashed bars*) and controls (*open bars*) rat pups. Western blot was performed using an anti-NFκB p65 antibody (65 KDa) (Santa Cruz Biotechnology, Inc, Santa Cruz, CA). Tubulin and histone H4 were used as controls for the respective fractions. A density-graphic analysis of the bands was performed. Data are represented as nuclear p65/total p65 ratio of Arbitrary Units (AU) (comparisons were analyzed with a Student's *t*-test; $*p < 0.05$)

hippocampus 8 h after birth, decreasing thereafter to almost negligible levels at 24 h (Fig. 3a). In telencephalon, translocation of p65 increased (>10×) from 1 to 8–24 h after birth in the controls, but in asphyxia-exposed animals, translocation of p65 was increased compared to the controls at 1 h, decreasing thereafter (Fig. 3c). Furthermore, TNF-α mRNA levels were increased in hippocampus 8 h after perinatal asphyxia (Fig. 4a), while IL-1β mRNA levels were only increased in mesencephalon, 24 h after the insult (Fig. 4b).

DNA damage and cell death elicited by perinatal asphyxia has the potential of activating PARP-1, promoting NFκB activation (see Skaper 2003; Gagne et al. 2008). In agreement, Ullrich et al. (2001) showed that the microglial migration towards the site of neuronal injury is controlled by PARP-1 overactivation, correlating with NFκB translocation. To evaluate the role of

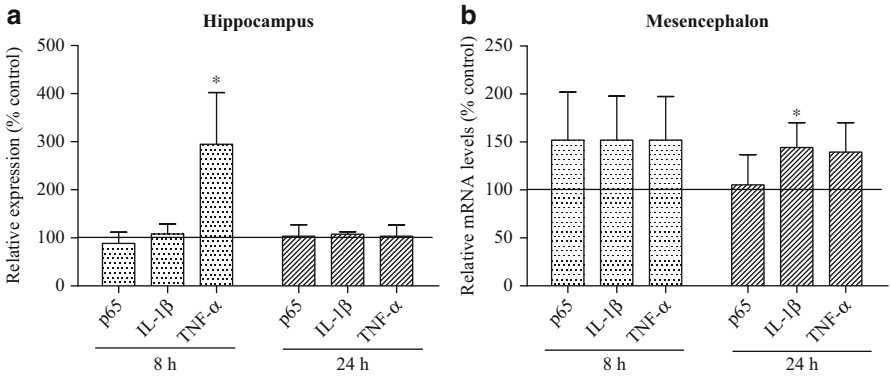

Fig. 4 *mRNA expression of inflammatory-related genes*. Total RNA was extracted with DNAzol, treated with DNAse, and subsequently verified for optical density 260/280 absorption ratios. The integrity of RNA was analyzed by denaturing gel electrophoresis. Reverse transcription was performed with oligo-dT primers from total RNA to obtain cDNA. Relative mRNA levels to GAPDH of p65, IL-1β, and TNF-α were measured by RT-qPCR in tissue samples from hippocampus (**a**) and mesencephalon (**b**). Figure shows the ratio asphyxia-exposed/control rats, at 8 (*stippled bars*) and 24 h (*dashed bars*) after birth. Results, in triplicates, were analyzed with a MxPro software (comparisons tested by a Student's *t*-test; $*p < 0.05$)

PARP-1 in inflammatory processes, PARP-1 $^{-/-}$ mice were challenged with lipopolysaccharides (LPS), finding that the knockout mice were resistant to the endotoxin shock compared with wild-type animals. The knockout mice also showed low levels of TNF-α, due to reduced transcriptional activity of NFκB (Petrilli et al. 2004).

Inhibition of PARP-1 downregulates the expression of proinflammatory cytokines and suppresses NFκB-dependent gene transcription in microglia. Kauppinen et al. (2006, 2009) demonstrated in a model of bilateral carotid occlusion-reperfusion in rats that treatment with the PARP-1 inhibitor PJ34 ([*N*-(6-oxo-5, 6-dihydrophenanthridin-2-yl)-*N*, *N*-dimethylacetamide.HCl]), 48 h later, rapidly suppressed the ischemia-induced microglial activation, enhancing long-term neuronal survival, neurogenesis, and spatial memory. Also, in a model of forebrain ischemia, PJ34 produced a near-complete inhibition of microglia activation and a significant reduction of neuronal death in the hippocampus (Hamby et al. 2007). Furthermore, it was reported that minocycline protects cortical neuron cultures against genotoxic agents causing DNA damage. Interestingly, minocycline also inhibits PARP-1 activity (Alano et al. 2006).

6 Epigenetics

PARP-1 activity is under control of and controls in a reciprocal way the activity of H1-histone, resulting in exclusion of H1 from a subset of PARP-regulated promoters and subsequent regulation of chromatin structure.

PARP itself can be poly-ADP-ribosylated (PARylated PARP), which is reversed by poly-ADP-ribose glycohydrolase (PARG). There is a link between poly-ADP-ribosylation and DNA methylation implying DNA silencing (Cohen-Armon et al. 2007). Specifically, it has been shown that PARG overexpression, or reduction of PARylated PARP, results in methylation of CpG islands in the DNA (cytosine-5-)-methyltransferase 1 (DNMT1) promoter (Zampieri et al. 2009). Gene silencing leads to widespread DNA hypomethylation, resulting in increased gene expression. Conversely, when PARylated PARP occupies the DNMT1 promoter, it protects its unmethylated state and DNMT transcription. Additionally, other transcription factors, such as p300, SRY, or E2F, are associated with PARP at the DNMT1 promoter (Rajamohan et al. 2009).

Thus, epigenetics is a factor to be considered when studying plastic changes elicited by metabolic insults occurring at birth. There is evidence that the molecular machinery that regulates histone acetylation and DNA methylation might be intimately involved in synaptic plasticity, including learning and memory. Importantly, dysfunction of epigenetic gene expression in the brain may result in neurodegenerative and psychiatric diseases (Sananbenesi and Fischer 2009). Conversely, drugs that increase histone acetylation (such as HDAC inhibitors) exhibit neuroprotective and neuro-regenerative properties in animal models of neurodegenerative diseases, enhancing cognitive functions (MacDonald and Roskams 2009).

7 A Therapeutic Target

The adverse consequences of long-term effects of overactive PARP-1 in response to asphyxia (Martin et al. 2005) have led to the notion that PARP-1 is a suitable target for therapeutic interventions preventing the long-term effects of perinatal asphyxia (see Herrera-Marschitz et al. 2011). Nicotinamide has been proposed as a prototype for counteracting PARP-1 overactivation (see Virag and Szabo 2002), replacing NADH/NAD^+ (Zhang et al. 1995), and protecting against oxidative stress (Yan et al. 1999; Wan et al. 1999; Sakakibara et al. 2000) and inflammation (Ducrocq et al. 2000).

It has been reported (Bustamante et al. 2003, 2007) that nicotinamide prevents several of the changes induced by perinatal asphyxia on monoamine contents and dopamine release monitored with in vivo microdialysis 3 months after birth, even if the treatment was delayed for 24 h, suggesting a clinically relevant therapeutic window, supporting the idea that nicotinamide can constitute a therapeutic strategy against the long-term deleterious consequences of perinatal asphyxia, as already proposed for several other pathophysiological conditions (see Virag and Szabo 2002). Nicotinamide has also been proposed as a treatment of Alzheimer's disease (Green et al. 2008).

The therapeutic doses of nicotinamide (0.8 mmol/kg, i.p.) used in the above-reported studies produce a long-lasting inhibition of PARP-1 activity measured in

brain and heart from asphyxia-exposed and control animals (Allende-Castro et al. 2012) and also a decrease of the number of apoptotic nuclei in hippocampus, increased by perinatal asphyxia (Morales et al. 2010).

The use of nicotinamide has, however, been challenged because of its low potency, limited cell uptake, and short cell viability, stimulating the search for more specific compounds, such as 3-aminobenzamide (Ducrocq et al. 2000; Hortobagyi et al. 2003; Koh et al. 2004); 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone (DPQ) (Takahashi et al. 1999); PJ34 (Abdelkarim et al. 2001); *N*-3-(4-oxo-3,4-dihydrophthalazin-1-yl)phenyl-4-(morpholin-4-yl) butanamide methane sulfonate monohydrate (ONO-1924H) (Kamanaka et al. 2004); 5-chloro-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl) propyl]-4(3H)-quinazoline (FR247304) (Iwashita et al. 2004); and 2-methyl-3,5,7,8-tetrahydrothiopyranol[4,3-d]pyrimidine-4-one (DR2313) (Nakajima et al. 2005). Ultrapotent novel PARP inhibitors are in clinical trials for reducing parenchymal cell necrosis following stroke and/or myocardial infarction, downregulating multiple pathways of inflammation and tissue injury following circulatory shock, colitis, or diabetic complications (Jagtap and Szabo 2005).

There is, however, concern about applying ultrapotent PARP inhibitors during development, since it has been shown that PARP is required for efficient repair of damaged DNA (Trucco et al. 1998; Schultz et al. 2003), suggesting that moderate PARP-1 inhibitors should be chosen for neuronal protection, whenever used for pediatric patients (Moonen et al. 2005; Geraets et al. 2006).

Nicotinamide is therefore an interesting molecule because of its low potency, which can be an advantage when used for developing animals, antagonizing the effects elicited by PARP-1 overactivation without impairing DNA repair or cell proliferation. Nicotinamide has already been tested in human clinical trials without showing any significant toxicity, although its therapeutic efficacy is still controversial (Macleod et al. 2004). Nicotinamide can constitute a lead for exploring compounds with similar or better pharmacological profiles.

8 An Experimental Model for Perinatal Asphyxia

A model for investigating the short- and long-term outcome of perinatal asphyxia was proposed at the Karolinska Institutet, Stockholm, Sweden, in the nineties (Bjelke et al. 1991; Andersson et al. 1992; Herrera-Marschitz et al. 1993). Asphyxia is induced at the time when the rats are delivered providing some features with clinical relevance: (i) it occurs at term, (ii) it is largely noninvasive, (iii) it allows studying peripheral and brain tissue in the same animal, (iv) it allows studying short- and long-term consequences of the insult in the same preparation, and (v) it is highly reproducible among laboratories. Lubec and coworkers in Vienna (Austria) (Lubec et al. 1997a, b; Seidl et al. 2000) have stressed the issue that the model allows to study the early phase of perinatal asphyxia, as observed in the clinical setup.

The model starts by evaluating the estral cycle of young female Wistar rats (~2 months of age), in order to plan for a programmed mating. A vaginal frottis is taken for evaluating the cycle, identifying proestrus, estrus, metaestrus, or diestrus. The female is then exposed to a male at the time of the proestrus for one night, evaluating thereafter for the presence of a vaginal clot. Thus, the time of delivery is calculated, supported by ethological and clinical observations, to predict the exact time of delivery (22 days). At the time of delivery, a first spontaneous birth can be observed before the dams are neck dislocated, and subjected to a caesarean section and hysterectomy. The uterine horns containing the fetuses are immediately immersed into a water bath at 37° C for various periods of time (0–22 min). Following asphyxia, the pups are removed from the uterine horns and resuscitated by cleaning from fluid and amniotic tissue, freeing the mouth and the nose. Pups exposed to caesarean delivery only (CS, 0 asphyxia) or to mild asphyxia (2–10 min) are rapidly resuscitated, without requiring anything else but removing fluid and amniotic tissue from the mouth. For pups exposed to longer periods of asphyxia (19–21 min), resuscitation implies expert and skilful handling, and it takes a long time (4–6 min) for a first gasping and even longer time for establishing a more or less regular breathing, always supported by gasping. After 60 min of caretaking, the pups are given to surrogate dams for nursing, pending further experiments. An Apgar scale is applied during the recovery period, similar to that observed in neonatal units, including parameters such as weight, sex, color of the skin, respiratory frequency, gasping, vocalization, muscular rigidity, and spontaneous movements (Dell'Anna et al. 1997; Morales et al. 2010; see Herrera-Marschitz et al. 2011). The Apgar evaluation is a critical parameter, because it assesses whether the pups are subjected to mild or to severe asphyxia, which is directly determined by the percentage of survival and recovery (Herrera-Marschitz et al. 1993, 1994). The Apgar evaluation also provides information about the condition shown by the caesarean-delivered control pups, which has to be similar to that shown by vaginally delivered pups. Thus, the Apgar evaluation is a requirement when using the present model of perinatal asphyxia, because it permits to compare results obtained by different laboratories and/or different treatments. The quality of the handling of the pups and the experience of the surrogate dam are important factors for the acceptance and nursing of both asphyxia-exposed and control pups.

The model has been useful for describing early molecular, metabolic, and physiological effects. Tissue sampling can be collected immediately after delivery, the time when the pups are removed from the uterine horns (0 min, with or without previous immersion into a water bath), or soon after reoxygenation (Lubec et al. 1997a, b; Seidl et al. 2000; Engidawork et al. 2001). Thus, energy-rich phosphates have been measured in brain and peripheral tissue immediately after delivery following short or long periods of perinatal asphyxia, demonstrating that adenosine triphosphate (ATP)/phosphocreatine (PCr) levels were first significantly decreased in kidneys (after 2 min of asphyxia), then in brain tissue (after 10 min), but in heart ATP dropped down after 20 min of asphyxia (Lubec et al. 1997a; Seidl et al. 2000; see Herrera-Marschitz et al. 2011).

9 Effect of Nicotinamide on the Long-Term Functional Consequences Elicited by Perinatal Asphyxia

Motor and cognitive alterations of variable severity, including cerebral palsy, seizures, spasticity, attention deficit, hyperactivity, mental retardation, and/or neuropsychiatric syndromes with delayed clinical onset have been associated to perinatal asphyxia (du Plessis and Volpe 2002; Van Erp et al. 2002; Kaufman et al. 2003; Vannuci and Hagberg 2004; Odd et al. 2009). With the proposed experimental model, several labs have investigated the behavioral effects associated to perinatal asphyxia, addressing motor function (Bjelke et al. 1991; Chen et al. 1995), emotion (Dell'Anna et al. 1991; Hoeger et al. 2000; Venerosi et al. 2004, 2006; Simola et al. 2008; Morales et al. 2010), and spatial memory (Boksa et al. 1995; Iuvone et al. 1996; Hoeger et al. 2000, 2006; Loidl et al. 2000; Van de Berg et al. 2003; Venerosi et al. 2004).

The issue whether perinatal asphyxia produces long-term effects on cognition has also been investigated. Novel object recognition was studied with a test applied under ethological-like conditions, devoid of any stressful cues or primary reinforcers, such as swimming, food deprivation, or electric shocks (Ennaceur and Delacour 1988). The animal is tested to discriminate between objects differing in shape and color, without any genuine significance to the rat or being previously associated to any rewarding or aversive stimuli. During a first session, two copies of the same object are presented to the rat for 4 min and then again, during a second session, when one of the previously presented objects is replaced by a novel one, similar in size, but different in shape and/or color. The rat has to recognize the novel object, spending longer time exploring the novel than that previously presented. The first and the second sessions are separated by different time intervals, for evaluating learning consolidation. A good memory would be able to recognize a previously presented object after a long time elapsing between a first and a second session, meaning that the animal would concentrate on exploring the novel object. Novel object recognition was studied using a 15 or 60 min interval, when the animals were 3 months old (Simola et al. 2008). No differences were observed between asphyxia-exposed (20 min asphyxia) and control animals when a 15 min interval elapsed between the first and the second session. Both asphyxia-exposed and control rats recognized the novel stimulus similarly well, spending longer time exploring the novel object. However, when 60 min elapsed between the first and second session, asphyxia-exposed animals spent less time exploring the novel object, indicating that asphyxia-exposed rats could not recognize its novelty. Conversely, asphyxia-exposed rats could not remember that one of the objects was already presented during the first session (Simola et al. 2008). This was a straightforward experiment showing a subtle consequence of hypoxia occurring at birth, impairing a cognitive function that shows up only after a proper challenge. It is very much reminiscent to the clinical experience revealing effects only when the child starts the primary school (see Odd et al. 2009; Strackx et al. 2010).

In a parallel study, the same condition was repeated, but a cohort of asphyxia-exposed and of control rats received 0.8 mmol/kg, i.p. of nicotinamide 1, 24, and 48 h after birth and evaluated 2–3 month later for the novel object recognition

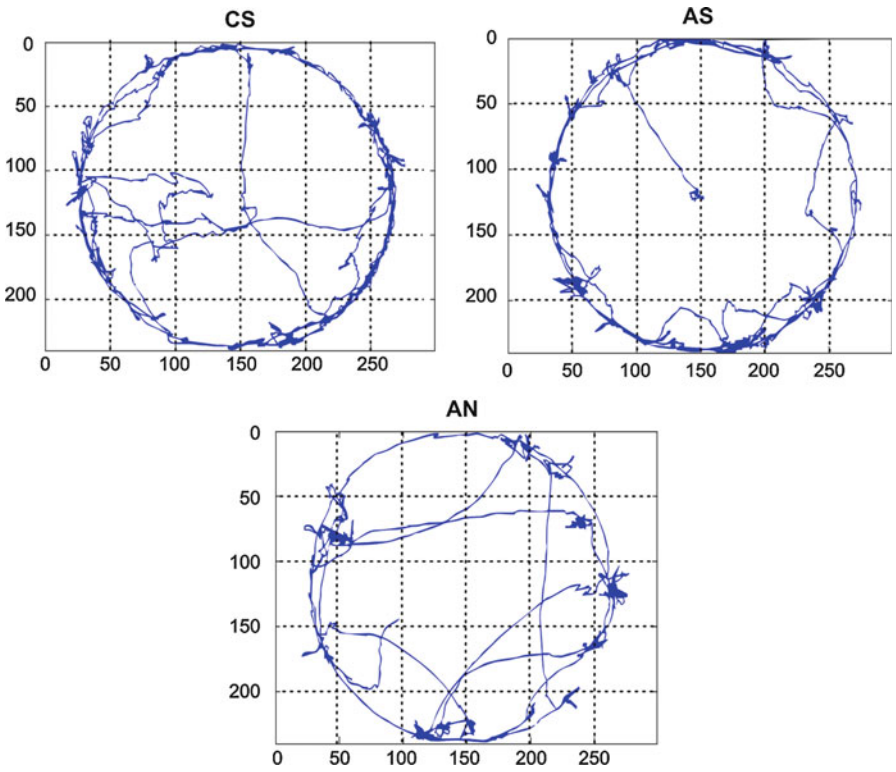

Fig. 5 *Reversal of the effect of perinatal asphyxia on exploratory behavior by neonatal nicotinamide treatment***.** Exploratory behavior was examined in a circular arena placed in a noise-free room. Arena was 140 cm in diameter painted in black, with a continuous wall of 15 cm high and placed 50 cm above the floor. A camera placed 250 cm over the arena was used for recording the rat performance. The camera was connected to a computerized processing unit (*CPU*) placed in the next room where the examiner stayed during the experiment. At 2–3 months of age, control (CS) and asphyxia-exposed (AS) rats treated with saline (3 × 0.1 ml/kg, i.p) or with nicotinamide (AN) (0.8 mmol/kg, i.p.; 1, 24 and 48 h after birth) were placed in the center of the arena free to explore for 5 min (Individual experiments taken from cohorts reported by Allende-Castro et al. 2012)

task. Nicotinamide did not affect the performance of the control animals, but prevented the effect of perinatal asphyxia, since the animals receiving nicotinamide performed identically as the controls on novel object recognition (Morales et al. 2010).

Asphyxia-exposed, treated, and control animals were tested in the first 2–3 months after birth for exploratory behavior, monitored with a video camera when animals were placed in a circular arena in a noise-free room. Each animal was left to explore the new environment for 5 min, monitoring the time expended in the center of the arena, exploiting the natural aversion of rodents for open spaces devoid of thigmotactic cues. A decreased time in the center of the arena was considered as a measure of anxiety (Perez de la Mora et al. 2012). Figure 5 shows the tracking of

a caesarean-delivered saline-treated (CS), a saline-treated asphyxia-exposed (AS), and a nicotine-treated asphyxia-exposed (AN) rat. As previously reported (Allende-Castro et al. 2012), the AS, but not CS or AN, rats avoided areas devoid of thigmotactic cues, indicating unconditioned fear and anxiety, also observed when analyzing the behavioral profile on an elevated plus maze of parallel experimental cohorts (Morales et al. 2010). Nicotinamide prevented the anxiety-related behavior in both experimental paradigms.

10 Pharmacodynamics and Pharmacokinetics of Nicotinamide

It was further investigated whether the effect of nicotinamide was related to PARP-1 inhibition using an enzymatic assay of PARP-1 activity, based on the accumulation of PAR polymers. It was found that a single dose of 0.8 mmol/kg, i.p. of nicotinamide decreased PARP-1 activity by ~70 % in brain (mesencephalon, telencephalon) and peripheral (heart) tissue of asphyxia-exposed and caesarean-delivered control pups, compared with the corresponding saline controls, 1–24 h after birth. A remarkable PARP-1 inhibition was still observed 24 h after the treatment. In vivo microdialysis experiments in ~8h-old pups allowed to monitor the distribution of a single dose of nicotinamide (0.8 mmol/kg, i.p.), in peripheral (subcutaneous) and brain (neostriatum) tissue (Allende-Castro et al. 2012), finding a rapid distribution of nicotinamide into the brain, detectable for longer than 6 h at a steady-state concentration of 20 μM. In the same study, theophylline was also investigated, only finding PARP-1 inhibition in peripheral tissue, in agreement with a poor distribution of a single dose of theophylline (0.14 nmol/kg, i.p.) into the brain compartment.

11 Conclusion

Perinatal asphyxia is still a health concern worldwide, a risk factor for several mental and neurological disorders with a delayed clinical onset. Hypoxia implies a severe energetic crisis, leading to death if reoxygenation is not promptly restored. The functional constraints produced by the lack of oxygen can be exacerbated by and during the reoxygenation period, implying oxidative stress, synthesis, and release of metabolic by-products delaying the onset of proper homeostasis and recovery. A leading role is played by HIF-1α, promoting the synthesis of proteins thought to minimize or compensate the damage induced by the hypoxic condition, but also to promote proinflammatory cytokines leading to cell death. A number of sentinel proteins are rapidly activated whenever there is a risk of genome damage, stimulating base excision repair. PARP-1 has been shown to play a pivotal role for repairing damaged DNA, but also for eliciting caspase-independent cell death when repairing is not viable, modulating pro- and anti-inflammatory signalling.

Furthermore, there is equilibrium between PARP-1 and SIRT proteins, regulating histone acetylation and DNA methylation, the core of epigenetic modification.

PARP-1 overactivation can lead to NAD^+ exhaustion, worsening the energy crisis, leading to the hypothesis that PARP-1 is a suitable target for therapeutic interventions preventing the long-term effects of perinatal asphyxia, nicotinamide being a prototype for counteracting PARP-1 overactivation.

The neuroprotection effect of nicotinamide has been studied in an experimental model of global perinatal asphyxia in rats. In this model, asphyxia is induced by immersing rat fetuses into a water bath for various periods of time. Following asphyxia, the pups are delivered, immediately treated, or given to surrogate dams for nursing, pending further experiments.

Following systemic administration, nicotinamide rapidly distributes into the brain and peripheral compartments reaching a steady-state concentration sufficient to inhibit PARP-1 activity for several hours. Nicotinamide prevents several of the long-term consequences elicited by perinatal asphyxia, supporting the idea that it can constitute a lead for exploring compounds with similar or better pharmacological profiles.

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References

Abdelkarim, G. E., Gertz, K., Harms, C., Katchanov, J., Dimagl, U., Szabo, C., & Endres, M. (2001). Protective effects of PJ34, a novel, potent inhibitor of poly(ADP-ribose) polymerase (PARP) in in vitro and in vivo models of stroke. *International Journal of Molecular Medicine, 7*, 255–260.

Akil, M., Edgar, C. L., Pierri, J. N., Casali, S., & Lewis, D. A. (2000). Decreased density of tyrosine hydroxylase-immunoreactive axons in the entorhinal cortex of schizophrenic subjects. *Biological Psychiatry, 47*, 361–370.

Alano, C. C., Kauppinen, T. M., Valls, A. V., & Swanson, R. A. (2006). Minocycline inhibits poly (ADP-ribose) polymerase-1 at nanomolar concentrations. *Proceedings of the National Academy Sciences USA, 103*, 9685–9690.

Allende-Castro, C., Espina-Marchant, P., Bustamante, D., Rojas-Mancilla, E., Neira, T., Gutierrez-Hernandez, M. A., Esmar, D., Valdes, J. L., Morales, P., Gebicke-Haerter, P. J., & Herrera-Marschitz, M. (2012). Further studies on the hypothesis of PARP-1 inhibition as a strategy for lessening the long-term effects produced by perinatal asphyxia: Effects of nicotinamide and theophylline on PARP-1 activity in brain and peripheral tissue. *Neurotoxicity Research, 22*, 79–90.

Amé, J.-C., Spenlehauer, C., & de Murcia, G. (2004). The PARP superfamily. *BioEssays, 26*, 882–893.

Andersson, K., Bjelke, B., Bolme, P., Ögren, S. O. (1992) Asphyxia-induced lesion of the rat hippocampus (CA1, CA3) and the nigro-striatal dopamine system. In J. Gross Hypoxia and Ischemia. CNS. Wissenschafliche Publikationen der Humboldt-Universität zu Berlin, B. Medizin 41:71–76.

Arvin, K. L., Han, B. H., Du, Y., Lin, S. Z., Paul, S. M., & Holtzman, D. M. (2002). Minocycline markedly protects the neonatal brain against hypoxic-ischemic injury. *Annals of Neurology, 52*, 54–61.

Bachus, S. E., Hyde, T. M., Herman, M. M., Egan, M. F., & Kleinman, J. E. (1997). Abnormal cholecystokinin mRNA levels in entorhinal cortex of schizophrenics. *Journal of Psychiatric Research, 31*, 233–256.

Bartley, J., Soltau, T., Wimborne, H., Kim, S., Martin-Sttudard, A., Hess, D., Hill, W., Waller, J., & Carrol, J. (2005). BrdU-positive cells in the neonatal mouse hippocampus following hypoxic-ischemic brain injury. *BMC Neuroscience, 6*, 15.

Basovich, S. N. (2010). The role of hypoxia in mental development and in the treatment of mental disorders. *Bioscience Trends, 4*, 288–296.

Berger, N. A. (1985). Poly (ADP-ribose) in the cellular response to DNA damage. *Radiation Research, 1001*, 4–15.

Bjelke, B., Andersson, K., Ögren, S. O., & Bolme, P. (1991). Asphyctic lesion: Proliferation of tyrosine hydroxylase immunoreactive nerve cell bodies in the rat substantia nigra and functional changes in dopamine transmission. *Brain Research, 543*, 1–9.

Boksa, P., Krishnamurthy, A., & Brooks, W. (1995). Effects of a period of asphyxia during birth on spatial learning in the rat. *Pediatric Research, 37*, 489–496.

Bustamante, D., Goiny, M., Åström, G., Gross, J., Andersson, K., & Herrera-Marschitz, M. (2003). Nicotinamide prevents the long-term effects of perinatal asphyxia on basal ganglia monoamine systems in the rat. *Experimental Brain Research, 148*, 227–232.

Bustamante, D., Morales, P., Torres-Pereyra, J., Goiny, M., & Herrera-Marschitz, M. (2007). Nicotinamide prevents the effect of perinatal asphyxia on dopamine release evaluated with in vivo microdialysis three months after birth. *Experimental Brain Research, 177*, 358–369.

Chen, Y., Ögren, S. O., Bjelke, B., Bolme, P., Eneroth, P., Gross, J., Loidl, F., Herrera-Marschitz, M., & Andersson, K. (1995). Nicotine treatment counteracts perinatal asphyxia-induced changes in the mesostriatal/limbic dopamine systems and in motor behaviour in the four-week old male rat. *Neuroscience, 68*, 531–538.

Chiappe-Gutierrez, M., Kitzmueller, E., Labudova, O., Fuerst, G., Hoeger, H., Hardmeier, R., Nohl, H., Gille, L., & Lubec, B. (1998). mRNA levels of the hypoxia inducible factor (HIF-1) and DNA repair genes in perinatal asphyxia of the rat. *Life Sciences, 63*, 1157–1167.

Cohen-Armon, M., Visochek, L., Rozendal, D., Kalal, A., Geistrikh, I., Klein, R., Bendetz-Nezer, S., Yao, Z., & Seger, R. (2007). DNA-independent PARP-1 activation by phosphorylated ERK2 increases Elk1 activity: A link to histone acetylation. *Molecular Cell, 25*, 297–308.

Correia, S. C., & Moreira, P. I. (2010). Hypoxia-inducible factor 1: A new hope to counteract neurodegeneration? *Journal of Neurochemistry, 112*, 1–12.

De Hann, M., Wyatt, J. S., Vargha-Khadem, F., Gadian, D., & Mishki, M. (2006). Brain and cognitive behavioural development after asphyxia at term birth. *Developmental Science, 9*, 350–358.

Dell'Anna, E., Calzolari, S., Milinari, M., Luvone, L., & Calimici, R. (1991). Neonatal anoxia induces transitory hyperactivity, permanent spatial memory deficits and CA1 cell density reduction in the developing rats. *Behavioural Brain Research, 45*, 125–134.

Dell'Anna, E., Chen, Y., Engidawork, E., Andersson, K., Lubec, G., Luthman, J., & Herrera-Marschitz, M. (1997). Delayed neuronal death following perinatal asphyxia in rat. *Experimental Brain Research, 115*, 105–115.

Denker, S., Ji, S., Lee, S. Y., Dingman, A., Derugin, N., Wendland, M., & Vexler, Z. S. (2007). Macrophages are comprised of resident brain microglia not infiltrating peripheral monocytes acutely after neonatal stroke. *Journal of Neurochemistry, 100*, 893–904.

Dommergues, M. A., Plaisant, F., Verney, C., & Gressens, P. (2003). Early microglia activation following neonatal excitotoxic brain damage in mice: A potential target for neuroprotection. *Neuroscience, 121*, 619–628.

du Plessis, A. J., & Volpe, J. J. (2002). Perinatal brain injury in the preterm and term newborn. *Current Opinion in Neurology, 15*, 151–157.

Ducrocq, S., Benjelloun, N., Plotkine, M., Ben-Ari, Y., & Charriaut-Marlangue, C. (2000). Poly (ADP-ribose) synthase inhibition reduces ischemic injury and inflammation in neonatal rat brain. *Journal of Neurochemistry, 74*, 2504–2511.

Ellenberger, T., & Tomkinson, A. E. (2008). Eukaryotic DNA ligases: Structural and functional insights. *Annual Review of Biochemistry, 77*, 313–338.

Engelhardt, B. (2003). Development of the blood–brain barrier. *Cell and Tissue Research, 314*, 119–129.

Engidawork, E., Loidl, F., Chen, Y., Kohlhauser, C., Stoeckler, S., Dell'Anna, E., Lubec, B., Lubec, G., Goiny, M., Gross, J., Andersson, K., & Herrera-Marschitz, M. (2001). Comparison between hypothermia and glutamate antagonism treatments on the immediate outcome of perinatal asphyxia. *Experimental Brain Research, 138*, 375–383.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. I: Behavioral data. *Behavioural Brain Research, 31*, 47–59.

Fähling, M. (2009). Surviving hypoxia by modulation of mRNA translation rate. *Journal of Cellular and Molecular Medicine, 13*, 2770–2779.

Foster, G. A. (1998). *Chemical neuroanatomy of the prenatal rat brain*. New York: Oxford University Press.

Gagne, J.-P., Isabelle, M., Lo, K. S., Bourassa, S., Hendzel, M. J., Dawson, V. L., Daeson, T. M., & Poirier, G. G. (2008). Proteome-wide identification of poly(ADP-ribose) binding proteins and poly(ADP-ribose)-associated protein complexes. *Nucleic Acids Research, 36*, 6959–6976.

Geraets, L., Moonen, H. J. J., Wouters, E. F. M., Bast, A., & Hageman, G. J. (2006). Caffeine metabolites are inhibitors of the nuclear enzyme poly(ADP-ribose)polymerase-1 at physiological concentrations. *Biochemical Pharmacology, 72*, 902–910.

Girard, S., Kadhim, H., Roy, M., Lavoie, K., Brochu, M. E., Larouche, A., & Sebire, G. (2009). Role of perinatal inflammation in cerebral palsy. *Pediatric Neurology, 40*, 168–174.

Green, A., Prager, A., & Stoudt, M. D. (1992). Relationships between DNA damage and the survival of radiosensitive mutant Chinese hamster cell lines exposed to gamma-radiation. Part 1: Intrinsic radiosensitivity. *International Journal of Radiation Biology, 61*, 465–472.

Green, K. N., Steffan, J. S., Martinez-Coria, H., Sun, X., Schreiber, S. S., Thompson, L. M., & LaFerla, F. M. (2008). Nicotinamide restores cognition in Alzheimer's disease transgenic mice via a mechanism involving sirtuin inhibition and selective reduction of Thr231-phosphotau. *The Journal of Neuroscience, 28*, 11500–11510.

Hagberg, H., Gilland, E., Bona, E., Hanson, L. A., Hahlin-Zoric, M., Biennow, M., Holst, M., McRae, A., & Söder, O. (1996). Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. *Pediatric Research, 40*, 603–609.

Hamby, A. M., Suh, S. W., Kauooinen, T. M., & Swanson, R. A. (2007). Use of a poly(ADP-ribose) polymerase inhibitor to suppress inflammation and neuronal death after cerebral ischemia-reperfusion. *Stroke, 38*, 632–636.

Herrera-Marschitz, M., Loidl, C. F., Andersson, K., & Ungerstedt, U. (1993). Prevention of mortality induced by perinatal asphyxia: Hypothermia or glutamate antagonism? *Amino Acids, 5*, 413–419.

Herrera-Marschitz, M., Loidl, C. F., You, Z.-B., Andersson, K., Silveira, R., O'Connor, W. T., & Goiny, M. (1994). Neurocircuitry of the basal ganglia studied by monitoring neurotransmitter release. Effects of intracerebral and perinatal asphyctic lesions. *Molecular Neurobiology, 9*, 171–182.

Herrera-Marschitz, M., Morales, P., Leyton, L., Bustamante, D., Klawitter, V., Espina-Marchant, P., Allende, C., Lisboa, F., Cunich, G., Jara-Caviedes, A., Neira, T., Gutierrez-Hernandez, M. A., Gonzalez-Lira, V., Simola, N., Schmitt, A., Morelli, M., Tasker, R. A., & Gebicke-Haerter, P. J. (2011). Perinatal asphyxia: Current status and approaches towards neuroprotective strategies, focus on sentinel proteins. *Neurotoxicity Research, 19*, 603–627.

Hoeger, H., Engelmann, M., Bernet, G., Seidl, R., Bubna-Littitz, H., Mosgoeller, W., Lubec, B., & Lubec, G. (2000). Long term neurological and behavioral effects of graded perinatal asphyxia in the rat. *Life Sciences, 66*, 947–962.

Hoeger, H., Engidawork, E., Stolzlechner, D., Bubna-Littitz, H., & Lubec, B. (2006). Long-term effect of moderate and profound hyperthermia on morphology, neurological, cognitive and behavioural functions in a rat model of perinatal asphyxia. *Amino Acids, 31*, 385–396.

Hökfelt, T., Ljungdahl, Å., Fuxe, K., & Johansson, O. (1974). Dopamine nerve terminals in the rat limbic cortex: Aspects of the dopamine hypothesis of schizophrenia. *Nature, 184*, 177–179.

Hortobagyi, T., Gorlach, C., Benyo, Z. L., Lacza, Z., Hortobagyi, S., Wahl, M., & Harkany, T. (2003). Inhibition of neuronal nitric oxide synthase-mediated activation of poly(ADP-ribose) polymerase in traumatic brain injury: Neuroprotection by 3-aminobenzamide. *Neuroscience, 121*, 983–990.

Iuvone, L., Geloso, M. C., & Dell'Anna, E. (1996). Changes in open field behavior, spatial memory, and hippocampal parvalbumin immunoreactivity following enrichment in rats exposed to neonatal anoxia. *Experimental Neurology, 139*, 25–33.

Iwashita, A., Tojo, N., Matsuura, S., Yamazaki, S., Kamijo, K., Ishida, J., Yamamoto, H., Hattori, K., Matsuoka, N., & Mutoh, S. (2004). A novel and potent Poly(ADP-Ribose) Polymerase-1 inhibitor, FR247304 (5-chloro-2-[3-(4-phenyl-3,6-dihydro-1(2H0-pyridinyl)propyl]-4(3H)-quinazolinone) attenuates neuronal damage in in vitro and in vivo models of cerebral ischemia. *The Journal of Pharmacology and Experimental Therapeutics, 310*, 425–436.

Iyer, N. V., Kotch, L. E., Agani, F., Leung, S. W., Laugner, E., Wenger, R. H., Gassmann, M., Gearhart, J. D., Lawler, A. M., Yu, A. Y., & Semenza, G. L. (1998). Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes & Development, 12*, 149–162.

Jagtap, P., & Szabo, C. (2005). Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nature Review, 4*, 421–440.

Kamanaka, Y., Kondo, K., Ikeda, Y., Kamoshima, W., Kitajima, T., Suzuki, Y., Nakamura, Y., & Umemura, K. (2004). Neuroprotective effects of ONO-1924H, an inhibitor of poly ADP-ribose polymerase (PARP), on cytotoxicity of PC12 cells and ischemic cerebral damage. *Life Sciences, 76*, 151–162.

Kaufman, S. A., Miller, S. P., Ferreiro, D. M., Glidden, D. H., Barkovich, A. J., & Partirdge, J. C. (2003). Encephalopathy as a predictor of magnetic resonance imaging abnormalities in asphyxiated newborns. *Pediatric Neurology, 28*, 342–346.

Kauppinen, T. M., Chan, W. Y., Suh, S. W., Wiggins, A. K., Huang, E. J., & Swanson, R. A. (2006). Direct phosphorylation and regulation of poly(ADP-ribose) polymerase-1 by extracellular signal-regulated kinases1/2. *Proceedings of the National Academy of Sciences USA, 103*, 7136–7141.

Kauppinen, T. M., Suh, S. W., Berman, A. E., Hamby, A. M., & Swanson, R. A. (2009). Inhibition of poly(ADP-ribose) polymerase suppresses inflammation and promotes recovery after ischemic injury. *Journal of Cerebral Blood Flow and Metabolism, 29*, 820–829.

Kniesel, U., Risau, W., & Wolburgh, H. (1996). Development of blood–brain barrier tight junctions in the rat cortex. *Brain Research and Development Brain Research, 96*, 229–240.

Koh, S.-H., Park, Y., Song, C. W., Kim, J. G., Kim, K., Kim, J., Kim, M.-H., Lee, S. R., Kim, D. W., Yu, H.-J., Chang, D., Hwang, S. J., & Kim, S. H. (2004). The effect of PARP inhibitor on ischemic cell death, its related inflammation and survival signals. *The European Journal of Neuroscience, 20*, 1461–1472.

Kolthur-Seetharam, U., Dantzer, F., McBurney, M. W., de Murcia, G., & Sassone-Corsi, P. (2006). Control of AIF-mediated cell death by the functional interplay of SIRT1 and PARP-1 in response to DNA damage. *Cell Cycle, 5*, 873–877.

Lawn, J. E., Kerber, K., Enweronu-Laryea, C., & Cousens, S. (2010). 3.6 million neonatal deaths-what is progressing and what is not? *Seminars in Perinatology, 34*, 371–386.

Lee, J. W., Beene, K., Nangie, L. A., Longo-Guess, C. M., Cook, S. A., Davisson, M. T., Sundberg, J. P., Schimmel, P., & Ackerman, S. L. (2006). Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. *Nature, 443*, 50–55.

Loidl, F., Gavilanes, A. W., Van Dijk, E. H., Vreuls, W., Blokland, A., Vies, J. S., Steinbusch, H. W., & Blanco, C. E. (2000). Effects of hypothermia and gender on survival and behaviour after perinatal asphyxia in rats. *Physiology and Behavior, 68*, 263–269.

Lubec, B., Mark, M., Herrera-Marschitz, M., Labudova, O., Hoeger, H., Gille, L., Nohl, H., Mosgoeller, W., & Lubec, G. (1997a). Decrease of heart protein kinase C and cyclin-dependent kinase precedes death in perinatal asphyxia of the rat. *The FASEB Journal, 11*, 482–492.

Lubec, B., Dell'Anna, E., Fang-Kircher, S., Mark, M., Herrera-Marschitz, M., & Lubec, G. (1997b). Decrease of brain protein kinase C, protein kinase A, and cyclin-dependent kinase correlating with pH precedes neuronal death in neonatal asphyxia of the rat. *Journal of Investigative Medicine, 45*, 284–294.

Lubec, B., Labudova, O., Hoeger, H., Kirchner, L., & Lubec, G. (2002). Expression of transcription factors in the brain of rats with perinatal asphyxia. *Biology of the Neonate, 81*, 266–278.

MacDonald, J. L., & Roskams, A. J. (2009). Epigenetic regulation of nervous system development by DNA methylation and histone deacetylation. *Progress in Neurobiology, 88*, 170–183.

Macleod, M. R., O'Collins, T., Howells, D. W., & Donnan, G. A. (2004). Pooling of animal experimental data reveals influence of study design and publication bias. *Stroke, 35*, 1203–1208.

Martin, S. S., Perez-Polo, J. R., Noppens, K. M., & Grafe, M. R. (2005). Biphasic changes in the levels of poly(ADPribose) polymerase-1 and caspase 3 in the immature brain following hypoxia-ischemia. *International Journal of Developmental Neuroscience, 23*, 673–686.

Moonen, H. J. J., Geraets, L., Vaarhorst, A., Wouters, E. F. M., Bast, A., & Hageman, G. J. (2005). Theophylline prevents NAD+ depletion via PARP-1 inhibition in human pulmonary epithelial cells. *Biochemical and Biophysical Research Communications, 338*, 1805–1810.

Morales, P., Fiedler, J. L., Andres, S., Berrios, C., Huaiquin, P., Bustamante, D., Cardenas, S., Parra, E., & Herrera-Marschitz, M. (2008). Plasticity of hippocampus following perinatal asphyxia: Effects on postnatal apoptosis and neurogenesis. *Journal of Neuroscience Research, 86*, 2650–2662.

Morales, P., Simola, N., Bustamante, D., Lisboa, F., Fiedler, J., Gebicke-Haerter, P., Morelli, M., Tasker, R. A., & Herrera-Marschitz, M. (2010). Nicotinamide prevents the effect of perinatal asphyxia on apoptosis, non-spatial working memory and anxiety in rats. *Experimental Brain Research, 202*, 1–14.

Nakajima, H., Kakui, N., Ohkuma, K., Ishikawa, M., & Hasegawa, T. (2005). A newly synthesized Poly(ADPRibose)Polymerase inhibitor, DR2313[2-methyl-3,5,7,8-tetrahydrothiopyranol[4,3-d]-pyrimidine-4-one]: Pharmacological profiles, neuroprotective effects and therapeutic time window in cerebral ischemia in rats. *The Journal of Pharmacology and Experimental Therapeutics, 312*, 472–481.

Northington, F. J., Ferreiro, D. M., Graham, E. M., Traystman, R. J., & Martin, L. J. (2001). Early neurodegeneration after hypoxia-ischemia in neonatal rat is necrosis while delayed neuronal death is apoptosis. *Neurobiology of Disease, 8*, 207–219.

Odd, D. E., Lewis, G., Whitelaw, A., & Gunnell, D. (2009). Resuscitation at birth and cognition at 8 years of age: A cohort study. *Lancet, 373*, 1615–1622.

Perez de la Mora, M., Gallegos-Cari, A., Crespo-Ramirez, M., Marcellino, D., Hansson, A. C., & Fuxe, K. (2012). Distribution of dopamine D2-like receptors in the rat amygdala and their role in the modulation of unconditioned fear and anxiety. *Neuroscience, 201*, 252–266.

Petrilli, V., Herced, Z., Hassa, P. O., Patel, N. S., Di Paola, R., Cortes, U., Dugo, L., Filipe, H. M., Thiemermann, C., Hottiger, M. O., Cuzzocrea, S., & Wang, Z. Q. (2004). Noncleavable poly (ADP-ribose) polymerase-1 regulates inflammation response in mice. *The Journal of Clinical Investigation, 114*, 1072–1081.

Qiao, M., Malisza, K. L., Del Biio, M. R., & Tuor, U. L. (2001). Correlation of cerebral hypoxic-ischemic T2 changes with tissue alterations in water content and protein extravasation. *Stroke, 32*, 958–963.

Rajamohan, S. B., Pillai, V. B., Gupta, M., Sundaresan, N. R., Birukov, K. G., Samant, S., Hottiger, M. A., & Gupta, M. P. (2009). SIRT promote cell survival under stress by deacetylation-dependent deactivation of poly(ADP) polymerase 1. *Molecular and Cellular Biology, 29*, 4116–4129.

Ransohoff, R. M., Kivisakk, P., & Kidd, G. (2003). Three or more routes for leukocyte migration into the central nervous system. *Nature Reviews Immunology, 3*, 569–581.

Robertson, N. J., Tan, S., Groenedaal, F., van Bel, F., Juul, S. E., Bennet, L., Derrick, M., Back, S. A., Valdez, R. C., Northington, F., Gunn, A. J., & Mallard, C. (2012). Which neuroprotective agents are ready for bench to bedside translation in the newborn infant? *Pediatrics, 160*, 544–552.

Sakakibara, Y., Mitha, A. P., Ogilvy, C. S., & Maynard, K. I. (2000). Post-treatment with nicotinamide (vitamin B(3)) reduces the infarct volume following permanent focal cerebral ischemia in female Sprague–Dawley and Wistar rats. *Neuroscience Letters, 281*, 111–114.

Sananbenesi, F., & Fischer, A. (2009). The epigenetic bottleneck of neurodegenerative and psychiatric diseases. *Biological Chemistry, 390*, 1145–1153.

Scheepens, A., Wassink, G., Piersma, M. J., Van de Berg, W. D. J., & Blanco, C. E. (2003). A delayed increase in hippocampus following global asphyxia in the neonatal rat. *Brain Research. Developmental Brain Research, 142*, 67–76.

Schultz, N., Lopez, E., Saleh-Gohari, N., & Helleday, T. (2003). Poly(ADP-ribose) polymerase (PARP-1) has a controlling role in homologous recombination. *Nucleic Acids Research, 31*, 4959–4964.

Seidl, R., Stoeckler-Ipsiroglu, S., Rolinski, B., Kohlhauser, C., Herkner, K. R., Lubec, B., & Lubec, G. (2000). Energy metabolism in graded perinatal asphyxia of the rat. *Life Sciences, 67*, 421–435.

Shalak, L. F., Laptook, A. R., Jafri, H. S., Ramilo, O., & Perlman, J. M. (2002). Clinical chorioamnionitis elevated cytokines and brain injury in term infants. *Pediatrics, 110*, 673–680.

Simola, N., Bustamante, D., Pinna, A., Pontis, S., Morales, P., Morelli, M., & Herrera-Marschitz, M. (2008). Acute perinatal asphyxia impairs non-spatial memory and alters motor coordination in adult male rats. *Experimental Brain Research, 185*, 595–601.

Skaper, S. D. (2003). Poly(ADP-ribosyl)ation enzyme-1 as a target for neuroprotection in acute central nervous system injury. *Current Drug Targets. CNS and Neurological Disorders, 2*, 279–291.

Strackx, E., Van den Hove, D. L., Prickaerts, J., Zimmermann, L., Steinbusch, H. W., Blanco, C. E., Gavilanes, A. W., & Vles, J. S. (2010). Fetal asphyctic preconditioning protects against perinatal asphyxia-induced behavioral consequences in adulthood. *Behavioural Brain Research, 208*, 343–351.

Sung, P., Bailly, V., Weber, C., Thompson, L. H., Prakash, L., & Prakash, S. (1993). Human xeroderma pigmentosum group D gene encodes a DNA helicase. *Nature, 365*, 852–855.

Takahashi, K., Pieper, A. A., Croul, S. E., Zhang, J., Snyder, S. H., & Greenberg, J. H. (1999). Post-treatment with an inhibitor of poly(ADP-ribose) polymerase attenuates cerebral damage in focal ischemia. *Brain Research, 829*, 46–54.

Trucco, C., Oliver, F. J., deMurcia, G., & Menissier de Murcia, J. (1998). DNA repair defect in poly(ADP-ribose) polymerase-deficient cell lines. *Nucleic Acids Research, 26*, 2644–2649.

Ullrich, O., Diestel, A., Evüpoglu, I. Y., & Nitsch, R. (2001). Regulation of microglial expression of integrins by poly(ADP-ribose) polymerase-1. *Nature Cell Biology, 3*, 1035–1042.

Van de Berg, W. D., Kwaijtaal, M., de Louw, A. J., Lissone, N. P., Schmitz, C., Faull, R. L., Blokland, A., Blanco, C. E., & Steinbusch, H. W. (2003). Impact of perinatal asphyxia on the GABAergic and locomotor system. *Neuroscience, 117*, 83–96.

Van Erp, T. G. M., Saleh, P. A., Rosso, P. A., Huttunen, M., Lönnqvist, J., Pirkola, T., Salonen, O., Valanne, L., Poutanen, V.-P., Standersköld-Nordenstam, C.-G., & Cannon, T. D. (2002). Contributions of genetic risk and fetal hypoxia to hippocampal volume in patients with schizophrenia or schizoaffective disorder, their unaffected siblings and healthy unrelated volunteers. *The American Journal of Psychiatry, 159*, 1514–1520.

Vannuci, S., & Hagberg, H. (2004). Hypoxia-ischemia in the immature brain. *The Journal of Experimental Biology, 207*, 3149–3154.

Venerosi, A., Valanzano, A., Cirulli, F., Alleva, E., & Calamandrei, G. (2004). Acute global anoxia during C-section birth affects dopamine-mediated behavioural responses and reactivity to stress. *Behavioural Brain Research, 154*, 155–164.

Venerosi, A., Cutuli, D., Chiarotti, F., & Calamandrei, G. (2006). C-section birth per se or followed by global asphyxia altered emotional behaviour in neonate and adult rats. *Behavioural Brain Research, 168*, 56–63.

Vexler, Z. S., & Yenari, M. A. (2009). Does inflammation after stroke affect the developing brain differently than adult brain? *Developmental Neuroscience, 31*, 378–393.

Virag, L., & Szabo, C. (2002). The Therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacological Reviews, 54*, 375–429.

Wan, F. J., Lin, H. C., Karg, B. H., Tseng, C. J., & Tung, C. S. (1999). D-amphetamine- induced depletion of energy and dopamine in the rat striatum is attenuated by nicotinamide pretreatment. *Brain Research Bulletin, 50*, 167–171.

Wyss, J. M., & Van Groen, T. (1992). Connections between the retrosplenial cortex and the hippocampal formation in the rat: A review. *Hippocampus, 2*, 1–11.

Wyss, M. T., Jolivet, R., Buck, A., Magistretti, P. J., & Weber, B. (2011). In vivo evidence for lactate as a neuronal energy source. *The Journal of Neuroscience, 31*, 7477–7485.

Yan, Q., Briehl, M., Crowley, C. L., Payne, C. M., Bernstein, H., & Bernstein, C. (1999). The NAD+ precursors, nicotinic acid and nicotinamide upregulate glyceraldehyde-3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase mRNA in Jurkat cells. *Biochemical and Biophysical Research Communications, 255*, 133–136.

Zampieri, M., Passananti, C., Calabrese, R., Perilli, M., Corbi, N., De Cave, F., Guastafierro, T., Bacalini, M. G., Reale, A., Amicosante, G., Calabrese, L., Zlatanova, J., & Caiafa, P. (2009). Parp 1 localizes within the Dnmt1 promoter and protects its unmethylated state by its enzymatic activity. *PLoS One, 4*, 4717.

Zhang, J., Pieper, A., & Snyder, S. H. (1995). Poly(ADP-ribose) synthase activation: An early indicator of neurotoxic DNA damage. *Journal of Neurochemistry, 65*, 1411–1414.

MPTP: Advances from an *Evergreen* Neurotoxin

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Abstract

Since its discovery in 1976, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models in rodents and nonhuman primates have continuously renewed to keep up with progresses of Parkinson's disease (PD) research. MPTP is able to reproduce almost all the clinical and neuropathological features of PD when administered to monkeys. In contrast, up to date no rodent model has been able to reproduce all PD features in one. Nevertheless, MPTP is a very versatile neurotoxin that can reproduce different aspects of PD pathology, depending upon the dose and regimen of administration. At the present time, a number of different MPTP models have been developed, allowing researchers to investigate either the classical PD neuropathology and neuroprotective mechanisms or known pathological processes underlining more recently discovered aspects of the disease, such as nonmotor symptoms. In this chapter primate and rodent MPTP models are reviewed, focusing mainly on the contribution that different MPTP protocols can offer to reproduce the multifaceted aspects of the disease.

Keywords

Model • MPTP • Parkinson • Primate • Rodent

1 Introduction

Preclinical investigation of Parkinson's disease (PD) neuropathology and testing of the neuroprotective strategies depends on the availability of reliable experimental animal models. However, modeling PD represents a sorely intricate task. Although several neurotoxin-based and genetic models of PD have been developed, most of them display limitations. Optimally, experimental models of PD should display face validity, reproducing the highest number of features of human PD; predictive validity is also sought, where the neuropathology should evolve progressively as in PD, and therapy responses should be comparable to the human disease; lastly, on a neuropathological view, a PD model should display construct validity, reproducing the complex interaction between neurons and surrounding cells. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is able to reproduce almost all the clinical and neuropathological features of PD when administered to monkeys (Wichmann and DeLong 2003). However, up to date no rodent model has been able to reproduce all of these features together. Depending on the

MPTP model, important limits may include lack of neurodegeneration, absence of motor symptoms despite a nigrostriatal degeneration, failure to reproduce the progressive, and slow nature of human pathology. Moreover, PD involves multiple brainstem nuclei and systemic pathologies, which is difficult to reproduce in a single animal model. On the other hand, MPTP has conveyed to be a very versatile neurotoxin, reproducing different aspects of PD pathology, depending upon the dose and regimen of administration adopted. Keeping this concept in mind and carefully pre-considering dosing regimens, MPTP models provide an incredible contribution to the understanding of the neuropathological mechanisms of PD and to the investigation of neuroprotective and disease-modifying strategies.

2 PD Symptoms and Neuropathology

Parkinson's disease is the most frequent neurodegenerative disorder after Alzheimer disease, affecting nearly 1 % of the population over 60s. Symptoms are mainly motor, including akinesia, rigidity, bradykinesia, postural abnormalities, and tremor. Furthermore, over the past decades substantial evidence has documented a plethora of nonmotor aspects of the disease. These may have either a central or an autonomic basis and include olfactory dysfunction, REM behavior disorders, chronic constipation, orthostatic intolerance, sweating, swallowing difficulties, and chronic fatigue. In addition, neuropsychiatric components such as depression, anxiety, cognitive decline, and dementia may often complicate the clinical picture. Most motor symptoms of PD are caused by the degeneration of nigrostriatal dopamine neurons. In addition, the involvement of brain areas other than the dopaminergic has been described since many years and is now gaining increasing attention for the understanding of biochemical mechanisms of nonmotor symptoms. Hence, PD is now recognized as a complex multisystem pathology, where neurodegeneration extends to brain areas such as the locus coeruleus, raphe nuclei, and dorsal motor nucleus of the vagus as well as to parts of the autonomic nervous system (Hawkes et al. 2009; Braak and Del Tredici 2008).

The appearance of motor symptoms reflects the nigrostriatal degeneration, rising when striatal DA drops to 20–30 % of physiological levels and when over 60 % of nigral neurons are lost. Most nonmotor symptoms also correlate with the advancing disease; however, some of them such as sleep behavior disorder and olfactory deficits precede the onset of motor symptoms by a number of years, being currently investigated as markers for early clinical diagnosis (Chaudhuri and Naidu 2008).

Alongside the degeneration of neurons of the substantia nigra *pars compacta* (SNc), cellular hallmarks of PD are the Lewy bodies and dystrophic neurites. Moreover, a number of malfunctioning cellular processes have been described, including oxidative stress, mitochondrial dysfunction, and impaired protein disposal system (Olanow 2007). In the past decade, several evidences have pointed to neuroinflammation as a key pathological feature and likely a hallmark of PD (Long-Smith et al. 2009). Postmortem studies have described reactive microglia around degenerating neurons in the SN, while inflammatory cytokines levels are elevated in the brain, cerebrospinal fluid, and serum of PD patients

(Blum-Degen et al. 1995; Dobbs et al. 1999; McGeer and McGeer 2008; Mogi et al. 1994). In PD, activated microglia are engaged in a vicious cycle called reactive gliosis, in which constant inflammation contributes to neuronal damage (Carta et al. 2011; Hirsch and Hunot 2009; Ransohoff and Cardona 2010; Schwartz et al. 2006).

3 MPTP Neurotoxicity

The use of MPTP to model PD is one of the few cases where the effect of a neurotoxin was discovered in humans first and then translated to an animal model. In 1982 a cluster of drug addicts in California were inadvertently intoxicated by MPTP, found as a by-product in a street preparation of an analog of the opioid meperidine. The parkinsonian syndrome shown was almost indistinguishable from PD, characterized by severe bradykinesia and rigidity, which were relieved by traditional antiparkinsonian therapies (Langston et al. 1983).

MPTP does not exert the neurotoxic effect by itself, but, after crossing the blood–brain barrier by virtue of its lipophilic nature, is rapidly oxidized to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP+) by monoamine oxidase B (MAO-B) in glia and serotonergic neurons (Fig. 1). It is then converted by spontaneous oxidation to the neurotoxic compound 1-methyl-4-phenylpyridinium (MPP+), which is a high-affinity substrate for the dopamine transporter (DAT). MPP+ can now effortlessly be taken up into dopaminergic cells and concentrated within mitochondria, following the mitochondrial transmembrane potential (Javitch et al. 1985; Mayer et al. 1986; Ramsay and Singer 1986; Ransom et al. 1987) (Fig. 1). At this level, MPP+ exerts its toxic effect by inhibiting the complex I of the electron transport chain and impairing oxidative phosphorylation (Nicklas et al. 1985). This event induces a decrease in ATP (Chan et al. 1991; Fabre et al. 1999) content and increase in ROS production that rapidly leads to cell damage and cell death (Hasegawa et al. 1990). Besides these direct effects on dopaminergic neurons, an important contribution to MPP+ toxicity is given by glial cells, which not only are responsible of MPP+ production but also are activated by MPP+ itself or by injury factors released by degenerating neurons, to upregulate inflammatory cytokines and the inducible nitric oxide synthase (iNOS) (Teismann et al. 2003; Youdim et al. 2002). Nitric oxide (NO) easily crosses cell membranes and diffuses toward surrounding dopaminergic neurons, thus contributing to oxidative stress, since peroxynitrite (ONOO-) derived from the reaction of NO with superoxide is highly reactive and induces nitration of proteins (Ischiropoulos and al-Mehdi 1995; Przedborski et al. 1996, Przedborski and Vila 2003).

4 Animal Species and Strand Sensitivity to MPTP Neurotoxicity

4.1 Rodents

In general, rodents are less susceptible than primates to MPTP toxicity. Among rodents, mice are mostly used, while rats are not recommended for

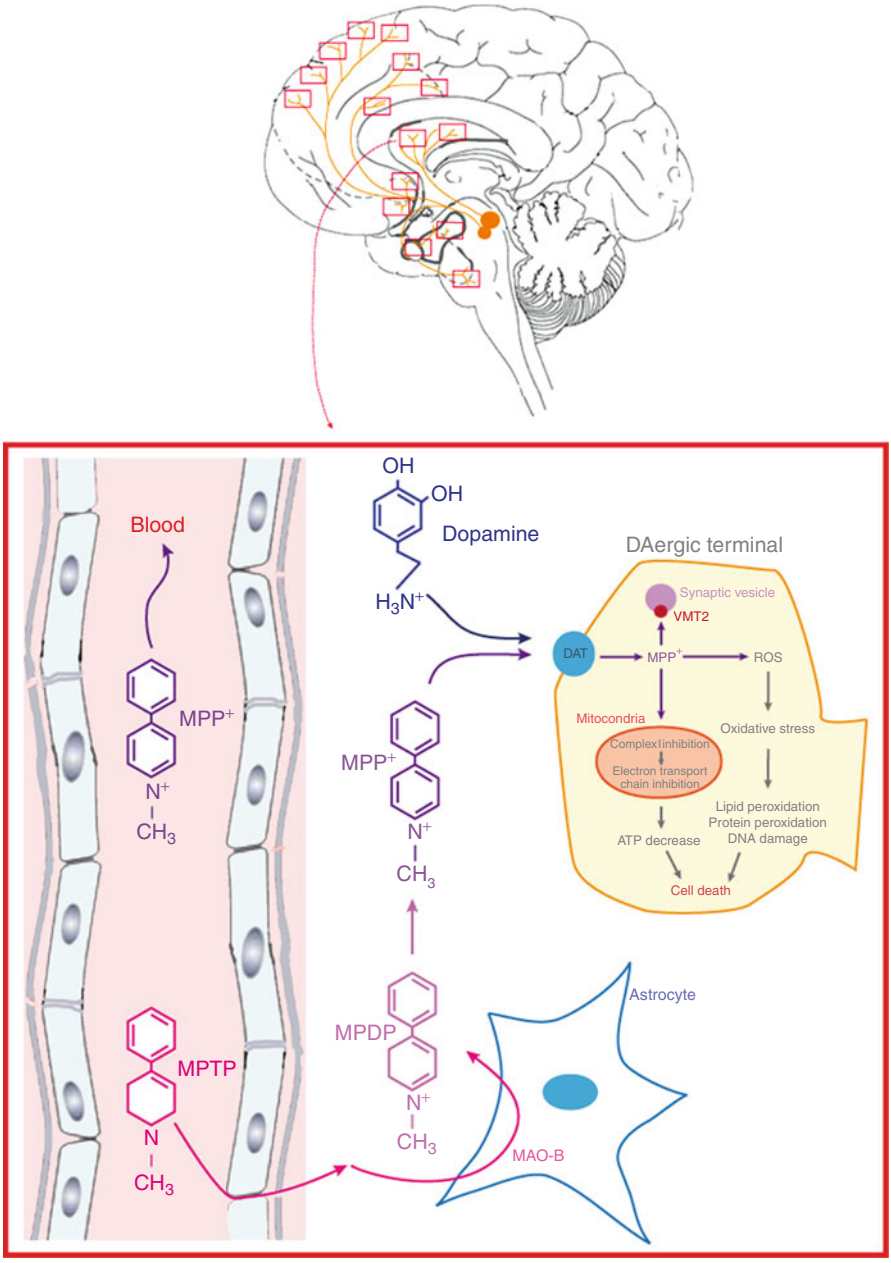

Fig. 1 Once MPTP has crossed the BBB, it is transformed to MPP+ inside the astrocytes through the action of MAO-B. Then, MPP+ enters DA neurons through the DAT and either inhibits the complex I of the mitochondria, inducing cell death, or enters into the synaptic vesicles through the VMAT-2. The levels of astrocytic MAO-B and the expression of DAT and VMAT-2 in DA neurons are important factors in the susceptibility to MPTP

MPTP models, as they display an elevated resistance to the neurotoxin (Giovanni et al. 1994; Heikkila et al. 1984; Sahgal et al. 1984). Variable susceptibility among mouse strains has also been found, the C57black/6 strain being the most sensitive to systemic MPTP injection and currently the most popular for PD research (Giovanni et al. 1994; Schmidt and Ferger 2001; Sedelis et al. 2000). Such differences among animal species and strains may be partially related to the metabolic processes that convert MPTP to toxic metabolites in the brain and periphery. MPTP clearance in the brain, and liver metabolism to nontoxic species, is slower in black mice as opposed to white mice and rats (Riachi et al. 1988). Furthermore, MAO activity in brain microvessels is lower in mice, leading to a reduced conversion of MPTP to MPP+ before entering the brain and to an enhanced capacity of MPTP to reach its sites of toxicity (Riachi et al. 1989). A higher VMAT2 density in DA neurons of rats with respect to mice has been also found (Staal and Sonsalla 2000). Based on that, a more efficient sequestration into synaptic vesicles has been suggested as a further mechanism of protection and reduced vulnerability of rats against MPP+ neurotoxicity. More recently, it has been suggested that variances in immunological backgrounds, or differences in glial and microglial cell number and ratio, might be major contributing factors in susceptibility differences to MPTP (Smeyne et al. 2005; Yasuda et al. 2008).

4.2 Primates

Apparently, all species of primates seem to be susceptible to the neurotoxin MPTP; however, species and primate families display some differences. For instance, old-world primates (those evolved in Africa, Asia, and small areas in Europe) such as baboons and macaques show a higher degree of susceptibility as compared to new-world primates (those evolved in America), such as capuchins monkeys, squirrel monkeys, or marmosets (Emborg 2007).

In order to achieve a stable parkinsonism in old-world primates such as baboons, macaques, green monkeys, and vervet monkeys, doses of MPTP range from 0.2–0.3 mg/kg to 0.6–1 mg/kg. However, 1.2 mg/kg, 2 mg/kg, or even higher doses should be used for new-world primates (Barcia et al. 2004a; Elsworth et al. 2000; Emborg 2007; Hantraye et al. 1996; Herrero et al. 1993b; Pearce et al. 1995; Perez-Otano et al. 1991; Varastet et al. 1994). These differences have led to believe that the higher degree in the phylogenetic scale, the more susceptibility to MPTP is shown. Hence, humans are highly susceptible to MPTP according to the small doses extrapolated from accidentally intoxicated individuals (Langston et al. 1983). We do not have data from great apes, such as gorillas, orangutans, or chimpanzees to compare with, but specific characteristics, described below, suggest that they may also present high susceptibility.

Different factors may contribute to the different susceptibility among primate species. Specifically, the levels of MAO-B and neuromelanin seem to play a main role. According to its higher susceptibility, primate brain contains high proportions of MAO-B (about 70–80 %) as compared with rodent brain that contains

predominantly MAO-A (Zesiewicz and Hauser 2002). On the other hand, neuromelanin, a pigment present in dopaminergic neurons, is contained in higher levels in the mesencephalic dopaminergic areas most susceptible to MPTP (Herrero et al. 1993b). In addition, elegant experiments have shown that chloroquine, a drug that blocks MPP+ binding to neuromelanin, protects monkeys from MPTP-induced dopaminergic degeneration (D'Amato et al. 1987). It is well known that MPTP susceptibility increases with age. Besides the aging effect on DA neurons itself, neuromelanin accumulation with age may also increase neuronal vulnerability to MPTP (Herrero et al. 1993a). It is noteworthy that within primates, there is a progressive increase of neuromelanin pigmentation as the phylogenetic relationship to man becomes closer (Marsden 1961; Scherer 1939), in line with an increased susceptibility to MPTP of phylogenetically higher primate species.

5 Currently Available In Vivo MPTP Models

Although a number of MPTP paradigms involving variable routes of administration have been developed to date, a main limit for all of these models is the failure of reproducing the complete and multifaceted PD pathology, particularly when working with rodents. For this reason, none of them are eligible as the best preclinical PD model, but each one should be regarded and carefully chosen for the specific purpose of the study, e.g., investigating motor or nonmotor disturbances, neurodegenerative and neuroprotective mechanisms, and neuroplastic changes.

5.1 Rodents

A variety of models are currently available, mostly in mice, since their higher sensitivity to systemic MPTP, including systemic administration (i.e., subcutaneous, intraperitoneal, or intranasal), or local injection (i.e., intraventricular or intranigral). Subcutaneous or intraperitoneal MPTP administration is used in mice only, while intracerebral injection or intranasal administration, allowing MPTP to bypass the BBB, is used in both mice and rats (Prediger et al. 2010; Yazdani et al. 2006). With regard to the protocol of administration, rodent models can be referred to as acute, subacute, or chronic. Acute models include single day-treatment paradigms. MPTP hydrochloride is administered via peripheral route and may involve a single dose of 20–40 mg/kg or a series of four doses (10–30 mg/kg) usually injected in 2 h intervals (Bezard et al. 1997a; Jackson-Lewis et al. 1995; Kohutnicka et al. 1998). A recent acute MPTP model involves the intranasal neurotoxin administration, where mice or rats receive small amounts (10–15 μl) of a MPTP solution directly in each nasal cavity (Prediger et al. 2006, 2010; Rojo et al. 2006). Subacute paradigms generally last about a week, with a daily administration protocol of doses ranging from 20 to 30 mg/kg (Carta et al. 2009; Tatton and Kish 1997; Vila et al. 2000). Chronic MPTP paradigms might be more reliable PD models, since they are able to mimic a higher number of PD features, including

the slow progressive pattern of neurodegeneration seen in human PD. Chronic protocols can last 3–5 weeks, with continuous or intermittent MPTP treatment. Intermittent treatment involves either daily or twice/week neurotoxin peripheral injection at doses 4–20 mg/kg (Bezard et al. 1997a; Lau et al. 1990; Petroske et al. 2001; Schintu et al. 2009). Concomitant administration of the clearance inhibitor probenecid (MPTPp model), by reducing brain and kidney excretion of MPTP, helps in keeping higher and more stable levels of the neurotoxin during treatment, resulting in a slowly progressing but persistent neurodegeneration (Lau et al. 1990; Petroske et al. 2001; Schintu et al. 2009). An alternative route of chronic neurotoxin administration consists in the continuous MPTP delivery via osmotic minipumps providing a daily dose of 30 mg/kg (Fornai et al. 2005).

5.2 Primates

The most common model used in primates to induce a parkinsonian syndrome is the intravenous administration of MPTP through the saphenous vein (Emborg 2007; Guridi et al. 1996). With this type of administration, a bilateral and stable parkinsonism can be achieved. Another commonly used protocol is the intracarotid administration, consisting of MPTP infusion through the carotid artery, leading to a hemi-parkinsonism, where animals only show symptoms of PD in one side of the body (contralateral to the injection side) (Emborg 2007; Kordower et al. 2000). The majority of animals display symptoms after 3–4 doses of MPTP, but some individuals may present a clear parkinsonian syndrome with even one dose (highly susceptible animals), while others may not show signs even after several doses (not susceptible animals) (Fig. 2). For this reason, it is important to evaluate and monitor each animal individually.

Both intrasaphenous and intracarotideal models show advantages as well as disadvantages. Parkinson's symptoms may be better measured in hemi-parkinsonian monkeys since the evaluation can be compared with the contralateral side of the same animal. On the other hand, the bilateral model, although trickier to detect small changes, mimics closer the clinical scenario observed in humans. However, in the bilateral model, one side is generally slightly more affected than the other, making it difficult to assess regular motor scores for parkinsonian monkeys. As a main advantage, the bilateral model with intrasaphenous infusion does not involve any surgery, being therefore a less complex and safer procedure for the animal.

The protocols of MPTP administration can be considered acute, subacute, subchronic, and chronic. Acute treatment is usually referred to a single dose of MPTP, with animals being sacrificed within 24 h, although some variations can be found in the literature. Subacute models may be more variable, being described as a short-term MPTP administration, with animals being sacrificed after few weeks (Garrido-Gil et al. 2009; Vazquez-Claverie et al. 2009a). A subchronic protocol is commonly referred to a MPTP treatment lasting few weeks to a month and sacrificing the animals within 3 months (Barcia et al. 2004a; Goldstein et al. 2003). The chronic protocol, also called remote, is usually referred to

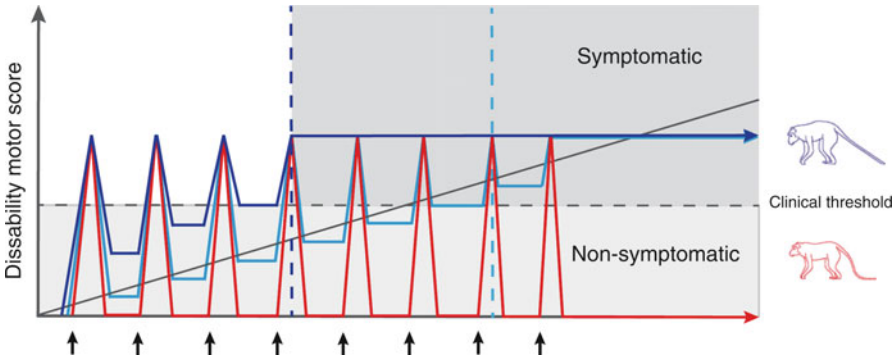

Fig. 2 The panel on the right shows the typical posture of a control longtail macaque (Macaca fascicularis) compared with a parkinsonian macaque. Note that the parkinsonian monkey shows a particular curvature of the limbs and trunk as well as rigidity of the tail. The panel on the left shows a chart with the typical curve of the motor score of a susceptible monkey compared with an asymptomatic one. After the systemic effects, the susceptible monkeys maintain stable parkinsonian symptoms, while asymptomatic animals recover completely after the systemic effects

a MPTP treatment lasting 3 months or more and sacrificing the monkeys within 6–35 months or even years after MPTP administration (Barcia et al. 2004b; Colosimo et al. 1992; Goldstein et al. 2003; Halliday et al. 2009; McGeer et al. 2003).

6 Motor and Nonmotor Symptoms

MPTP administration induces a short-term and transient systemic effect both in monkeys and in rodents, which is independent from its ability to alter locomotor activity but may be a confusing factor for the detection of PD symptoms. Systemic effect may include piloerection, hypersalivation, hypokinesia and seizures, tachycardia, hyperventilation, and hypothermia that may require the use of a heater and recover within 24–48 h. Hours after MPTP administration, primates may also show some episode of vomiting and lack of appetite that may result in loss of body weight. When working with MPTP, it is essential to respect the time of quarantine to reach a full elimination of any residual MPP+ by the urine and feces.

6.1 Rodents

The induction and detection of motor impairments in mice are profoundly affected by the schedule of MPTP delivery and behavioral tests applied, for which reason results have been variable and sometimes ambiguous (Table 1). Acute MPTP paradigms convey a reduction, no changes, or even an increase in locomotor activity (Colotla et al. 1990; Fredriksson and Archer 1994; Luchtman et al. 2009;

Table 1 Effect of acute, subacute, and chronic MPTP protocols (see text for details) on mice motor activity or on nonmotor symptoms typical of PD. Motor activity was measured as spontaneous activity or via more specific tests such as the rotarod or the beam-walking test. ↑ and ↓ indicate, respectively, an increase and a decrease with respect to the motor performance of drug-naive mice; = indicates no differences

MPTP protocol	Motor activity		Nonmotor symptoms
Acute	Colotla et al. 1990	↑	Anderson et al. 2007 (*enteric*)
	Fredriksson and Archer 1994	↓	Monaghan et al. 2010 (*cognitive*)
	Rousselet et al. 2003	=	Prediger et al. 2010 (*olfactory, cognitive*)
			Tian et al. 2008 (*enteric*)
Subacute	Arai et al. 1990	↓	Laloux et al. 2008 (*sleep*)
	Chia et al. 1996	↑	
	Petroske et al. 2001	=	
	Willis and Donnan 1987	=	
Chronic	Bezard et al. 1997b	=	Schintu et al. 2009 (*olfactory*)
	Fornai et al. 2005	↓	
	Petroske et al. 2001	↓	
	Rousselet et al. 2003	↑	
	Schintu et al. 2009	↓	

Quinn et al. 2007; Rousselet et al. 2003; Sedelis et al. 2000; Tillerson et al. 2002). In any case, the motor impairment induced by acute MPTP recovers nearly completely after 3–7 days postinjection (Schmidt and Ferger 2001). Subacute MPTP treatments have also given contradictory results. When mice are treated for a period ranging from 5 days to few weeks, no changes (Petroske et al. 2001; Willis and Donnan 1987), decrease (Arai et al. 1990), or increase (Chia et al. 1996; Luchtman et al. 2009) in locomotor activity has been reported.

After a series of improvements and protocol adjustments, chronic MPTP models are now able to provide more consistent results. In a first attempt of reproducing the disease in chronic nature, MPTP was given daily at a very low dose for 20 days (Bezard et al. 1997a). Unfortunately, despite a progressive decline of TH immunoreactivity, the MPTP-treated mice did not display parkinsonian motor abnormalities after this dosing regimen (Bezard et al. 1997b). In the last decade, the chronic MPTPp protocol, firstly proposed by Lau in 1990 and widely characterized by several studies thereafter, brought a significant improvement to PD models (Lau et al. 1990). First, the MPTPp administration schedule (twice/week) allows to perform motor tests between injections, offering the useful advantage of monitoring motor symptoms in the presence of an ongoing neurodegeneration. When tested along the treatment, mice display an increasing impairment in motor performance, suggesting the development of progressive nigrostriatal degeneration (Schintu et al. 2009). Moreover, MPTPp-treated mice display a reduced motor performance when tested 3 weeks to 6 months after treatment, suggesting persistent neurodegeneration (Petroske et al. 2001; Schintu et al. 2009). Confirming dopaminergic degeneration, amphetamine-induced locomotor activity is reduced after 4 weeks from neurotoxin treatment (Al-Jarrah et al. 2007). As an alternative model, the continuous MPTP

delivery through minipumps was reported to dramatically depress spontaneous activity in the open field test (Fornai et al. 2005). However, constant brain levels of MPTP obtained by this route may induce some nonspecific systemic effects, including depressed motor behavior that may confound the assessment of PD symptoms.

A further burst to the use of MPTP models in PD research comes from recent studies, highlighting that specific protocols may be suitable for studying nonmotor aspects of the disease (Table 1). The possibility of mimicking symptoms associated with the early premotor phases of PD has been deeply explored in the intranasal MPTP infusion model (Prediger et al. 2006, 2010). In this model, animals develop selective symptoms such as olfactory dysfunction and cognitive deficits, while not displaying a clear motor impairment, suggesting that this MPTP protocol may provide a useful model of premotor symptoms of PD. Interestingly, in the MPTPp model, olfactory deficits appear after the first neurotoxin administration, preceding motor symptoms, accordingly with the graded progression of PD neuropathology (Hawkes et al. 2009; Haehner et al. 2011; Schintu et al. 2009). Cognitive impairment was reported after an acute intraperitoneal MPTP administration as well (Monaghan et al. 2010). Among other nonmotor symptoms, sleep alterations have been reported after a subacute MPTP treatment (Laloux et al. 2008). Peripheral pathologies associated with PD have been sparely investigated. Interestingly, behavioral and electrophysiological defects in the enteric nervous system, associated with a significant reduction of TH-positive neurons in the same site, were reported after acute MPTP intoxication in mice (Anderson et al. 2007; Tian et al. 2008). Certainly more efforts are sought to evaluate the possible contribution of MPTP models to the understanding of peripheral aspects of PD pathology; nevertheless, these few studies support the interesting possibility that MPTP may provide a useful tool to investigate on the graded neuropathology hypothesis of the disease (Hawkes et al. 2009).

6.2 Primates

The evidence of major motor symptoms of PD after MPTP intoxication of young drug addicts prompted researchers to test the neurotoxin in nonhuman primates. Since then, the MPTP model has been used both as a pathogenic and as a clinical symptomatic model in monkeys for its peculiarity of mimicking almost all of the clinical hallmarks of the disease (Bezard and Przedborski 2011), including the side effects induced by dopamine replacement with levodopa and/or dopaminergic agonists (Blanchet et al. 2004; Herrero et al. 1995; Jenner 2003). After MPTP intoxication, monkeys develop akinesia/bradykinesia, rigidity, and freezing phenomena. They also may show tremor in the arms and hands and occasionally in the head. Differently from humans, who show resting tremor, MPTP-intoxicated monkeys show a tremor that appears with action. Eventually, monkeys may display occasional falls and vertical saccadic eye movements. After MPTP exposure, a stable parkinsonism may be observable through the years (Halliday et al. 2009). Although some animal may improve slightly during the observation, parkinsonism

is usually irreversible. However, as described above, it is important to consider that some individuals are not susceptible to MPTP, and after the recovery of systemic symptoms (24–48 h), no apparent motor alterations may be seen. MPTP-treated monkeys also show a postural alteration that is characterized by the curvature of the limbs and trunk and a peculiar rigidity of the tail (Fig. 2).

MPTP-treated monkeys also show similarities with PD patients with regard to nonmotor symptoms. Similarly to the patients, MPTP-treated monkeys present alterations in the heart sympathetic innervation (Goldstein et al. 2003; Li et al. 2002; Yuste et al. 2012), enteric nervous system (Chaumette et al. 2009), retina (Cuenca et al. 2005), as well as sleep disorders (Almirall et al. 1999) and modifications in the circadian rhythm of secretion of hormones such as prolactin and melatonin (Barcia et al. 2003). Moreover, when the schedule of MPTP intoxication is chronic and slow, fronto-executive cognitive impairments are evident even before and independent of the motor symptoms (Schneider and Pope-Coleman 1995). The first cognitive symptoms include significant impairment in performing short- and medium-duration delay trials, suggesting attentional deficits that disrupt the encoding of behaviorally relevant information to be kept in short-term memory. These symptoms have been also reported in asymptomatic MPTP-treated monkeys. Moreover, after repeated administration of low doses of MPTP for long periods, parkinsonian monkeys develop significant impairment in planning, cognitive flexibility, and attention set shifting (Decamp and Schneider 2004). Altogether, these evidences point to the MPTP primate model as a useful tool for studying the progression of parkinsonian symptoms (Storvik et al. 2010) as well as for the understanding of the broad range of systems that are affected in PD (Olanow et al. 2011).

Importantly, the MPTP primate model faithfully reproduces L-DOPA-induced dyskinesia. Similarly to humans, L-DOPA administration in parkinsonian primates brings about recovery of motor symptoms, while the prolonged L-DOPA treatment causes the appearance of dyskinesia. The MPTP primate model gives the unique opportunity to replicate a dyskinesia nearly identical to parkinsonian patients (Ahmed et al. 2010; Clarke et al. 1987; Herrero et al. 1995).

7 Neuropathological Markers of PD in MPTP Models

While nigrostriatal degeneration can be achieved by different protocols of MPTP administration, the dosing regimen and schedule of administration are the main determinants for modeling histological features of PD, such as α-synuclein-positive inclusion bodies, chronic neuroinflammation, and neuronal death by apoptotic mechanisms.

7.1 Neurodegeneration in Rodents

Classically, MPTP leads to the selective degeneration of nigrostriatal dopamine neurons. Striatal dopamine terminals are extremely sensitive to MPTP toxicity, and

Table 2 Effect of different MPTP protocols on striatal dopamine levels (DA), TH immunoreactivity (TH-IR), histological staining, and the appearance of apoptotic cells in the substantia nigra pars compacta (SNc). ↓ indicates a decrease in the measured parameter, = indicates no changes with respect to drug-naïve mice, - means not analyzed

MPTP protocol		DA (Str)	TH-IR (SNc)	Histological staining (SNc)	Apoptotic nuclei (SNc)
Acute	Jackson-Lewis et al. 1995	–	↓	↓	No
	Hallman et al. 1985	↓	=	–	–
	Heikkila et al. 1984	↓	↓	–	–
	Ricaurte et al. 1986	↓	=	=	–
Subacute	Carta et al. 2009	–	↓	↓	–
	Petroske et al. 2001	↓	=	=	–
	Tatton and Kish. 1997	–	↓	↓	Yes
	Vila et al. 2000	–	↓	–	Yes
	Willis and Donnan 1987	↓	=	–	–
Chronic	Alvarez-Fischer et al. 2008	↓	↓	–	–
	Bezard et al. 1997a, b	–	↓	–	–
	Fornai et al. 2005	↓	↓	–	–
	Novikova et al. 2006	↓	↓	–	Yes
	Petroske et al. 2001	↓	↓	↓	–
	Rousselet et al. 2003	↓	↓	–	–
	Schintu et al. 2009	↓	↓	↓	–

even a single dose leads to a rapid decrease of striatal dopamine and DOPAC levels (Hallman et al. 1985; Jackson-Lewis et al. 1995; Schintu et al. 2009) (Table 2). Moreover, an important aspect that has been revaluated in recent years is the MPTP-induced depletion of catecholamines in areas other than the striatum. MPTP-induced decrease of dopamine and/or noradrenaline in the olfactory bulb and prefrontal cortex, associated with the appearance of typical PD nonmotor symptoms, supports the interesting perspectives of using MPTP to model nonmotor aspects of the disease (Dluzen 1992; Dluzen and Kefalas 1996; Hallman et al. 1985; Luchtman et al. 2009; Prediger et al. 2010; Tanila et al. 1998).

As compared to striatal terminals, degeneration of cell bodies in the SNc is a more challenging task. MPTP toxicity toward dopaminergic cell bodies is age dependent, particularly after acute protocols (Ricaurte et al. 1987). Moreover, contradictory results have been reported on nigral lesion after acute (Hallman et al. 1985; Heikkila et al. 1984; Jackson-Lewis et al. 1995; Ricaurte et al. 1986), subacute (Carta et al. 2009; Petroske et al. 2001; Vila et al. 2000; Willis and Donnan 1987), and chronic (Alvarez-Fischer et al. 2008; Bezard et al. 1997a; Fornai et al. 2005; Petroske et al. 2001; Rousselet et al. 2003; Schintu et al. 2009) MPTP regimens (Table 2). To this regard, the possible lack of correlation between the loss of TH-positive immunoreactivity and loss of Nissl-stained cells is a pivotal

factor to be considered (Bian et al. 2007; Jackson –Lewis et al. 1995; Seniuk et al. 1990). For instance, after acute MPTP treatment, the number of TH-positive neurons decreases rapidly, while the effective loss of Nissl-stained cells only begins at 12 h postinjection and continues up to 4 days (Jackson-Lewis et al. 1995). Therefore, neurodegeneration is best shown through histological direct evidence. Accordingly, a lack of correlation between motor impairment and loss of nigral TH-positive neurons has been often reported. Importantly, the rate and mechanism of cell death is also strictly dependent on the MPTP schedule and dosing (Table 2). After acute and subacute protocols, cell death occurs rapidly. In contrast, chronic MPTP, particularly when associated with a clearance inhibitor, induces a slower and progressive loss of DA neurons (Alvarez-Fischer et al. 2008; Petroske et al. 2001; Schintu et al. 2009). Furthermore, neuronal loss is a transient phenomenon after short-term regimens, while on the other hand, it is long lasting after chronic regimens, being stable up to 6 months after the last MPTP administration (Petroske et al. 2001; Schintu et al. 2009). Finally, acute MPTP protocols result in a rapid non-apoptotic, likely necrotic cell death, while subacute and chronic regimens, where lower MPTP doses are delivered over days or weeks, lead to preferential cell death by apoptotic mechanisms (Jackson-Lewis et al. 1995; Novikova et al. 2006; Tatton and Kish 1997; Vila et al. 2000).

7.2 Neurodegeneration in Primates

The degeneration of the SNc in MPTP-treated monkeys presents a region-dependent vulnerability, similarly to humans. The ventrolateral part is the most susceptible, displaying the highest degree of dopaminergic degeneration (80–90 %). This area is equivalent to the α + β area defined in humans, presenting very similar features, and it is equally vulnerable in humans and in all families of primates. Other mesencephalic areas are less susceptible and appear to be partially protected against degeneration. The ventral tegmental area (VTA), the dorsal and lateral part of the SNc, presents a 50 % of dopaminergic loss upon MPTP intoxication, while the periaqueductal gray matter (PAG) and periretrorubral area A8 shows 10 % or less dopaminergic degeneration (Herrero et al. 1993b). These differences in regional susceptibility may be due to several factors such as the level of neuromelanin, as described above, as well as the levels of calbindin, DAT, and vesicular monoamine transporter-2 (VMAT-2) that also show regional differences within dopaminergic areas. Accordingly, dopamine neurons of most susceptible mesencephalic areas display high levels of DAT and low levels of VMAT-2, showing the highest cytoplasmic accumulation of MPP+ (Dauer and Przedborski 2003).

The loss of DA neurons of the SNc in MPTP-treated monkeys mimics and matches with the scenario observed in human PD. Usually, there is a fine correlation between the level of dopaminergic cell loss caused by MPTP and motor alterations (Barcia et al. 2011; Mounayar et al. 2007). However, this correlation may have a range of variation depending on the adaptation, recovery, and compensatory mechanisms that the animal may develop in response to MPTP.

In contrast with DA neurons, the degeneration of dopaminergic fibers in the striatum seems to differ between humans and nonhuman primates. In PD patients, the putamen is the most affected striatal area. However, MPTP seems to affect to a higher extent the caudate rather than the putamen in primates (Moratalla et al. 1992; Snow et al. 2000). Moreover, other brain regions may be affected by MPTP in primates as, for instance, the locus coeruleus (Forno et al. 1986; Herrero et al. 1993a) or the dorsal raphe nucleus (Perez-Otaño et al. 1991). It has also been reported that the damage and alteration of Purkinje cells of the cerebellum may correlate with nigral degeneration (Heman et al. 2012; Necchi et al. 2004). Although the cerebellar activity is altered in PD patients, histological evidences in this nucleus are elusive.

7.3 Alpha-Synuclein/Lewy Body Deposits in Rodents

Ubiquitin- and α-synuclein-positive Lewy bodies have not been observed with any MPTP treatment in mice, despite the evidence that α-synuclein is involved in MPTP neurotoxicity in this species (Dauer et al. 2002; Shimoji et al. 2005). Nevertheless, an increase of α-synuclein-positive neurons in the SNc of mice treated with a subacute MPTP regimen has been reported (Vila et al. 2000). Moreover, in mice chronically treated with MPTP either i.p. or through minipumps, alpha-synuclein-immunoreactive inclusions have been reported in a few neurons of the SNc by two studies (Fornai et al. 2005; Meredith et al. 2002) but not by other studies (Alvarez-Fischer et al. 2008; Shimoji et al. 2005), leaving this issue controversial.

7.4 Alpha-Synuclein/Lewy Body Deposits in Primates

α-Synuclein aggregation after the administration of MPTP occurs in both old-world (Kowall et al. 2000) and new-world primates (Purisai et al. 2005). This phenomenon has been related with the dopaminergic cell death, although the mechanism linking α-synuclein aggregation with neuronal damage is unclear (Sulzer 2010). Despite α-synuclein accumulation, studies performed in acute MPTP-treated primates have revealed that this does not result in the formation of Lewy bodies (Kowall et al. 2000; Purisai et al. 2005). Since these experiments have been performed in acute protocols of MPTP administration, it has been postulated that a longer period of years may be required for Lewy body formation. However, studies performed in monkeys bearing parkinsonism from over a decade failed to reveal any Lewy body formation (Halliday et al. 2009), which suggests that this phenomenon may be an exclusive feature of the human disease.

7.5 Neuroinflammation in Rodents

A number of studies have reported a neuroinflammatory response in mice treated either acutely (Kurkowska-Jastrzebska et al. 1999), subacutely (Carta et al. 2009),

or chronically (Schintu et al. 2009) with MPTP. However, the mouse MPTP model presents some limitations in reproducing the inflammatory scenario that characterize PD. In vivo and ex vivo observations in parkinsonian patients, as well as in MPTP-treated primates, have shown a chronic and persistent inflammatory response, where activated microglia is engaged in the auto-fueling and detrimental cycle named reactive gliosis (Hirsch and Hunot 2009). In contrast, the neuroinflammatory response in mice is transient and tightly related to the neurotoxin administration (Schintu et al. 2009).

Nevertheless, MPTP studies in mice have contributed to highlight the involvement of neuroinflammation in dopaminergic degeneration. Upon MPTP administration, neuroinflammation has been mainly described as microglia activation associated with the overproduction of inflammatory cytokines. Acute and subacute MPTP intoxication causes a rapid and transient microglial activation in the striatum and in the SNc that returns to basal levels within 14 days post-intoxication (Carta et al. 2009; Kurkowska-Jastrzebska et al. 1999). Moreover, a transient increase in either protein or mRNA for inflammatory cytokines in both areas was reported shortly after MPTP injection (Ciesielska et al. 2003; Kurkowska-Jastrzebska et al. 1999; Lofrumento et al. 2011; Pattarini et al. 2007). The chronic administration of MPTP in association with probenecid induces a gradual increase of microglial activation and density (Meredith et al. 2005; Novikova et al. 2006; Schintu et al. 2009). Interestingly, activated microglia persist longer after chronic MPTP intoxication as compared to shorter protocols, being still detectable up to 6 months posttreatment (Meredith et al. 2005; Schintu et al. 2009). In addition, microglia undergoe changes in morphology and phenotype upon this protocol of MPTP administration, gradually switching to more activated states, associated with altered cytokines production (Pisanu et al. in preparation).

7.6 Neuroinflammation in Primates

Different from mice, the inflammatory response caused by MPTP in primates is persistent and may be perpetuated over the years (Barcia et al. 2004b; McGeer et al. 2003). This inflammatory response is characterized by an increase of proinflammatory cytokines in the blood and brain, similar to PD patients, and can be clearly detected at the cellular level (Barcia et al. 2005, 2011; Hunot and Hirsch 2003). From a histological point of view, the inflammatory response is characterized by microglia and astroglia activation specifically in the SNc, appearing particularly higher in the $\alpha + \beta$ area where DA cell loss is higher (Barcia et al. 2004b). After MPTP intoxication, microglial cells display a bigger cell body and a higher number of processes and ramifications and overexpress human leukocyte antigen-DR (HLA-DR) in their membrane (Barcia et al. 2009; McGeer et al. 2003). These features are very similar or even identical to the scenario observed in humans intoxicated with MPTP and in PD patients (Langston et al. 1999; McGeer et al. 1988). Importantly, the level of activation appears very similar in acute and chronic protocols of MPTP administration (Vazquez-Claverie et al. 2009b). On the other

hand, astrocytes also appear over-reactive in MPTP-treated primates similarly to humans, showing a bigger cell body and increased number of ramifications (Barcia et al. 2009; Forno et al. 1992). Particularly, the glial fibrillary acidic protein (GFAP) is overexpressed in reactive astrocytes over the dopaminergic pathway, and again, it is particularly increased in the α + β area of the SNc, where DA loss is highest. Importantly, the levels of cytokines in the blood and brain seem to contribute to the inflammatory response (Barcia et al. 2011; De Lella Ezcurra et al. 2010; Pott Godoy et al. 2008). Particularly, IFN-γ and TNF-α are thought to be involved in the mechanisms of microglial and astroglial activation and may be key cytokines in the neuroinflammatory response occurring in dopaminergic degeneration (Barcia et al. 2011; De Lella Ezcurra et al. 2010; Mount et al. 2007).

8 Conclusion

MPTP administration to primates reproduces almost all of the clinical hallmarks of PD, being used as a pathogenic as well as clinical symptomatic model of the disease. Importantly, MPTP-induced parkinsonism in monkeys is usually irreversible, and the neurodegeneration progresses even after withdrawal of neurotoxin. On the other hand, to reproduce PD by MPTP administration in rodents represents a more intricate task. Up to date, no MPTP rodent model has been able to reproduce all PD features in one. Nevertheless, a number of protocols have been developed that can reproduce different aspects of PD pathology, depending upon the dose and regimen of MPTP administration. Therefore, while rodent models allow to investigate on motor and nonmotor symptoms and on PD neuropathology, a careful evaluation of the most appropriate protocol for the study purpose has to be made in advance. Keeping this concept in mind, MPTP models of PD have provided and continue to give a great contribution to the understanding of disease neuropathology and to the development of new symptomatic and disease-modifying compounds.

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References

Ahmed, M. R., Berthet, A., Bychkov, E., Porras, G., Li, Q., Bioulac, B. H., Carl, Y. T., Bloch, B., Kook, S., Aubert, I., Dovero, S., Doudnikoff, E., Gurevich, V. V., Gurevich, E. V., & Bezard, E. (2010). Lentiviral overexpression of GRK6 alleviates L-dopa-induced dyskinesia in experimental Parkinson's disease. *Science Translational Medicine, 2*(28), 28r–ra28.

Al-Jarrah, M., Pothakos, K., Novikova, L., Smirnova, I. V., Kurz, M. J., Stehno-Bittel, L., & Lau, Y. S. (2007). Endurance exercise promotes cardiorespiratory rehabilitation without neurorestoration in the chronic mouse model of parkinsonism with severe neurodegeneration. *Neuroscience, 149*(1), 28–37.

Almirall, H., Pigarev, I., de la Calzada, M. D., Pigareva, M., Herrero, M. T., & Sagales, T. (1999). Nocturnal sleep structure and temperature slope in MPTP treated monkeys. *Journal of Neural Transmission, 106*, 1125–1134.

Alvarez-Fischer, D., Guerreiro, S., Hunot, S., Saurini, F., Marien, M., Sokoloff, P., Hirsch, E. C., Hartmann, A., & Michel, P. P. (2008). Modelling Parkinson-like neurodegeneration via osmotic minipump delivery of MPTP and probenecid. *Journal of Neurochemistry, 107*(3), 701–711.

Anderson, G., Noorian, A. R., Taylor, G., Anitha, M., Bernhard, D., Srinivasan, S., & Greene, J. G. (2007). Loss of enteric dopaminergic neurons and associated changes in colon motility in an MPTP mouse model of Parkinson's disease. *Experimental Neurology, 207*(1), 4–12.

Arai, N., Misugi, K., Goshima, Y., & Misu, Y. (1990). Evaluation of a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated C57 black mouse model for parkinsonism. *Brain Research, 515*(1–2), 57–63.

Barcia, C., Bautista, V., Sanchez-Bahillo, A., Fernandez-Villalba, E., Navarro-Ruis, J. M., Barreiro, A. F., Poza, Y. P. M., & Herrero, M. T. (2003). Circadian determinations of cortisol, prolactin and melatonin in chronic methyl-phenyl-tetrahydropyridine-treated monkeys. *Neuroendocrinology, 78*, 118–128.

Barcia, C., De Pablos, V., Bautista-Hernandez, V., Sanchez-Bahillo, A., Fernandez-Barreiro, A., Poza, M., & Herrero, M. T. (2004a). Measurement of motor disability in MPTP-treated macaques using a telemetry system for estimating circadian motor activity. *Journal of Neuroscience Methods, 134*, 59–64.

Barcia, C., Sanchez Bahillo, A., Fernandez-Villalba, E., Bautista, V., Poza, Y. P. M., Fernandez-Barreiro, A., Hirsch, E. C., & Herrero, M. T. (2004b). Evidence of active microglia in substantia nigra pars compacta of parkinsonian monkeys 1 year after MPTP exposure. *Glia, 46*, 402–409.

Barcia, C., de Pablos, V., Bautista-Hernandez, V., Sanchez-Bahillo, A., Bernal, I., Fernandez-Villalba, E., Martin, J., Banon, R., Fernandez-Barreiro, A., & Herrero, M. T. (2005). Increased plasma levels of TNF-alpha but not of IL1-beta in MPTP-treated monkeys one year after the MPTP administration. *Parkinsonism & Related Disorders, 11*, 435–439.

Barcia, C., Ros, C. M., Carrillo, M. A., Ros, F., Gomez, A., de Pablos, V., Bautista-Hernandez, V., Sanchez-Bahillo, A., Villalba, E. F., & Herrero, M. T. (2009). Increase of secondary processes of microglial and astroglial cells after MPTP-induced degeneration in substantia nigra pars compacta of non human primates. *Journal of Neural Transmission, 73*, 253–258.

Barcia, C., Ros, C. M., Annese, V., Gomez, A., Ros-Bernal, F., Aguado-Yera, D., Martinez-Pagan, M. E., de Pablos, V., Fernandez-Villalba, E., Herrero, M., Barcia, C., Ros, C. M., Annese, V., Gomez, A., Ros-Bernal, F., Aguado-Yera, D., Martinez-Pagan, M. E., de Pablos, V., Fernandez-Villalba, E., & Herrero, M. T. (2011). IFN-gamma signaling, with the synergistic contribution of TNF-alpha, mediates cell specific microglial and astroglial activation in experimental models of Parkinson's disease. *Cell death & disease, 2*, e142.

Bezard, E., & Przedborski, S. (2011). A tale on animals models of Parkinson's disease. *Movement Disorders, 26*, 993–1002.

Bezard, E., Dovero, S., Bioulac, B., & Gross, C. (1997a). Effects of different schedules of MPTP administration on dopaminergic neurodegeneration in mice. *Experimental Neurology, 148*(1), 288–292.

Bezard, E., Dovero, S., Bioulac, B., & Gross, C. E. (1997b). Kinetics of nigral degeneration in a chronic model of MPTP-treated mice. *Neuroscience Letters, 234*(1), 47–50.

Bian, G. L., Wei, L. C., Shi, M., Wang, Y. Q., Cao, R., & Chen, L. W. (2007). Fluoro-Jade C can specifically stain the degenerative neurons in the substantia nigra of the 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine-treated C57BL/6 mice. *Brain Research, 1150*, 55–61.

Blanchet, P. J., Calon, F., Morissette, M., Hadj Tahar, A., Bélanger, N., Samadi, P., Grondin, R., Grégoire, L., Meltzer, L., Di Paolo, T., & Bédard, P. J. (2004). Relevance of the MPTP primate model in the study of dyskinesia priming mechanisms. *Parkinsonism & Related Disorders, 10*(5), 297–304.

Blum-Degen, D., Müller, T., Kuhn, W., Gerlach, M., Przuntek, H., & Riederer, P. (1995). Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. *Neuroscience Letters, 202*, 17–20.

Braak, H., & Del Tredici, K. (2008). Invited Article: Nervous system pathology in sporadic Parkinson disease. *Neurology, 70*(20), 1916–1925.

Carta, A. R., Kachroo, A., Schintu, N., Xu, K., Schwarzschild, M. A., Wardas, J., & Morelli, M. (2009). Inactivation of neuronal forebrain A receptors protects dopaminergic neurons in a mouse model of Parkinson's disease. *Journal of Neurochemistry, 111*(6), 1478–1489.

Carta, A. R., Frau, L., Pisanu, A., Wardas, J., Spiga, S., & Carboni, E. (2011). Rosiglitazone decreases peroxisome proliferator receptor-gamma levels in microglia and inhibits TNF-alpha production: New evidences on neuroprotection in a progressive Parkinson's disease model. *Neuroscience, 194*, 250–261.

Chan, P., DeLanney, L. E., Irwin, I., Langston, J. W., & Di Monte, D. (1991). Rapid ATP loss caused by 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine in mouse brain. *Journal of Neurochemistry, 57*, 348–351.

Chaudhuri, K. R., & Naidu, Y. (2008). Early Parkinson's disease and non-motor issues. *Journal of Neurology*, *255*(Suppl. 5), 33–38.

Chaumette, T., Lebouvier, T., Aubert, P., Lardeux, B., Qin, C., Li, Q., Accary, D., Bezard, E., Bruley des Varannes, S., Derkinderen, P., et al. (2009). Neurochemical plasticity in the enteric nervous system of a primate animal model of experimental Parkinsonism. *Neurogastroenterology and Motility, 21*, 215–222.

Chia, L. G., Ni, D. R., Cheng, L. J., Kuo, J. S., Cheng, F. C., & Dryhurst, G. (1996). Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 5,7-dihydroxytryptamine on the locomotor activity and striatal amines in C57BL/6 mice. *Neuroscience Letters, 218*(1), 67–71.

Ciesielska, A., Joniec, I., Przybyłkowski, A., Gromadzka, G., Kurkowska-Jastrzebska, I., Członkowska, A., & Członkowski, A. (2003). Dynamics of expression of the mRNA for cytokines and inducible nitric synthase in a murine model of the Parkinson's disease. *Acta Neurobiologiae Experimentalis (Warsaw), 63*(2), 117–126.

Clarke, C. E., Sambrook, M. A., Mitchell, I. J., & Crossman, A. R. (1987). Levodopa-induced dyskinesia and response fluctuations in primates rendered parkinsonian with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Journal of the Neurological Sciences, 78*(3), 273–280.

Colosimo, C., Granata, R., Del Zompo, M., Piccardi, M. P., Perretta, G., & Albanese, A. (1992). Chronic administration of MPTP to monkeys: Behavioural morphological and biochemical correlates. *Neurochemistry International, 20*(Suppl), 279S–285S.

Colotla, V. A., Flores, E., Oscos, A., Meneses, A., & Tapia, R. (1990). Effects of MPTP on locomotor activity in mice. *Neurotoxicology and Teratology, 12*(4), 405–407.

Cuenca, N., Herrero, M. T., Angulo, A., de Juan, E., Martinez-Navarrete, G. C., Lopez, S., Barcia, C., & Martin-Nieto, J. (2005). Morphological impairments in retinal neurons of the scotopic visual pathway in a monkey model of Parkinson's disease. *The Journal of Comparative Neurology, 493*, 261–273.

D'Amato, R. J., Benham, D. F., & Snyder, S. H. (1987). Characterization of the binding of N-methyl-4-phenylpyridine, the toxic metabolite of the parkinsonian neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, to neuromelanin. *Journal of Neurochemistry, 48*, 653–658.

Dauer, W., & Przedborski, S. (2003). Parkinson's disease: Mechanisms and models. *Neuron, 39*, 889–909.

Dauer, W., Kholodilov, N., Vila, M., Trillat, A. C., Goodchild, R., Larsen, K. E., Staal, R., Tieu, K., Schmitz, Y., Yuan, C. A., Rocha, M., Jackson-Lewis, V., Hersch, S., Sulzer, D., Przedborski, S., Burke, R., & Hen, R. (2002). Resistance of alpha -synuclein null mice to the parkinsonian neurotoxin MPTP. *Proceedings of the National Academy of Sciences of the United States of America, 99*(22), 14524–14529.

De Lella Ezcurra, A. L., Chertoff, M., Ferrari, C., Graciarena, M., & Pitossi, F. (2010). Chronic expression of low levels of tumor necrosis factor-alpha in the substantia nigra elicits progressive neurodegeneration, delayed motor symptoms and microglia/macrophage activation. *Neurobiol of Disease*, *37*(3), 630–640.

Decamp, E., & Schneider, J. S. (2004). Attention and executive function deficits in chronic low dose MPTP-treated non-human primates. *European Journal of Neuroscience, 20*, 1371–1378.

Dluzen, D. E. (1992). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) reduces norepinephrine concentrations in the olfactory bulbs of male mice. *Brain Research, 586*(1), 144–147.

Dluzen, D. E., & Kefalas, G. (1996). The effects of intranasal infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) upon catecholamine concentrations within olfactory bulbs and corpus striatum of male mice. *Brain Research, 741*(1–2), 215–219.

Dobbs, R. J., Charlett, A., Purkiss, A. G., Dobbs, S. M., Weller, C., & Peterson, D. W. (1999). Association of circulating TNF-alpha and IL-6 with ageing and parkinsonism. *Acta Neurologica Scandinavica, 100*, 34–41.

Elsworth, J. D., Taylor, J. R., Sladek, J. R., Jr., Collier, T. J., Redmond, D. E., Jr., & Roth, R. H. (2000). Striatal dopaminergic correlates of stable parkinsonism and degree of recovery in old-world primates one year after MPTP treatment. *Neuroscience, 95*, 399–408.

Emborg, M. E. (2007). Nonhuman primate models of Parkinson's disease. *ILAR Journal//National Research Council, Institute of Laboratory Animal Resources, 48*, 339–355.

Fabre, E., Monserrat, J., Herrero, A., Barja, G., & Leret, M. L. (1999). Effect of MPTP on brain mitochondrial H_2O_2 and ATP production and on dopamine and DOPAC in the striatum. *Journal of Physiology and Biochemistry, 55*, 325–331.

Fornai, F., Schlüter, O. M., Lenzi, P., Gesi, M., Ruffoli, R., Ferrucci, M., Lazzeri, G., Busceti, C. L., Pontarelli, F., Battaglia, G., Pellegrini, A., Nicoletti, F., Ruggieri, S., Paparelli, A., & Südhof, T. C. (2005). Parkinson-like syndrome induced by continuous MPTP infusion: Convergent roles of the ubiquitin-proteasome system and alpha-synuclein. *Proceedings of the National Academy of Sciences of the United States of America, 102*(9), 3413–3418.

Forno, L. S., Langston, J. W., DeLanney, L. E., Irwin, I., & Ricaurte, G. A. (1986). Locus ceruleus lesions and eosinophilic inclusions in MPTP-treated monkeys. *Annals of Neurology, 20*(4), 449–455.

Forno, L. S., DeLanney, L. E., Irwin, I., Di Monte, D., & Langston, J. W. (1992). Astrocytes and Parkinson's disease. *Progress in Brain Research, 94*, 429–436.

Fredriksson, A., & Archer, T. (1994). MPTP-induced behavioural and biochemical deficits: A parametric analysis. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 7*(2), 123–132.

Garrido-Gil, P., Belzunegui, S., San Sebastian, W., Izal-Azcarate, A., Lopez, B., Marcilla, I., & Luquin, M. R. (2009). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure fails to produce delayed degeneration of substantia nigra neurons in monkeys. *Journal of Neuroscience Research, 87*, 586–597.

Giovanni, A., Sieber, B. A., Heikkila, R. E., & Sonsalla, P. K. (1994). Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Part 1: Systemic administration. *Journal of Pharmacology and Experimental Therapeutics, 270*, 1000–1007.

Goldstein, D. S., Li, S. T., Holmes, C., & Bankiewicz, K. (2003). Sympathetic innervation in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine primate model of Parkinson's disease. *Journal of Pharmacology and Experimental Therapeutics, 306*, 855–860.

Guridi, J., Herrero, M. T., Luquin, M. R., Guillen, J., Ruberg, M., Laguna, J., Vila, M., Javoy-Agid, F., Agid, Y., Hirsch, E., & Obeso, J. A. (1996). Subthalamotomy in parkinsonian monkeys. Behavioural and biochemical analysis. *Brain, 119*(Pt 5), 1717–1727.

Haehner, A., Hummel, T., & Reichmann, H. (2011). Olfactory loss in Parkinson's disease. *Parkinsons Disease, 2011*, 450939.

Halliday, G., Herrero, M. T., Murphy, K., McCann, H., Ros-Bernal, F., Barcia, C., Mori, H., Blesa, F. J., & Obeso, J. A. (2009). No Lewy pathology in monkeys with over 10 years of severe MPTP Parkinsonism. *Movement Disorders, 24*, 1519–1523.

Hallman, H., Lange, J., Olson, L., Strömberg, I., & Jonsson, G. (1985). Neurochemical and histochemical characterization of neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on brain catecholamine neurons in the mouse. *Journal of Neurochemistry, 44*(1), 117–127.

Hantraye, P., Brouillet, E., Ferrante, R., Palfi, S., Dolan, R., Matthews, R. T., & Beal, M. F. (1996). Inhibition of neuronal nitric oxide synthase prevents MPTP-induced parkinsonism in baboons. *Nature Medicine, 2*, 1017–1021.

Hasegawa, E., Takeshige, K., Oishi, T., Murai, Y., & Minakami, S. (1990). 1-Methyl-4-phenylpyridinium (MPP+) induces NADH-dependent superoxide formation and enhances NADH-dependent lipid peroxidation in bovine heart submitochondrial particles. *Biochemical and Biophysical Research Communications, 170*, 1049–1055.

Hawkes, C. H., Del Tredici, K., & Braak, H. (2009). Parkinson's disease: The dual hit theory revisited. *Annals of the New York Academy of Sciences, 1170*, 615–622.

Heikkila, R. E., Hess, A., & Duvoisin, R. C. (1984). Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. *Science, 224*(4656), 1451–1453.

Heman, P., Barcia, C., Gomez, A., Ros, C. M., Ros-Bernal, F., Yuste, J. E., de Pablos, V., Fernandez-Villalba, E., Toledo-Cardenas, M. R., & Herrero, M. T. (2012). Nigral degeneration correlates with persistent activation of cerebellar Purkinje cells in MPTP-treated monkeys. *Histology and Histopathology, 27*(1), 89–94.

Herrero, M. T., Hirsch, E. C., Kastner, A., Ruberg, M., Luquin, M. R., Laguna, J., Javoy-Agid, F., Obeso, J. A., & Agid, Y. (1993a). Does neuromelanin contribute to the vulnerability of catecholaminergic neurons in monkeys intoxicated with MPTP? *Neuroscience, 56*, 499–511.

Herrero, M. T., Hirsch, E. C., Kastner, A., Luquin, M. R., Javoy-Agid, F., Gonzalo, L. M., Obeso, J. A., & Agid, Y. (1993b). Neuromelanin accumulation with age in catecholaminergic neurons from *Macaca fascicularis* brainstem. *Developmental Neuroscience, 15*, 37–48.

Herrero, M. T., Augood, S. J., Hirsch, E. C., Javoy-Agid, F., Luquin, M. R., Agid, Y., Obeso, J. A., & Emson, P. C. (1995). Effects of L-DOPA on preproenkephalin and preprotachykinin gene expression in the MPTP-treated monkey striatum. *Neuroscience, 68*(4), 1189–1198.

Hirsch, E. C., & Hunot, S. (2009). Neuroinflammation in Parkinson's disease: A target for neuroprotection? *Lancet Neurology, 8*(4), 382–397.

Hunot, S., & Hirsch, E. C. (2003). Neuroinflammatory processes in Parkinson's disease. *Annals of Neurology*, *53*(Suppl. 3), S49–S58.

Ischiropoulos, H., & al-Mehdi, A. B. (1995). Peroxynitrite-mediated oxidative protein modifications. *FEBS Letters, 364*, 279–282.

Jackson-Lewis, V., Jakowec, M., Burke, R. E., & Przedborski, S. (1995). Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurodegeneration, 4*(3), 257–269.

Javitch, J. A., D'Amato, R. J., Strittmatter, S. M., & Snyder, S. H. (1985). Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Uptake of the metabolite N-methyl-4-phenylpyridinium by dopamine neurons explain selective toxicity. *Proceedings of the National Academy of Science United States of America, 82*, 2173–2177.

Jenner, P. (2003). The MPTP-treated primate as a model of motor complications in PD: Primate model of motor complications. *Neurology, 61*, S4–S11.

Kohutnicka, M., Lewandowska, E., Kurkowska-Jastrzebska, I., Członkowski, A., & Członkowska, A. (1998). Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Immunopharmacology, 39*(3), 167–180.

Kordower, J. H., Emborg, M. E., Bloch, J., Ma, S. Y., Chu, Y., Leventhal, L., McBride, J., Chen, E. Y., Palfi, S., Roitberg, B. Z., Brown, W. D., Holden, J. E., Pyzalski, R., Taylor, M. D., Carvey, P., Ling, Z., Trono, D., Hantraye, P., Deglon, N., & Aebischer, P. (2000). Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science, 290*(5492), 767–773.

Kowall, N. W., Hantraye, P., Brouillet, E., Beal, M. F., McKee, A. C., & Ferrante, R. J. (2000). MPTP induces alpha-synuclein aggregation in the substantia nigra of baboons. *Neuroreport, 11*, 211–213.

Kurkowska-Jastrzebska, I., Wrońska, A., Kohutnicka, M., Członkowski, A., & Członkowska, A. (1999). MHC class II positive microglia and lymphocytic infiltration are present in the substantia nigra and striatum in mouse model of Parkinson's disease. *Acta Neurobiologiae Experimentalis*, *59*, 1–8.

Laloux, C., Derambure, P., Kreisler, A., Houdayer, E., Bruezière, S., Bordet, R., Destée, A., & Monaca, C. (2008). MPTP-treated mice: Long-lasting loss of nigral TH-ir neurons but not paradoxical sleep alterations. *Experimental Brain Research, 186*(4), 635–642.

Langston, J. W., Ballard, P., Tetrud, J. W., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science, 219*, 979–980. New York, NY.

Langston, J. W., Forno, L. S., Tetrud, J., Reeves, A. G., Kaplan, J. A., & Karluk, D. (1999). Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. *Annals of Neurology*, *46*, 598–605.

Lau, Y. S., Trobough, K. L., Crampton, J. M., & Wilson, J. A. (1990). Effects of probenecid on striatal dopamine depletion in acute and long-term 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice. *General Pharmacology, 21*(2), 181–187.

Li, S. T., Dendi, R., Holmes, C., & Goldstein, D. S. (2002). Progressive loss of cardiac sympathetic innervation in Parkinson's disease. *Annals of Neurology, 52*, 220–223.

Lofrumento, D. D., Saponaro, C., Cianciulli, A., De Nuccio, F., Mitolo, V., Nicolardi, G., & Panaro, M. A. (2011). MPTP-induced neuroinflammation increases the expression of pro-inflammatory cytokines and their receptors in mouse brain. *Neuroimmunomodulation, 18*(2), 79–88.

Long-Smith, C. M., Sullivan, A. M., & Nolan, Y. M. (2009). The influence of microglia on the pathogenesis of Parkinson's disease. *Progress in Neurobiology, 89*, 277–287.

Luchtman, D. W., Shao, D., & Song, C. (2009). Behavior, neurotransmitters and inflammation in three regimens of the MPTP mouse model of Parkinson's disease. *Physiology and Behavior, 98* (1–2), 130–138.

Marsden, C. D. (1961). Pigmentation in the nucleus substantiae nigrae of mammals. *Journal of Anatomy, 95*, 256–261.

Mayer, R. A., Kindt, M. V., & Heikkila, R. E. (1986). Prevention of the nigrostriatal toxicity of 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine by inhibitors of 3,4-dihydroxyphenethylamine transport. *Journal of Neurochemistry, 47*, 1073–1079.

McGeer, P. L., Itagaki, S., Boyes, B. E., & McGeer, E. G. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology*, *38*, 1285–1291.

McGeer, P. L., & McGeer, E. G. (2008). Glial reactions in Parkinson's disease. *Movement Disorders, 23*, 474–483.

McGeer, P. L., Schwab, C., Parent, A., & Doudet, D. (2003). Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. *Annals of Neurology, 54*, 599–604.

Meredith, G. E., Totterdell, S., Petroske, E., Santa Cruz, K., Callison, R. C., Jr., & Lau, Y. S. (2002). Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease. *Brain Research, 956*(1), 156–165.

Meredith, G. E., Dervan, A. G., & Totterdell, S. (2005). Activated microglia persist in the substantia nigra of a chronic MPTP mouse model of Parkinson's disease. In J. P. Bolam, C. A. Ingham, & P. J. Magill (Eds.), *The basal ganglia VIII* (pp. 341–347). Singapore: Springer.

Mogi, M., Harada, M., Kondo, T., Riederer, P., Inagaki, H., Minami, M., & Nagatsu, T. (1994). Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. *Neuroscience Letters, 180*, 147–150.

Monaghan, M. M., Leddy, L., Sung, M. L., Albinson, K., Kubek, K., Pangalos, M. N., Reinhart, P. H., Zaleska, M. M., & Comery, T. A. (2010). Social odor recognition: A novel behavioral model for cognitive dysfunction in Parkinson's disease. *Neurodegenerative Diseases, 7*(1–3), 153–159.

Moratalla, R., Quinn, B., DeLanney, L. E., Irwin, I., Langston, J. W., & Graybiel, A. M. (1992). Differential vulnerability of primate caudate-putamen and striosome-matrix dopamine systems to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proceedings of the National Academy of Sciences of the United States of America, 89*(9), 3859–3863.

Mounayar, S., Boulet, S., Tande, D., Jan, C., Pessiglione, M., Hirsch, E. C., Feger, J., Savasta, M., Francois, C., & Tremblay, L. (2007). A new model to study compensatory mechanisms in MPTP-treated monkeys exhibiting recovery. *Brain, 130*(Pt 11), 2898–2914.

Mount, M. P., Lira, A., Grimes, D., Smith, P. D., Faucher, S., Slack, R., Anisman, H., Hayley, S., & Park, D. S. (2007). Involvement of interferon-gamma in microglial-mediated loss of dopaminergic neurons. *Journal of Neuroscience*, *27*(12), 3328–3337.

Necchi, D., Soldani, C., Ronchetti, F., Bernocchi, G., & Scherini, E. (2004). MPTP-induced increase in c-Fos- and c-Jun-like immunoreactivity in the monkey cerebellum. *European Journal of Histochemistry, 48*(4), 385–392.

Nicklas, W. J., Vyas, I., & Heikkila, R. E. (1985). Inhibition of NADH- inked oxidation in brain mitochondria by MPP+, a metabolite of the neurotoxin MPTP. *Life Sciences, 36*, 2503–2508.

Novikova, L., Garris, B. L., Garris, D. R., & Lau, Y. S. (2006). Early signs of neuronal apoptosis in the substantia nigra pars compacta of the progressive neurodegenerative mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid model of Parkinson's disease. *Neuroscience, 140*(1), 67–76.

Olanow, C. W. (2007). The pathogenesis of cell death in Parkinson's disease. *Movement Disorders, 22*(Suppl 17), S335–S342.

Olanow, C. W., Stochi, F., & Lang, A. E. (2011). *Parkinson's disease: Non-motor and non-dopaminergic features*. New York: Wiley.

Pattarini, R., Smeyne, R. J., & Morgan, J. I. (2007). Temporal mRNA profiles of inflammatory mediators in the murine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrimidine model of Parkinson's disease. *Neuroscience, 145*(2), 654–668.

Pearce, R. K., Jackson, M., Smith, L., Jenner, P., & Marsden, C. D. (1995). Chronic L-DOPA administration induces dyskinesias in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated common marmoset (Callithrix Jacchus). *Movement Disorders, 10*, 731–740.

Perez-Otano, I., Herrero, M. T., Oset, C., De Ceballos, M. L., Luquin, M. R., Obeso, J. A., & Del Rio, J. (1991). Extensive loss of brain dopamine and serotonin induced by chronic administration of MPTP in the marmoset. *Brain Research, 567*, 127–132.

Petroske, E., Meredith, G. E., Callen, S., Totterdell, S., & Lau, Y. S. (2001). Mouse model of Parkinsonism: A comparison between subacute MPTP and chronic MPTP/probenecid treatment. *Neuroscience, 106*(3), 589–601.

Pott Godoy, M. C., Tarelli, R., Ferrari, C. C., Sarchi, M. I., & Pitossi, F. J. (2008). Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. *Brain*, *131*, 1880–1894.

Prediger, R. D., Batista, L. C., Medeiros, R., Pandolfo, P., Florio, J. C., & Takahashi, R. N. (2006). The risk is in the air: Intranasal administration of MPTP to rats reproducing clinical features of Parkinson's disease. *Experimental Neurology, 202*(2), 391–403.

Prediger, R. D., Aguiar, A. S., Jr., Rojas-Mayorquin, A. E., Figueiredo, C. P., Matheus, F. C., Ginestet, L., Chevarin, C., Bel, E. D., Mongeau, R., Hamon, M., Lanfumey, L., & Raisman-Vozari, R. (2010). Single intranasal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in C57BL/6 mice models early preclinical phase of Parkinson's disease. *Neurotoxicity Research, 17*(2), 114–129.

Przedborski, S., & Vila, M. (2003). The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model: A tool to explore the pathogenesis of Parkinson's disease. *Annals of the New York Academy of Sciences, 991*, 189–198.

Przedborski, S., Jackson-Lewis, V., Yokoyama, R., Shibata, T., Dawson, V. L., & Dawson, T. M. (1996). Role of neuronal nitric oxide in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 4565–4571.

Purisai, M. G., McCormack, A. L., Langston, W. J., Johnston, L. C., & Di Monte, D. A. (2005). Alpha-synuclein expression in the substantia nigra of MPTP-lesioned non-human primates. *Neurobiology of Disease, 20*, 898–906.

Quinn, L. P., Perren, M. J., Brackenborough, K. T., Woodhams, P. L., Vidgeon-Hart, M., Chapman, H., Pangalos, M. N., Upton, N., & Virley, D. J. (2007). A beam-walking apparatus to assess behavioural impairments in MPTP-treated mice: Pharmacological validation with R-(−)-deprenyl. *Journal of Neuroscience Methods, 164*(1), 43–49.

Ramsay, R. R., & Singer, T. P. (1986). Energy-dependent uptake of N-methyl-4-phenylpyridinium, the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, by mitochondria. *Journal of Biological Chemistry, 261*, 7585–7587.

Ransohoff, R. M., & Cardona, A. E. (2010). The myeloid cells of the central nervous system parenchyma. *Nature, 468*, 253–262.

Ransom, B. R., Kunis, D. M., Irwin, I., & Langston, J. W. (1987). Astrocytes convert the parkinsonism inducing neurotoxin, MPTP, to its active metabolite, MPP+. *Neuroscience Letters, 75*, 323–328.

Riachi, N. J., Harik, S. I., Kalaria, R. N., & Sayre, L. M. (1988). On the mechanisms underlying 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity II. Susceptibility among mammalian species correlates with the toxin's metabolic patterns in brain microvessels and liver. *Journal of Pharmacology and Experimental Therapeutics, 244*(2), 443–448.

Riachi, N. J., LaManna, J. C., & Harik, S. I. (1989). Entry of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine into the rat brain. *Journal of Pharmacology and Experimental Therapeutics, 249*, 744–748.

Ricaurte, G. A., Langston, J. W., Delanney, L. E., Irwin, I., Peroutka, S. J., & Forno, L. S. (1986). Fate of nigrostriatal neurons in young mature mice given 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: A neurochemical and morphological reassessment. *Brain Research, 376*(1), 117–124.

Ricaurte, G. A., Irwin, I., Forno, L. S., DeLanney, L. E., Langston, E., & Langston, J. W. (1987). Aging and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced degeneration of dopaminergic neurons in the substantia nigra. *Brain Research, 403*(1), 43–51.

Rojo, A. I., Montero, C., Salazar, M., Close, R. M., Fernández-Ruiz, J., Sánchez-González, M. A., de Sagarra, M. R., Jackson-Lewis, V., Cavada, C., & Cuadrado, A. (2006). Persistent penetration of MPTP through the nasal route induces Parkinson's disease in mice. *European Journal of Neuroscience, 24*(7), 1874–1884.

Rousselet, E., Joubert, C., Callebert, J., Parain, K., Tremblay, L., Orieux, G., Launay, J. M., Cohen-Salmon, C., & Hirsch, E. C. (2003). Behavioral changes are not directly related to striatal monoamine levels, number of nigral neurons, or dose of parkinsonian toxin MPTP in mice. *Neurobiology of Disease, 14*(2), 218–228.

Sahgal, A., Andrews, J. S., Biggins, J. A., Candy, J. M., Edwardson, J. A., Keith, A. B., Turner, J. D., & Wright, C. (1984). N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) affects locomotor activity without producing a nigrostriatal lesion in the rat. *Neuroscience Letters, 48*(2), 179–184.

Scherer, H. J. (1939). Melanin pigmentation of the substantia nigra in primates. *The Journal of Comparative Neurology, 71*, 91–98.

Schintu, N., Frau, L., Ibba, M., Garau, A., Carboni, E., & Carta, A. R. (2009). Progressive dopaminergic degeneration in the chronic MPTPp mouse model of Parkinson's disease. *Neurotoxicity Research, 16*(2), 127–139.

Schmidt, N., & Ferger, B. (2001). Neurochemical findings in the MPTP model of Parkinson's disease. *Journal of Neural Transmission, 108*, 1263–1282.

Schneider, J. S., & Pope-Coleman, A. (1995). Cognitive deficits precede motor deficits in a slowly progressing model of parkinsonism in the monkey. *Neurodegeneration, 4*(3), 245–255.

Schwartz, M., Butovsky, O., Brück, W., & Hanisch, U. K. (2006). Microglial phenotype: Is the commitment reversible? *Trends in Neurosciences, 29*, 68–74.

Sedelis, M., Hofele, K., Auburger, G. W., Morgan, S., Huston, J. P., & Schwarting, R. K. (2000). MPTP susceptibility in the mouse: Behavioral, neurochemical, and histological analysis of gender and strain differences. *Behavior Genetics, 30*(3), 171–182.

Seniuk, N. A., Tatton, W. G., & Greenwood, C. E. (1990). Dose-dependent destruction of the coeruleus-cortical and nigral-striatal projections by MPTP. *Brain Research, 527*(1), 7–20.

Shimoji, M., Zhang, L., Mandir, A. S., Dawson, V. L., & Dawson, T. M. (2005). Absence of inclusion body formation in the MPTP mouse model of Parkinson's disease. *Brain Research. Molecular Brain Research, 134*(1), 103–108.

Smeyne, M., Jiao, Y., Shepherd, K. R., & Smeyne, R. J. (2005). Glia cell number modulates sensitivity to MPTP in mice. *Glia, 52*, 144–152.

Snow, B. J., Vingerhoets, F. J., Langston, J. W., Tetrud, J. W., Sossi, V., & Calne, D. B. (2000). Pattern of dopaminergic loss in the striatum of humans with MPTP induced parkinsonism. *Journal of Neurology, Neurosurgery, and Psychiatry, 68*(3), 313–316.

Staal, R. G., & Sonsalla, P. K. (2000). Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4-phenylpyridinium neurotoxicity in vivo in rat striata. *Journal of Pharmacology and Experimental Therapeutics, 293*, 336–342.

Storvik, M., Arguel, M. J., Schmieder, S., Delerue-Audegond, A., Li, Q., Qin, C., Vital, A., Bioulac, B., Gross, C. E., Wong, G., Nahon, J. L., & Bezard, E. (2010). Genes regulated in MPTP-treated macaques and human Parkinson's disease suggest a common signature in prefrontal cortex. *Neurobiology of Disease, 38*(3), 386–394.

Sulzer, D. (2010). Clues to how alpha-synuclein damages neurons in Parkinson's disease. *Movement Disorders, 25*, S27–S31.

Tanila, H., Björklund, M., & Riekkinen, P. (1998). Cognitive changes in mice following moderate MPTP exposure. *Brain Research Bulletin, 45*(6), 577–582.

Tatton, N. A., & Kish, S. J. (1997). In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labelling and acridine orange staining. *Neuroscience, 77*(4), 1037–1048.

Teismann, P., Tieu, K., Cohen, O., Choi, D. K., Wu du, C., Marks, D., Vila, M., Jackson-Lewis, V., & Przedborski, S. (2003). Pathogenic role of glial cells in Parkinson's disease. *Movement Disorders, 18*, 121–129.

Tian, Y. M., Chen, X., Luo, D. Z., Zhang, X. H., Xue, H., Zheng, L. F., Yang, N., Wang, X. M., & Zhu, J. X. (2008). Alteration of dopaminergic markers in gastrointestinal tract of different rodent models of Parkinson's disease. *Neuroscience, 153*(3), 634–644.

Tillerson, J. L., Caudle, W. M., Reverón, M. E., & Miller, G. W. (2002). Detection of behavioral impairments correlated to neurochemical deficits in mice treated with moderate doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Experimental Neurology, 178*(1), 80–90.

Varastet, M., Riche, D., Maziere, M., & Hantraye, P. (1994). Chronic MPTP treatment reproduces in baboons the differential vulnerability of mesencephalic dopaminergic neurons observed in Parkinson's disease. *Neuroscience, 63*, 47–56.

Vazquez-Claverie, M., Garrido-Gil, P., San Sebastian, W., Belzunegui, S., Izal-Azcarate, A., Lopez, B., Marcilla, I., & Luquin, M. R. (2009a). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) does not elicit long-lasting increases in cyclooxygenase-2 expression in dopaminergic neurons of monkeys. *Journal of Neuropathology and Experimental Neurology, 68*, 26–36.

Vazquez-Claverie, M., Garrido-Gil, P., San Sebastian, W., Izal-Azcarate, A., Belzunegui, S., Marcilla, I., Lopez, B., & Luquin, M. R. (2009b). Acute and chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administrations elicit similar microglial activation in the substantia nigra of monkeys. *Journal of Neuropathology and Experimental Neurology, 68*, 977–984.

Vila, M., Vukosavic, S., Jackson-Lewis, V., Neystat, M., Jakowec, M., & Przedborski, S. (2000). Alpha-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the parkinsonian toxin MPTP. *Journal of Neurochemistry, 74*(2), 721–729.

Wichmann, T., & DeLong, M. R. (2003). Pathophysiology of Parkinson's disease: The MPTP primate model of the human disorder. *Annals of the New York Academy of Sciences, 991*, 199–213.

Willis, G. L., & Donnan, G. A. (1987). Histochemical, biochemical and behavioural consequences of MPTP treatment in C-57 black mice. *Brain Research, 402*(2), 269–274.

Yasuda, Y., Shimoda, T., Uno, K., Tateishi, N., Furuya, S., Yagi, K., Suzuki, K., & Fujita, S. (2008). The effects of MPTP on the activation of microglia/astrocytes and cytokine/chemokine levels in different mice strains. *Journal of Neuroimmunology, 204*, 43–51.

Yazdani, U., German, D. C., Liang, C. L., Manzino, L., Sonsalla, P. K., & Zeevalk, G. D. (2006). Rat model of Parkinson's disease: Chronic central delivery of 1-methyl-4-phenylpyridinium (MPP+). *Experimental Neurology, 200*(1), 172–183.

Youdim, M. B., Grunblatt, E., Levites, Y., Maor, G., & Mandel, S. (2002). Early and late molecular events in neurodegeneration and neuroprotection in Parkinson's disease MPTP model as assessed by cDNA micro-array; the role of iron. *Neurotoxicity Research, 4*, 679–689.

Yuste, J. E., Echeverry, M. B., Ros-Bernal, F., Gomez, A., Ros, C. M., Campuzano, C. M., Fernandez-Villalba, E., & Herrero, M. T. (2012). 7-Nitroindazole down-regulates dopamine/DARPP-32 signaling in neostriatal neurons in a rat model of Parkinson's disease. *Neuropharmacology*, *63*, 1258–1267.

Zesiewicz, T. A., & Hauser, R. A. (2002). Monoamine oxidase inhibitors. In S. A. Factor & W. J. Weiner (Eds.), *Parkinson's disease: Diagnosis and clinical management*. New York: Demos Medical Publishing.

Nanomedicines for Nervous System Diseases

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Abstract

The need for the development of nanomedicines for the treatment of degenerative and inflammatory diseases of the nervous system is of immediate importance. Nanoparticles are drug carriers that can specifically target diseased brain subregions and thus enhance therapeutic efficacy. Multifunctional nanoparticles delivering pharmaceutical agents serve a dual purpose: While they facilitate drug delivery they also provide imaging of cell dynamics and pharmacokinetic assessments. Polymer science and nanotechnology could be used to improve diagnostic and therapeutic outcomes. Despite their promise in combating disease, nanomedicines have yet to make their mark as broadly viable pharmaceuticals. The translation of nanoformulation technology for clinical therapeutic and diagnostic applications shows both real potential and inherent obstacles. However, safety concerns remain operative for their potential broad clinical implications, tempering any translational potential.

Keywords

Cell-based drug delivery • Nanomedicines • Nanoparticles • Neuroimmunology • Neuroinflammatory disease

1 Introduction

A range of insults can affect the nervous system that include infection, trauma, vascular insufficiency, misfolded aggregated protein-induced degeneration, toxins and metabolic dysregulations (de Vries et al. 1997, 2012; Frank-Cannon et al. 2009; Graves and Vernino 2012; Kanwar et al. 2012a; Rosenberg 1997). All lead to activation of the immune system that serves as a key factor in both the etiology and progressive nature of a number of neurological diseases (de Vries et al. 1997, 2012; Frank-Cannon et al. 2009; Rosenberg 1997). Such immune responses can serve as disease propagators and healers. The former leads to sustained phenotype switches for glia (microglia and astrocytes) with the secretion of a broad range of inflammatory mediators that include excitotoxins, pro- and anti-inflammatory cytokines, and chemokines (Ransohoff and Engelhardt 2012). These result in alterations in phagocytosis, intracellular killing, clearance, and antigen presentation. Ultimately compromise of the blood-brain barrier (BBB) ensues with extravasation of toxic proteins, altered glial secretions, and neurotoxicity (de Vries et al. 1997; Kanwar et al. 2012a; O'Callaghan et al. 2008; Rosenberg 1997). While complex in nature, each component step for disease provides opportunities for therapeutic intervention (Kanwar et al. 2012b). In all, the targeting of such interventional strategies with facilitation of drug penetrance across the BBB could lead to improved therapeutic outcomes in central nervous system (CNS) disease (Kanwar et al. 2012a).

1.1 Current Treatment Strategies

Treatment of Alzheimer's and Parkinson's diseases (AD and PD, respectively), amyotrophic lateral sclerosis (ALS), a range of microbial infections and multiple sclerosis (MS) is limited to ameliorating symptoms and as such poses continuous challenges to clinicians and clinical investigators (de Vries et al. 1997, 2012; Kanwar et al. 2012a). Simply stated, conventional treatments are palliative. Curative therapies are available, for the most part, only for infectious diseases (Elman et al. 2007; Nowacek et al. 2009a). Even with the potential of disease cure, microbial resistance remains an ever significant concern. Furthermore, accurate diagnosis often precludes early therapeutic intervention. Complicating treatment for neurodegenerative, neuroinfectious, and neuroinflammatory diseases is poor penetration of many drugs across the BBB. To this end, considerable efforts are being placed on developing novel strategies for improving drug penetrance and delivery to sites within the brain parenchyma that are disease targets as well as developing improved methods for early disease diagnosis (Alam et al. 2010).

1.2 Urgent Need for Nanomedicines

Considerable emphasis is being placed on the future development of therapeutic drug formulations that target the CNS and halt ongoing toxic, metabolic, and inflammatory events that affect the tempo and progression of neurodegenerative diseases (Graves and Vernino 2012; Ulbrich and Lamprecht 2010). Nanotechnology offers one important means toward such a goal. The ability of nanoparticles to traverse the BBB can be exploited to enhance drug entry into the CNS (Fernandes et al. 2010; Kanwar et al. 2012b). Nanoparticles targeted to the CNS can be efficient delivery strategies to improve the efficacy of commonly used medicines along with developing new therapeutics intended to reach the site of disease action (Kanwar et al. 2012b; Metcalfe and Fahmy 2012; Silva 2005; Ulbrich and Lamprecht 2010). In addition, nanoparticle-based imaging systems can provide earlier and more effective diagnosis of subtle brain dysfunctions (Kanwar et al. 2012b; Stoll and Bendszus 2010).

2 Nanoparticle Compositions

Disorders of the CNS that include AD, PD, ALS, and stroke are rapidly increasing in disease prevalence and incidence (Brown et al. 2005; Checkoway et al. 2011; Mayeux 2003). Therapies that can change the course of disease would have substantial impact as AD alone costs more than $100 billion/year in the United States for medical and institutional care. Such numbers will certainly double in the next decades (ADI 2012). Currently, disease diagnosis is made, for the most part, by clinical observation as it has for more than 100 years (de Carvalho et al. 2008;

Jack et al. 2011; Li et al. 1991; Rao et al. 2006; Yew and Cheng 2009). Drugs, vaccines, or regenerative proteins present "real" possibilities to positively alter disease outcomes.

Delivery of drugs to treat CNS diseases poses particular challenges. Barriers to CNS drug delivery include CNS structure, function, and physiology. To enter into the brain parenchyma, drugs must cross the endothelial cell lining of the microvasculature (Nowacek et al. 2009a). Under physiologic conditions, this barrier selectively regulates intracellular and paracellular exchange of nutrients, macromolecules, and cells between the blood and the CNS. Permeability of many drugs through the BBB is very low even though the capillary surface area is large. Thus, the BBB plays an indispensable role in regulating drug CNS concentrations (Alam et al. 2010).

Development of ways to overcome the barriers to CNS drug delivery is of clinical relevance. Delivery of drugs by nanocarrier systems is a strategy that could overcome barriers to CNS drug delivery, including poor BBB penetration, extensive binding to plasma proteins, and the presence of drug efflux pumps that diminish drug levels crossing the BBB into the CNS parenchyma (Bhaskar et al. 2010; Kanwar et al. 2012b; Nowacek et al. 2009a). Various nanotechnology-based systems are being developed to overcome barriers to drug delivery for improving CNS treatments. These systems are discussed in relation to their potential use in neuroinflammatory and neurodegenerative disorders.

2.1 Liposomes

Liposomes are spherical vesicles composed of a lipid bilayer membrane surrounding an aqueous inner core (Fernandes et al. 2010; McMillan et al. 2011). Their high biocompatibility, low immunogenicity, and extended circulation time contribute to their use as drug-delivery systems. Hydrophilic drugs can be loaded into the aqueous core, while hydrophobic molecules can be carried in the lipid membrane for delivery to the target site. However, low loading capacity, relatively quick release profiles of hydrophobic drugs, and lesser physical stability than polymeric formulations limit their applications for specific drugs (Liu et al. 2010). Inclusion of cholesterol into the liposomal membranes can improve stability of the bilayer, coating with polyethylene glycol (PEG) chains can enhance the circulation time of liposomal formulations in vivo by reducing uptake by the reticuloendothelial system, and adding targeting moieties on the liposome surface can improve delivery to the target site (Fernandes et al. 2010; McMillan et al. 2011; Micheli et al. 2012). The mechanism by which liposomes transverse the BBB is still not understood but is proposed to be achieved by passive diffusion through the brain capillary endothelial cells, by endothelial cell endocytosis, or by fusion with the endothelial cell membrane (Fernandes et al. 2010). Improved CNS drug delivery and reduced toxicity have been observed in animal models using liposomal systems for antifungals, antiretrovirals, antiepileptics, anti-ischemia drugs, and chemotherapeutics (Wong et al. 2012). Cationic liposomes are being explored to improve delivery of nucleic acids to target cells for gene therapy (Fernandes et al. 2010).

2.2 Solid Lipid Nanoparticles and Nanocapsules

Other lipid-based systems that show potential for enhanced drug delivery across the BBB include solid lipid nanoparticles and lipid nanocapsules (Fernandes et al. 2010; Wong et al. 2012). These systems usually use triglycerides and phospholipids to encase the drug and are stabilized using surfactants. Solid lipid nanoparticles containing nitrendipine or 5-fluoro-2-deoxyuridine improved penetration of the drugs into the brain up to twofold (Fernandes et al. 2010; Manjunath and Venkateswarlu 2006; Wang et al. 2002). Indomethacin-loaded nanocapsules reduced tumor size and decreased morbidity and mortality in rats implanted with C6 glioma (Bernardi et al. 2009).

2.3 Polymeric Micelles

Polymeric micelles can be used to deliver poorly soluble drugs with high bioavailability (Gaucher et al. 2010; Kanwar et al. 2012a; Kedar et al. 2010). Polymeric micelles have an anisotropic water distribution with a hydrophilic corona and hydrophobic core. Amphiphilic block polymers containing poly (ethylene) oxide-polypropylene oxide are most commonly used in assembly of polymeric micelles for targeted drug delivery. Kabanov et al. demonstrated CNS delivery of micelles formed by polymeric block polymers surrounding a non-covalently bound drug core (Manickam et al. 2012; Sharma et al. 2008). One of the major important advantages of this system is the ability of the micellar formulations to be used in applications involving drug delivery across the BBB, since the micellar formulations are able to transport the active agents into body compartments where the free drug alone cannot penetrate (Manickam et al. 2012; Sharma et al. 2008). They also have a low critical micelle concentration and slow rate of dissociation that allows the loaded drugs to remain in the system for a longer period of time and provides greater drug delivery to the target site (Batrakova et al. 2011; Fernandes et al. 2010; McMillan et al. 2011).

2.4 Polymeric Nanoparticles

Polymeric nanoparticles are prepared using a variety of polymers. Intended drugs can be dissolved or encapsulated to obtain nanospheres or nanocapsules by attaching the drug to the surface of the nanoparticle (Gupta and Jain 2010; Mallipeddi and Rohan 2010). Nanocapsules are fabricated by confining the drug to a cavity surrounded by a polymer membrane, and nanospheres are matrix systems formed by a uniform dispersion of the drug (Gupta and Jain 2010). The development of biodegradable polymers and their use for various drug-delivery applications have been described recently. The most widely used nontoxic polymeric materials include polyvinyl alcohol (PVA), polyacrylic acid (PAA), polyacrylamide, PEG, and polycaprolactone (Gupta and Jain 2010). These polymers

could break down easily into biologically acceptable molecules. Polymeric nanoparticles may cross the BBB by passing through tight junctions; enhanced binding, endocytosis, and transcytosis by endothelial cells; or inhibition of endothelial cell efflux transporters (Fernandes et al. 2010; Kanwar et al. 2012a; Khalil et al. 2011; Wong et al. 2012).

2.5 Dendrimers

Dendrimers are macromolecules with a defined branched architecture. They are assembled in layers, or generations, and are composed of a series of repeated branched chains around a central core with many identical functional groups on the exterior (Boas and Heegaard 2004). Drugs or other molecules can be carried within the void spaces in the interior of the structures, while attachment of functional groups on the exterior can provide targeting to specific cells and tissues (Fernandes et al. 2010; McMillan et al. 2011). The ability to pass through the BBB is greatly influenced by the molecular architecture (Fernandes et al. 2010).

2.6 Fullerenes, Carbon Nanotubes, and Carbon Nanofibers

Biomedical application of fullerenes and carbon nanotubes has been limited due to their poor water solubility. Functionalization has increased their water solubility and utility for use in nanomedicine. Use of functional moieties such as dendrimers, amino acids, peptides, proteins and growth factors, liposomes, polyamines, polymers, carboxyl groups, and gadolinium has expanded their application for drug delivery, reactive oxygen species quenching, promotion of neurite outgrowth, and imaging. Carbon nanofibers have potential for design of neural prosthetics (Kanwar et al. 2012b; McMillan et al. 2011).

3 Nanoparticles for Imaging and Diagnostics

Nanoparticles are used for neuroimaging and are increasingly being used as tools to help identify biomarkers of disease and monitor the success of stem cell treatment (Kanwar et al. 2012b; McMillan et al. 2011; Stoll and Bendszus 2010). Liposomes, dendrimers, magnetic nanoparticles, and quantum dots are being developed for single photon emission computed tomography, positron emission tomography, magnetic resonance imaging (MRI), fluorescence microscopy, computed tomography, and ultrasound. Of importance, these nanoparticles can be targeted to specific cell types and proteins to enhance sensitivity and improve detection of early markers of disease including BBB integrity and secretion of pro-inflammatory markers (Kanwar et al. 2012b). Incorporating multiple contrast agents into a single particle can provide for multiple imaging modalities, while incorporating drug and contrast agents into one particle can enable in vivo tracking of the drug distribution (Bhaskar et al. 2010; McMillan et al. 2011). Iron particles are commonly used as contrast agents in the

form of superparamagnetic iron oxide (SPIO) or ultrasmall superparamagnetic iron oxide (USPIO) (Bhaskar et al. 2010) and provide a means to track the cells and determine CNS infiltration by MRI (Stoll and Bendszus 2010). SPIO have been used successfully in experimental models of MS, stroke, and other CNS disorders to track the migration of circulating monocytes/macrophages over the course of inflammation (Bhaskar et al. 2010; Stoll and Bendszus 2010).

4 Neuroimmunology and Nanomedicine

4.1 Innate and Adaptive Immune Responses

Neuroinflammation is centered on microglial activation (de Vries et al. 1997, 2012). In the presence of an activating stimulus, microglial surface receptor expression and production and secretion of immunomodulatory factors is altered (reviewed by de Vries et al. (2012) and Frank-Cannon et al. (2009)). Glial production of specific pro-inflammatory mediators such as tumor necrosis factor (TNF) and interleukins-1 (IL-1), -2, -4, -5, -8, -10, and -12 alter brain capillary endothelial cell function and promote leukocyte infiltration into the CNS (Benoit et al. 2008; de Vries et al. 1997; Persidsky et al. 2006; Ransohoff and Engelhardt 2012). Secretion of interleukins, such as IL-6 and -8, can promote both leukocyte infiltration and neuroinflammation (Benoit et al. 2008; de Vries et al. 1997; Persidsky et al. 2006). Glial production of interferon gamma (IFN-γ) and macrophage colony–stimulating factor (MCSF) stimulates release of eicosanoids, reactive oxygen and reactive nitrogen species, and chemokines, such as RANTES and macrophage inhibitory proteins-1 alpha and -1 beta (MIP-1α and MIP-1β) (de Vries et al. 1997; Persidsky et al. 2006). Infiltration of neutrophils, monocytes, and antigen-specific B and T cells into the CNS is accelerated by the expression of intercellular adhesion molecule-1 (ICAM-1), ICAM-2, and vascular cell adhesion molecule-1 (VCAM-1) on brain capillary endothelial cells (de Vries et al. 1997; Persidsky et al. 2006) and their interactions with lymphocyte functional antigen-1 (LFA-1) and very late antigen-4 (VLA-4) (de Vries et al. 1997; Kanwar et al. 2012a; Lyck and Engelhardt 2012). IL-1 beta (IL-1β) is one example of the range of inflammatory factors that affects endothelial cell expression of adhesion molecules, enhances chemoattractant secretions by astrocytes, and promotes inflammatory cell migration (Brabers and Nottet 2006). Chronic activation of this system designed to fight CNS inflammation in disease or injury is believed to underlie the pathobiology of neurodegenerative diseases (de Vries et al. 1997, 2012).

4.2 Cell-Based Drug Delivery

The application of cells as targeted carriers for nanomedicines is a developing concept. Because of their ability to migrate to sites of disease and inflammation, immunocytes, mononuclear phagocytes (MP; monocytes, macrophages, dendritic

cells), lymphocytes, neutrophils, and stem cells are attractive as drug carriers (Batrakova et al. 2011; McMillan et al. 2011). They can also act as Trojan horses for delivery of drug cargo across biologic barriers such as the BBB. For cells to be effective drug carriers, however, a number of criteria must be fulfilled. First, the cells must migrate to the site of injury or disease in sufficient numbers. For CNS disorders, this means that the cells must be able to cross the BBB. Second, the cells must release their drug cargo intact at the disease site. Thus, the drug must be carried in non-degrading compartments within the cell and be released from the cell continuously at the site of action. Finally, carriage of the drug cargo should not adversely affect the cell's normal function. The potential for cell-based drug delivery has been demonstrated by our own studies. Macrophages loaded with indinavir nanoparticles and injected intravenously into severe combined immunodeficient (SCID) mice migrated to a site of HIV-encephalitis (HIVE) in the brain, reduced the level of infection, and provided enhanced drug levels at the site of infection (Dou et al. 2009). As a second example, in an experimental model of Parkinson's disease, macrophages loaded with catalase nanozymes were injected into 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)-treated mice and provided enhanced catalase levels in the brains of these mice (Batrakova et al. 2007; Brynskikh et al. 2010). Drug loading of neural stem cells and their use as drug-delivery vehicles to areas of CNS disease is also being explored (Martinez-Serrano and Bjorklund 1998; Martinez-Serrano et al. 1996; McMillan et al. 2011; Muller et al. 2006). The success of a nanocarrier for use in a cell-based delivery system is determined by a number of factors. Drug nanocarriers are commonly composed of a polymeric outer shell with an inner core for drug carriage. The charge state of the outer polymeric shell can directly influence the uptake of the nanoparticles by immunocytes and stem cells through cell surface receptor interactions and stability of the nanoparticles inside the cells (Batrakova et al. 2011; McMillan et al. 2011). As an example, nanozymes containing superoxide dismutase were protected inside macrophages when the nanoparticles were made using positively charged block copolymers but not negatively charged block copolymers (Batrakova et al. 2011; Zhao et al. 2011). The composition of the outer polymeric coating can also influence the toxicity of the nanoparticles to the cells (Batrakova et al. 2011; McMillan et al. 2011). We have demonstrated that nanoformulations of the antiretroviral drug ritonavir, which enter cells through clathrin-mediated endocytosis, are trafficked to and stored in recycling endosomes and can be released intact (Fig. 1) (Kadiu et al. 2011). Of particular interest these compartments are the same compartments where the human immunodeficiency virus-1 (HIV-1) replicates (Kadiu and Gendelman 2011).

While these experiments demonstrate the potential for cell-based drug delivery, they require pre-loading of cells with nanoparticles and injection into donors. A more practical, clinically useful system would provide for administration of drug nanoparticles to the diseased patient; uptake of the nanoparticles by host MPs, immunocytes, or lymphocytes; and storage within these cells to provide (a) sustained drug release and (b) delivery of the drug nanoparticles to sites of CNS disease. We have recently demonstrated this possibility using polymeric crystalline

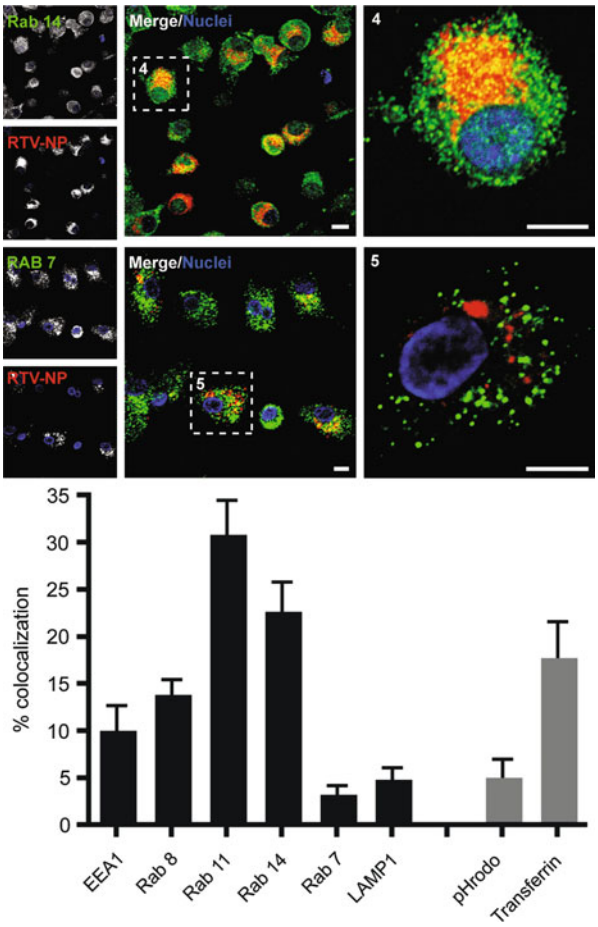

Fig. 1 Immunohistological identification of nanoparticle subcellular localization. Confocal microscopy confirmed distribution of RTV-NP within recycling endosomal (Rab 14) rather than late endosomal (Rab 7) compartments. Note that RTV-NPs are *red* and compartments are *green*, and *yellow* signifies compartment marker-particle overlap in all panels. Pearson's co-localization coefficients indicate RTV-NPs are preferentially distributed to Rab11 and Rab14 recycling endosomes compared with early endosomes, Rab8 or Rab7 endosomes, and lysosomes. Analysis of distribution of RTV-NP within acidified (degrading) compartments, identified by pHrodo-dextran beads, revealed minimal overlap, indicating RTV-NPs likely bypass degradation within the cell and are primarily recycled for release. High RTV-NP co-localization with transferrin also indicates that particles are most likely recycled. Measure bars equal 1 μm. Graphical data represent the mean ± standard error of the mean for n = 3. *NP* Nanoparticle, *RTV* Ritonavir (Figure reprinted with permission from Kadiu et al. (2011))

nanoparticles of the antiretroviral drugs atazanavir (ATV) and ritonavir (RTV) (Fig. 2). Polymer-coated crystalline nanoparticles of antiretroviral drugs (nanoART) are taken up by macrophages in culture, retained inside the cells for up to 15 days, and demonstrate antiretroviral efficacy over this time period

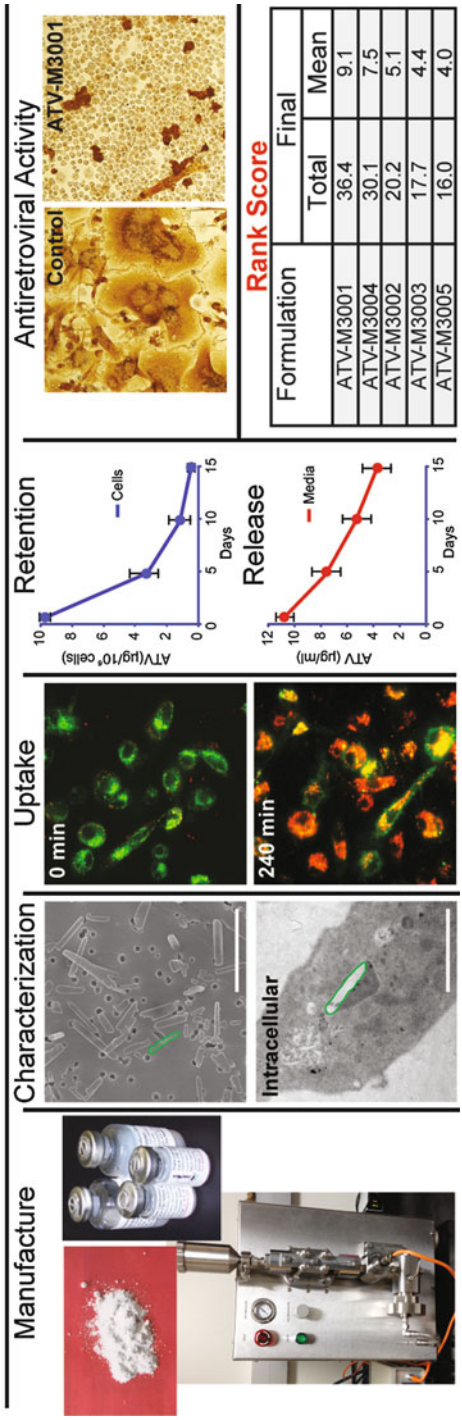

Fig. 2 Schematic for manufacture, characterization, cell-based screening, and scoring of nanoformulated antiretrovirals (nanoART). Crystalline antiretroviral drug is coated with polymer surfactant by high-pressure homogenization and characterized by size, polydispersity, zeta potential, and shape. NanoART is screened in cell-based assays for macrophage uptake, retention, release, and antiretroviral efficacy. The formulations are rank-scored for selection of those suitable for animal testing, as described by Nowacek et al. (2011)

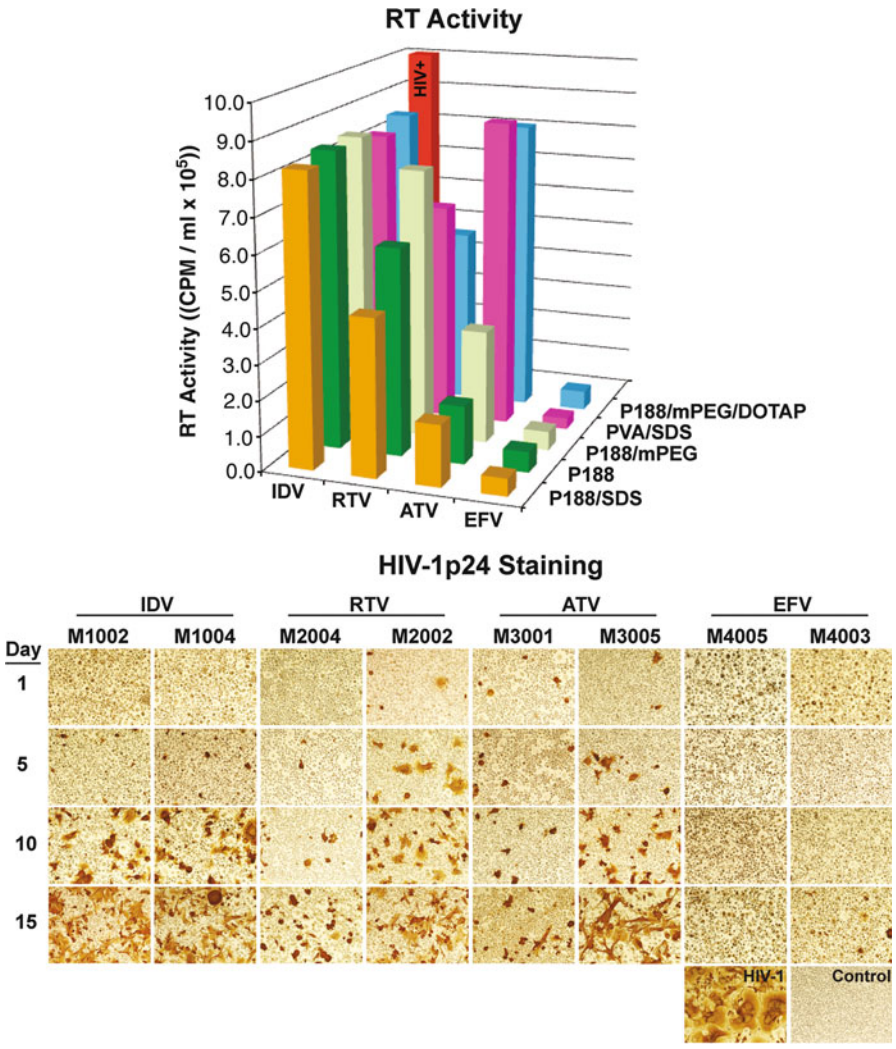

Fig. 3 **Antiretroviral efficacy of nanoART**. Comparison of antiretroviral effects in monocyte-derived macrophages challenged with HIV-1_{ADA} 15 days after pretreatment with nanoART, as measured by reverse transcriptase (RT) activity and HIV-1p24 antigen staining 10 days after viral challenge. RT activities were measured by ^{3}H-TTP incorporation. Data represent mean for n = 8 determinations/treatment (Figure reprinted with permission from Nowacek et al. (2011))

(Fig. 3) (Balkundi et al. 2011; Nowacek et al. 2009b, 2010, 2011). When administered subcutaneously to mice, nanoART (250 mg/kg, ATV/RTV) provided sustained plasma ATV concentrations (>100 ng/ml) and liver and spleen levels exceeding 1,000 ng/g (Roy et al. 2012) for up to 2 weeks. Predominant sequestration of nanoART inside liver Kupffer cells was demonstrated in mice

treated with dye-labeled nanoART followed by isolation and selection of Kupffer cells by in situ collagenase digestion and CD11b + cell sorting (Gautam et al. 2013). Of significance, weekly administration of nanoART reduced HIV-1 viral load to nearly undetectable levels in a humanized mouse model of chronic HIV infection (Dash et al. 2012). Targeting the folate receptor on macrophages using a folate-modified polymer coating improved by up to twofold in vitro macrophage uptake and antiretroviral efficacy of nanoART and enhanced mouse plasma and tissue drug levels up to fivefold compared to non-targeted nanoART (Puligujja et al. 2013).

5 Degenerative, Inflammatory, and Infectious Diseases of the Nervous System

5.1 AD, PD, and ALS

5.1.1 Pathobiology of Diseases

AD, PD, and ALS are progressive neurodegenerative disorders that usually develop later in life. The etiologies of these diseases are different but have in common deposition of mutated proteins and chronic neuroinflammation (Kanwar et al. 2012b; Nowacek et al. 2009a; Re et al. 2012b). AD is characterized by losses in memory and cognitive function and pathologically by neurofibrillary tangles formed by hyperphosphorylated tau protein and extracellular β-amyloid protein (Aβ) plaques, considered the cause of neuronal loss. PD is marked by degeneration of dopaminergic neurons in the substantia nigra and characterized pathologically by cytoplasmic inclusions called Lewy bodies composed of mutated α-synuclein. ALS is marked by the selective loss of motor neurons in the brain and spinal cord leading to paralysis. The majority of ALS cases are sporadic; however, the familial form has been linked to missense mutations in superoxide dismutase 1 (SOD1), which forms intracellular deposits that inhibit chaperone and proteasome activity.

5.1.2 Nanoparticle-Based Therapies and Diagnostics

Approved therapies for these diseases are mainly symptomatic. AD treatments include acetylcholinesterase inhibitors to prevent breakdown of acetylcholine and the use of antioxidants and anti-inflammatory drugs (Kanwar et al. 2012a). Treatments for PD include dopamine mimetics and monoamine oxidase inhibitors to reduce dopamine degradation (Kanwar et al. 2012a). The only currently approved treatment for ALS is riluzole, a sodium channel blocker that inhibits stimulation of glutamate receptors and is used to slow the progression of the disease. None of these treatments are curative, and there is a grave need for novel therapeutic and early diagnostic approaches.

Development of nanotherapeutic-based treatments for each of these diseases is ongoing (Kanwar et al. 2012b; Nowacek et al. 2009a). However, while some are promising, none have reached clinical trials. Again, many, but not all, of the nanoparticle-based systems are designed to enhance the effectiveness and reduce the toxicity of traditional treatments. Use of nanoparticles for treating AD has

focused on three strategies: prevention of Aβ aggregation in the CNS and blood; reducing transition-metal-induced production of reactive oxygen species; and improved delivery of acetylcholinesterase inhibitors to the CNS (Nowacek et al. 2009a; Re et al. 2012a). Polyamidoamine (PAMAM) dendrimers, and nanoliposomes decorated with phosphatidic acid, cardiolipin, anti-Aβ antibody or a curcumin derivative can inhibit in vitro aggregation of Aβ, and C_{60} fullerenes were reported to prevent cognitive impairments induced by Aβ aggregation in rats (Nowacek et al. 2009a; Re et al. 2012a). Aberrant homeostasis of transition metals in the CNS has been associated with oxidative insult to neurons and promotion of Aβ aggregation. The use of metal chelators has been proposed, but is complicated by their systemic toxicity and inability to cross the BBB. To improve the therapeutic efficacy and CNS delivery of chelators and reduce their unwanted systemic toxicity, nanoparticle systems are being developed (Cui et al. 2005; Liu et al. 2009a,b). Nanoparticle systems to improve delivery of anticholinesterase inhibitors to the brain, such as polysorbate 80-coated poly(*n*-butylcyanoacrylate) nanoparticles to deliver tacrine and poly(DL-lactide-co-glycolic acid) (PLGA) microspheres to deliver huperzine-A, are also under development (Chu et al. 2007; Nowacek et al. 2009a; Re et al. 2012a; Wang et al. 2007; Wilson et al. 2008).

Nanoparticles for treatment of PD are being developed to treat loss of dopamine and dopaminergic neurons and reduce microglia-activated reactive oxygen species production (Nowacek et al. 2009a; Re et al. 2012a). To deliver dopamine to the CNS, chitosan nanoparticles containing dopamine adsorbed to the surface were developed and provided reduced toxicity and increased dopamine levels in the striatum (Trapani et al. 2011). Solid lipid nanoparticles containing bromocriptine, a dopamine agonist, improved performance in behavioral tests when administered to rats (Esposito et al. 2008). Lactoferrin-modified nanoparticles containing human neurotrophic factor were used for gene therapy in a rat model of PD and provided improved locomotor activity, reduced neuronal loss, and increased brain dopamine levels.

Fewer nanoparticle treatments are under development for ALS than for AD and PD; however, the approaches being used are similar (Nowacek et al. 2009a; Re et al. 2012b). Solid lipid nanoparticles containing riluzole exhibit high drug loading, a greater ability to deliver drug to the brain, and a higher efficacy compared to free drug (Bondi et al. 2010). In addition, PLGA nanoparticles containing SOD1 and carboxyfullerene SOD mimetics show protection from hydrogen-peroxide-induced damage in neuronal cultures, (Ali et al. 2008; Reddy et al. 2008) which opens the way for these systems to be tested in animal models of ALS.

A cell-based Trojan horse approach for treatment of neurodegenerative disease has been developed by Batrakova and coworkers (2007, 2011; Brynskikh et al. 2010). Catalase was packaged into nanoparticles composed of a block copolymer complex with polyethyleneimine-PEG (termed nanozymes) and loaded into mouse bone marrow macrophages in vitro. The macrophages loaded with nanozymes were adoptively transferred into isologous mice treated with MPTP as a model of acute PD. Nanozyme-loaded macrophages were able to cross the BBB and deliver active

catalase to sites of damage to a greater extent that free catalase (Batrakova et al. 2007), providing proof that this concept is feasible and could be applied to other neurodegenerative disorders.

Nanotechnology also offers tools for early disease diagnosis. Ultrasensitive assay systems for detecting low concentrations of AD pathogenic proteins using gold nanoparticles have been developed by Georganopoulou et al. (2005) and Neely et al. (2009). Amyloid plaques in brains of AD transgenic mice were detected using Aβ-coupled iron oxide particles and MRI (Yang et al. 2011). For early diagnosis of PD, sensitive in vitro nanoparticle-based systems are being developed for detection of α-synuclein (An et al. 2010; Yu and Lyubchenko 2009). MRI has also been used to track CD4+ lymphocytes loaded with SPIO nanoparticles and injected into a rat model of ALS to detect regions of pathology in the CNS (Bataveljic et al. 2011; Machtoub et al. 2011).

5.2 Multiple Sclerosis

5.2.1 Pathobiology of Disease

MS is a progressive inflammatory autoimmune disorder of the CNS characterized by demyelination and degeneration of neuronal axons (Goldenberg 2012; Lassmann and van Horssen 2011). Destruction of oligodendrocytes occurs upon infiltration of lymphocytes into the CNS and initiation of inflammation and autoimmune reactions (Kanwar et al. 2012a). The disease can be manifest as a relapsing remitting MS, characterized by recurring attacks of acute focal neurological deficits or exacerbation of existing deficits followed gradually by partial or full recovery, or a progressive steady decline without remission (Goldenberg 2012). The disease course includes distinct exacerbations with no symptom-free remissions; instead, patients will experience progressive acceleration of disability, accumulating irreversible neurologic deficits.

The pathology of MS was originally defined by the presence of focal white matter lesions, characterized by primary demyelination with partial preservation of axons and reactive astrocytic scar formation (Bo et al. 2003; Nylander and Hafler 2012). The disease pathology changes with time. For patients in the early stages of disease experiencing clinical relapses and remissions, inflammatory demyelination leads to the formation of focal plaques predominantly located in the white matter (Rovira and Leon 2008). In latter stages of the disease, widespread demyelination in the cerebral and cerebellar cortex is seen as well as diffuse degenerative changes throughout the entire white and gray matter. Long-standing severe disease results in profound brain and spinal cord atrophy, with extreme tissue loss and dilatation of cerebral ventricles (Androdias et al. 2010; Kutzelnigg et al. 2005; Popescu et al. 2011). In a substantial number of patients, the disease eventually subsides and inflammation as well as neurodegeneration decline to levels seen in age-matched controls. During stages of active disease, both in the relapsing as well as in the progressive stages, profound oxidative tissue injury is seen. In early stages, oxidative damage is mainly driven by inflammation, resulting in an oxidative burst in microglia and macrophages. In the progressive stage, active neurodegeneration occurs against a background of mild-to-moderate inflammation, and oxidative

damage appears to be augmented by release of iron from intracellular stores. In the latter stages, the presence of iron in the brain in the absence of additional oxidative bursts from microglia does not lead to further neurodegeneration; however, patients may be affected by concomitant diseases such as stroke or AD.

5.2.2 Current Treatments

Traditional treatment for MS is aimed at shortening the duration and frequency of acute exacerbations and providing symptomatic relief. Most currently approved MS therapeutics are immunosuppressants with the exception of beta interferons and glatiramer acetate as disease-modifying drugs for relapsing remitting MS (Gasperini and Ruggieri 2012; Silva 2005; Tubaro et al. 2012). The mechanism by which these drugs act involves downregulation of T-cell proliferation and migration, reduction in antigen presentation, and shifting the immune response to T-helper 2 (Th2) cells (Devonshire and Verdun di Cantogno 2011). The necessity for repeated subcutaneous or intramuscular injections of these drugs can lead to suboptimal long-term adherence in some patients and can negatively affect clinical outcomes. Other treatments target immune suppression by inducing apoptosis in lymphocytes (mitoxantrone) or T and B cells (cyclophosphamide) or using corticosteroids to suppress the immune response. While these are commonly used for treatment of relapsing remitting MS, they have limited application in the treatment of other forms of MS because of undesirable side effects.

5.2.3 Nanoparticle-Based Experimental Systems

Nanoparticle-based treatments for MS are being developed that seek to not only modulate the immune response, but also promote neuroprotection and myelin regeneration (Amedei et al. 2012; Dharamkar 2008; Ellis-Behnke 2007; Silva 2005; Ulbrich and Lamprecht 2010). PEG-coated liposomal formulations of the traditional MS therapeutics, prednisolone and minocycline, are being developed for enhanced delivery of drug to inflammatory lesions in the CNS and to minimize serious systemic side effects (Hu et al. 2009; Linker et al. 2008; Schmidt et al. 2003a, b). PEG-coated liposomal prednisolone administered intravenously to rats with experimental autoimmune encephalomyelitis (EAE), an animal model of MS, provided a 4.5-fold higher concentration of drug in the spinal cord in comparison to rats without disease (Schmidt et al. 2003a, b). High levels of prednisolone in the CNS were observed only in EAE rats and were accompanied by low levels of free drug in the serum. In other studies Linker et al. (2008) observed that liposomal methylprednisolone and PEG-liposomal prednisolone had superior efficacy to methylprednisolone pulse therapy in EAE rats through second or third relapses. Studies by Hu et al. (2009) demonstrated that PEG-liposomal minocycline, administered every 5 days, was as effective as daily injections of non-formulated minocycline at reducing CNS manifestations in EAE rats. These results suggest that long-acting nanoformulations of glucocorticoids and minocycline can improve pharmacokinetics and drug dosing while reducing unwanted side effects in MS treatment.

Nanoparticle systems that deliver non-traditional therapeutics for MS are also being developed. Therapeutics that aid neuroprotection and neuroregeneration are of

particular interest. A water-soluble fullerene derivative ABS-75 attached to an *N*-methyl-D-aspartate (NMDA) receptor antagonist was designed to contain both antioxidant (fullerene) and anti-excitotoxic (NMDA receptor antagonism) properties (Basso et al. 2008). Fullerene ABS-75 reduced the clinical progression of EAE in a mouse model immunized with myelin oligodendrocyte glycoprotein (MOG). This was accompanied by reduced axonal loss and demyelination and inhibition of oxidative injury, CD11b+ cell infiltration, and CCL2 expression in the spinal cord. Nanoparticles have also been proposed as a way to deliver myelin to demyelinated cells (Dharamkar 2008) and as scaffolds for neuroregeneration (Ellis-Behnke 2007; Silva 2005).

In addition to drug delivery, nanoparticles are being used to aid in the development of stem cell therapies for inducing local neurogenesis or myelogenesis in CNS lesions. Mesenchymal stem cells (MSCs), which can give rise to neural-, glial-, and astrocytic-like cells in vitro, were labeled with the SPIO MRI contrast agent ferumoxides (Feridex) and injected intrathecally and intravenously into MS patients (Karussis et al. 2010). MRI revealed the presence of MSCs in the occipital horns of the ventricles, indicating the possible migration of cells into the meninges, subarachnoid space, and spinal cord. In addition, an increase in CD4+ CD25+ T cells and a decrease in lymphocyte proliferation and expression of CD40+, CD83+, CD86+ and HLA-DR on myeloid dendritic cells indicated immunomodulatory effects of MSC transplantation. Further studies by Cohen et al. (2010) demonstrated that uptake of ferumoxides by neural precursor cells (NPCs) produced no adverse effects on in vitro cell survival or differentiation or in vivo functional response, suggesting that tracking of ferumoxides-labeled NPCs by MRI is a viable noninvasive method for monitoring transplantation.

A hallmark of the immune response in disorders such as MS is a deficit in the numbers of T regulatory (Treg) cells (Buckner 2010; Wing and Sakaguchi 2010). In an effort to enhance Treg numbers, PEG-coated gold nanoparticles were used to co-administer the aryl hydrocarbon receptor activator, 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), and a T-cell epitope from MOG (MOG_{35-55}) to promote the generation of Tregs by dendritic cells (Yeste et al. 2012). When administered to EAE mice, the ITE-$MOG_{35–55}$ nanoparticles expanded the $FoxP3^+$ Treg population and suppressed the development of disease.

5.3 Microbial Infections

5.3.1 Bacterial Meningitis

Pathobiology of Disease

Meningitis is an inflammation of the brain and spinal cord caused by chemical irritation, drug allergies, tumors, fungal infections, and infectious agents (Yogev and Tan 2011). Viral meningitis, most commonly caused by a number of enteroviruses, but also by herpes viruses and West Nile virus, occurs frequently but usually resolves without treatment. Treatment with antivirals is required for herpes virus infections (Stahl et al. 2011a, b).

Bacterial meningitis is more severe and can result in brain damage or even death if not treated. The most frequent causative agents are *Hemophilus influenzae, Neisseria meningitidis* and *Streptococcus pneumoniae*. The only clinically available vaccine is against *H. influenzae* (McIntyre et al. 2012; Schuchat et al. 1997; Yogev and Tan 2011) and has been very effective in eliminating this disease when introduced into a population (Schuchat et al. 1997; Yogev and Tan 2011). Effective antibiotics are available, although the mortality rate in the very young and the elderly remains high. However, the increasing development of antibiotic-resistant organisms is of great concern. The pathogenesis of bacterial meningitis involves the infiltration of bacteria into the subarachnoid space. Bacterial degradation products stimulate the release of pro-inflammatory cytokines, such as TNFα and IL-1β, and prostaglandins by leukocytes, microglial cells, astrocytes, and endothelial cells, leading to increased BBB permeability. Infiltration of neutrophils and leakage of plasma proteins produces further damage (de Vries et al. 1997). Antibiotics can halt bacterial replication but are not effective alone in reducing the inflammatory events that contribute to brain damage and long-term disability (Borchorst and Moller 2012; de Vries et al. 1997; van de Beek et al. 2012). Adjunctive therapy with corticosteroids to reduce inflammation is recommended, but the incidence of detrimental side effects is high (Borchorst and Moller 2012). Thus, there is a need for new therapeutics that can penetrate the BBB and deliver traditional and novel antibiotics and adjuncts to the site of infection and inflammation while reducing the incidence and severity of side effects.

Complicating effective treatment of the disease are the time-consuming methods used for diagnosis, which can delay initiation of therapy and contribute to morbidity and mortality. Current diagnostic methods use conventional microscopy and bacterial culture of blood and cerebrospinal fluid followed by confirmation and serotype identification by polymerase chain reaction (PCR) (Bronska et al. 2006; Reddy et al. 2012; Rosenstein et al. 2001; Tunkel et al. 2004). New methods to speed diagnosis and expedite drug therapy are sorely needed.

Nanoparticle-Based Therapies

A number of experimental nanoparticle-based systems for diagnosis and treatment of infectious meningitis have been described. A recent study by Reddy et al. (2012) described the development of an acoustic wave immunosensor for meningococcal antigen that is enhanced using gold nanoparticles. Cell surface outer membrane protein 85 (OMP85) of *N. meningitidis* was bound to gold nanoparticles and quantitated by interaction with antibody immobilized on the surface of a polyvinylidene fluoride–coated quartz crystal microbalance. Binding of OMP85 to the gold nanoparticles allowed detection of as little as 312 ng/ml OMP85 and would provide detection of antigen in blood or CSF with minimal sample preparation.

Development and efficacy of self-assembled cationic antimicrobial peptide nanoparticles has been described (Liu et al. 2009c; Wang et al. 2010) for treatment of *Staphylococcus aureus* and *Cryptococcus neoformans* meningitis in rabbits. Cholesterol-conjugated G_3R_6TAT (CG_3R_6TAT) self-assembled in deionized

water into cationic core/shell nanoparticles with TAT-anchored shells. The nanoparticles crossed the BBB and were equally as effective as vancomycin and amphotericin B in treating *S. aureus* and *C. neoformans* meningitis, respectively (Liu et al. 2009c; Wang et al. 2010). In addition, they did not impair liver and kidney functions or induce blood electrolyte imbalances. Of critical importance antimicrobial peptide nanoparticles show promise for treating drug-resistant bacterial, yeast, and fungal meningitis infections. Recently, a different nanoparticle-based approach to combat development of drug-resistant *S. aureus* was described by Chakraborty et al. (2012). Folic acid–tagged chitosan nanoparticles were used to effectively deliver vancomycin into a drug-resistant strain of *S. aureus* in an in vitro study and promote bacterial lysis. Using the folic acid–conjugated chitosan nanocarrier provided an effective way of delivering vancomycin across the thicker cell wall in vancomycin-resistant bacterial strains (Chakraborty et al. 2012).

Adjunctive nanoparticle-based therapies have also been described. Tsao et al. (1999) demonstrated that *Escherichia coli*–induced meningitis could be inhibited by carboxyfullerene (C60). Treatment of *E. coli*-infected mice with a water-soluble malonic acid derivative of C60 reduced mortality, decreased levels of TNFα and IL-1β in the brain, and inhibited increases in BBB permeability and neutrophil infiltration (Tsao et al. 1999).

5.3.2 Human Immunodeficiency Virus

Pathobiology of Disease

HIV-associated neurocognitive disorders (HAND) comprise a spectrum of disease states from clinically asymptomatic to overt cognitive and behavioral impairments to full-blown dementia (HIVE). HIVE is fueled by immune activation of CNS cells and virotoxins, including viral proteins, cytokines, and chemokines that are secreted by infected brain macrophages and microglia (Kaul 2009; Shapshak et al. 2011). Following HIV infection, the resulting early inflammation and secreted viral proteins induce a breach in the BBB integrity, allowing HIV and infected mononuclear phagocytes to invade the CNS. Virus and toxic products secreted by infected brain mononuclear phagocytes and glial cells induce neuroinflammation, neurodegeneration, and encephalopathy. The neuropathology of HIVE is characterized by neuronal loss, glial activation, presence of multinucleated giant cells, perivascular mononuclear infiltration, myelopathy, and myelin pallor. In addition to neuronal loss, aberrant sprouting, and dystrophic synaptodendritic connections, HIVE is also characterized by extensive damage to microvessels of the blood-brain barrier caused by infiltration of HIV-infected cells and inflammatory cells into the brain (Gelman et al. 2006, 2012).

The mechanism by which HIV infection leads to neuroinflammation and encephalitis is multifactorial and has been shown to involve direct viral infection of brain mononuclear phagocytes and dysfunction and injury of brain endothelial cells, astrocytes, oligodendrocytes, and neurons, induced by HIV itself and secreted

viral factors (Byrd et al. 2012; Noorbakhsh et al. 2005; Pacifici et al. 2013; Silva et al. 2012). HIVE generally occurs in the late stages of HIV infection and is associated with low levels of CD4+ T cells and high plasma viral loads. It involves continued CNS inflammation and neuronal and astrocyte injury induced by the virus, secreted viral proteins, and secreted cytokines and chemokines (Pacifici et al. 2013).

Current Treatments

With the advent of effective combination antiretroviral therapy (cART) (Table 1) and the longer survival of infected patients, the pathogenesis of HIVE has been transformed from a subacute, neuroinflammatory disorder with florid neurological alterations, to a chronic and protracted condition. Advances in ART drugs and treatment regimens have improved virological control and greatly increased the life expectancy of infected individuals. However, cART has not eradicated virus from protected sites, such as the brain and lymphoid tissue, nor has it eliminated the incidence of milder forms of HAND (Valcour et al. 2011). With the need for continued life-long daily doses of cART, occurrence of adverse side effects, and lack of adherence to treatment regimens, the development of resistant strains has become a cause for concern (Chen et al. 2002; Chulamokha et al. 2005; Fellay et al. 2001; Hawkins 2006; Shehu-Xhilaga et al. 2005). Thus, there is need for novel adjunctive treatments that address HIV-associated neurological deficits and improved antiretroviral therapies to reduce the incidence of side effects and simplify dosing regimens (Mahajan et al. 2012; Nowacek and Gendelman 2009).

Nanoparticle-Based Experimental Systems

Various nanoparticle-based systems have been explored for improving antiretroviral therapy. Efavirenz (EFV)-poloxamine/poloxamer polymeric micelles were synthesized as nanocarriers via oral administration, and displayed promising physical stability and pharmacokinetics (Seremeta et al. 2013). Intranasal administration of poly(ethylene oxide)-poly(propylene oxide) polymeric micelles loaded with EFV enhanced bioavailability of the drug in the CNS fourfold and increased the relative exposure index (ratio between the area under the curve in the CNS and plasma) fivefold with respect to the same system administered intravenously (Chiappetta et al. 2013). Polybutylcyanoacrylate and methylmethacrylate-sulfopropymethacrylate nanoparticles have been reported to increase delivery of ART drugs to the CNS (Mulik et al. 2012). The disadvantages of polymeric nanoparticles include residual contamination, polymer initiation, toxic monomers, and stability issues. Shibata et al. (2013) produced biodegradable combination antiretroviral PLGA nanoparticles containing EFV and lopinavir by high-pressure homogenization. These nanoparticles also efficiently inhibited in vitro HIV-1 infection and transduction. Tenofovir, a nucleotide analog reverse transcriptase inhibitor, was embedded in PLGA and chitosan nanocarriers and demonstrated sustained in vitro release, suggesting that these particles could be effective and attractive drug carriers (Belletti et al. 2012).

Table 1 FDA-approved antiretroviral (ARV) drugs for HIV-1 treatment

ARV class	ARV agent	Abbreviation	Recommended as initial therapy
Non-nucleoside reverse transcriptase inhibitors			
	Efavirenz	EFV	Yes
	Nevirapine	NVP	Yes
	Rilpivirine	RPV	Yes
	Delavirdine	DLV	No
	Etravirine	ETR	No
Nucleoside analog reverse transcriptase inhibitors			
	Emtricitabine	FTC	Yes
	Lamivudine	3TC	Yes
	Abacavir	ABC	Yes
	Zidovudine	ZDV	Yes
	Didanosine	ddI	No
	Stavudine	d4T	No
	Apricitabine	ATC	Phase III trials
Nucleotide analog reverse transcriptase inhibitor			
	Tenofovir disoproxil fumarate	TDF	Yes
Protease inhibitors			
	Atazanavir	ATV	Yes
	Darunavir	DRV	Yes
	Fosamprenavir	FPV	Yes
	Lopinavir	LPV	Yes
	Saquinavir	SQV	Yes
	Ritonavir	RTV	Yes (as boost)
	Indinavir	IDV	No
	Nelfinavir	NFV	No
	Tipranavir	TPV	No
Integrase strand transfer inhibitors			
	Elvitegravir	EVG	Yes
	Raltegravir	RAL	Yes
CCR5 antagonist			
	Maraviroc	MRV	Yes
Fusion inhibitor			
	Enfuvirtide	T20	No
CYP3A4 inhibitor			
	Cobicistat	COBI	Yes (as boost)

Cell-Based Nanoparticle Delivery for HIV-1 Treatment

Use of macrophages as Trojan horses to improve ART dosing by acting as drug reservoirs and providing delivery to sites of residual viral infection is being developed in our own laboratories (Balkundi et al. 2011; Nowacek et al. 2009b, 2010, 2011). We have developed polymer-coated crystalline nanoformulations of antiretrovirals (nanoART) that can be taken up by macrophages and remain inside the cells for an extended period of time (Dou et al. 2009; Nowacek et al. 2011). These formulations were manufactured by high-pressure homogenization or wet milling of crystalline drug using various surfactants including poloxamer 188 (P188), poloxamer 407 (P407), 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethyleneglycol)-2000] (DSPE-mPEG), PVA, and PLGA. NanoART was efficiently and rapidly taken up by human monocyte–derived macrophages (MDM) in culture. Drug (atazanavir (ATV), ritonavir (RTV), or EFV) was retained inside the cells for up to 14 days and inhibited HIV-1 replication in macrophages in a dose-dependent manner (Balkundi et al. 2011; Nowacek et al. 2009b, 2010, 2011). NanoART-loaded macrophages could deliver drug to sites of HIV-1 infection in the brain in an animal model of HIVE. Importantly, nanoART (P188-ATV/RTV) administered subcutaneously at weekly doses (250 mg/kg each drug) provided sustained plasma and tissue ATV levels above the minimal effective concentration, suppressed viral replication in spleen and brain, and maintained CD4+ T-cell numbers in a humanized mouse model of chronic HIV-1 infection (Dash et al. 2012). These studies are an important step in the development of a cell-based delivery system for ART drugs (Balkundi et al. 2010; Nowacek et al. 2009b).

Our group has also developed nanoART targeted to macrophage receptors. Decorating nanoART with folic acid (to target macrophage folate receptors) elicited a >2-fold concentration-dependent enhancement of macrophage uptake and retention in vitro when compared to replicate non-decorated nanoART (Puligujja et al. 2013). Antiretroviral activity, measured by inhibition of HIV reverse transcriptase activity and HIV-1p24 staining, was also enhanced twofold. These activities paralleled greater nanoART retention in recycling endosomes, providing a stable cell-based drug depot. We also observed that by actively targeting the nanoART to macrophages through folate modification, plasma and tissue ATV levels could be maintained at levels above the minimum effective concentration after a single intramuscular injection (50 mg/kg) in mice (Puligujja et al. 2013).

We are also developing a novel nanoparticle system to provide a noninvasive assessment of ART tissue distribution by MRI. Small magnetite ART (SMART) particles were manufactured by encasing ATV and SPIO (magnetite) in PLGA, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, and DSPE-mPEG. SMART particles were efficiently taken up by MDM in culture and retained in the cells for up to 15 days. The levels of drug and magnetite in the cells mirrored the drug/magnetite ratio of the SMART particles (Guo et al. 2013). As shown in Fig. 4, parenteral administration of SMART particles to mice demonstrated that

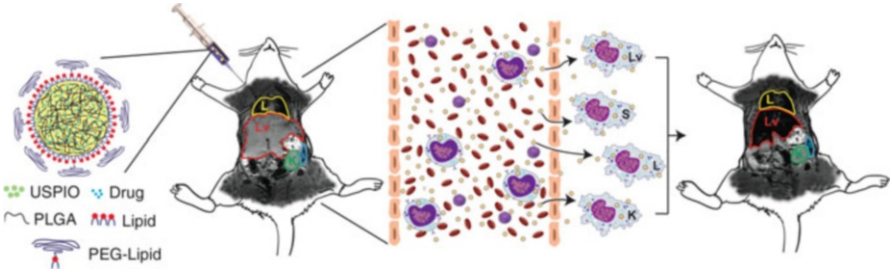

Fig. 4 Small magnetitie-containing antiretroviral (SMART) nanoparticles can be used to assess drug biodistribution. SMART particles, parentally administered to mice, are taken up by circulating monocytes/macrophages and accumulate in tissues, including liver (Lv), spleen (S), lungs (L), and kidneys (K). Magnetic resonance imaging (MRI) is used to monitor biodistribution of the magnetite-/drug-containing SMART particles (Figure reprinted with permission from Guo et al. (2013))

magnetite and drug biodistribution paralleled one another. SMART nanoparticles could facilitate the evaluation of drug biodistribution and provide rapid in vivo assessment of nanoparticles that target cell and tissue viral reservoirs. These findings can serve to improve and speed pharmacokinetic parameters in nanoparticle-based systems and to bring nanotechnology closer to human clinical application.

6 Nanotoxicology

Most neuroinflammatory nanomedicine studies have focused on drug delivery of nanoparticles, but the consequent adverse effects or toxicity issues of the nanocarriers cannot be ignored. Toxicity depends on various parameters of nanoparticles including morphology, size, charge, surface area, material solubility, dose, route, and duration of administration (Kanwar et al. 2012b; Liu et al. 2012). Smaller nanoparticles exhibit superior translocation kinetics; however, their smaller size could result in toxicological effects caused by the trade-off between drug potency and immunologic surveillance (Beyerle et al. 2010; Bhaskar et al. 2010; Chen and Schluesener 2008; Tian et al. 2006). The physicochemical properties of nanosized materials differ with respect to their larger counterparts, increasing the potential for toxic interactions (Nel et al. 2006, 2009). Nonbiological nanoparticulate carriers larger than 200 nm can cause cell membrane ruffling, cytoskeletal rearrangement and stimulate endocytic machinery, promoting uptake by phagocytic cells (Harding and Song 1994; Paulo et al. 2011; Pichai and Ferguson 2012). Furthermore, some studies show that nanoparticles can be pro-oxidant and pro-inflammatory (Stoeger et al. 2006, 2009). Certain types of nanoparticles may stimulate the release of cytokines and chemokines such as IL-6, IL-1β, TNFα, reactive oxygen species, and C-reactive

protein with subsequent activation of mitogen-activated protein kinase (MAPK), redox-activated transcription factors, the transcription factor nuclear factor kappa B (NFkB), and activating protein-1 (AP-1). Long-term exposure to nanoparticles may thus result in chronic inflammation that could accelerate atherosclerosis (Fernandes et al. 2010). Data are limited, however, on the potential toxicities of long-term nanoparticle exposure and possible neurological consequences of these drug-delivery systems (Bhaskar et al. 2010; Kanwar et al. 2012b).

Although nanomedicines for treatment of neuroinflammation have shown promise, further studies on the safety of these systems are needed. To reduce the potential for adverse effects, a couple of different strategies are being used in the development of nanoparticle drug carrier systems. Novel biodegradable or biocompatible polymers are being used in the preparation of nanoparticles to reduce their toxicity and allow them to escape the reticuloendothelial system, as well as to aid drug release. Biocompatible polymers such as polylactic acid, polybutylcyanoacrylate, and polycaprolactone are being used in the preparation of nanomedicines to decrease toxicity (Kanwar et al. 2012b; Nel et al. 2006). In addition, surface functional modification of nanomedicines to actively target nanoparticles to sites of disease is being increasingly used to decrease nonspecific interactions and toxicities (Koo et al. 2005; McMillan et al. 2011).

7 Conclusion

Treatments of neurodegenerative and neuroinflammatory disorders require drugs acting on the CNS. Thus, development of novel nanoparticle systems for drug delivery across the BBB is a promising and urgent industry aimed at improving disease outcomes (Bhaskar et al. 2010; Fernandes et al. 2010; Kanwar et al. 2012a, b; Re et al. 2012b). Nanoparticles may be employed as drug carrier systems that assist in therapeutic targeting to the CNS. Multifunctional nanoparticles allow delivery of pharmaceutical agents into the brain and provide for neuroimaging of cell dynamics and tracking of nanoparticle delivery. Such nanocarriers and/or their combination with other drugs will drive the search for targeting specific areas in the brain and thus enhance therapies. Nanomedicine has yet to make its mark in clinical studies; however, nanoparticle-based drugs are gaining considerable interest in the pharmaceutical industry. The translation of nanoparticles for clinical use in therapeutic and diagnostic applications is promising amid recent developments. However, even though nanoparticle-based diagnostic and therapeutic agents for neuroinflammatory diseases have shown great potential, there continues to be concerns about the safety of these particles. Thus, it is also necessary to gain a better understanding of the potential neurotoxicity associated with nanoparticle-based therapies.

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References

ADI. (2012). World Alzheimer report 2012. Overcoming the stigma of dementia. In: *Alzeimers Disease International* http://www.alz.org/documents_custom/world_report_2012_final.pdf. Accessed June 13, 2013.

Alam, M. I., Beg, S., Samad, A., Baboota, S., Kohli, K., Ali, J., Ahuja, A., & Akbar, M. (2010). Strategy for effective brain drug delivery. *European Journal of Pharmaceutical Sciences, 40*, 385–403.

Ali, S. S., Hardt, J. I., & Dugan, L. L. (2008). SOD activity of carboxyfullerenes predicts their neuroprotective efficacy: A structure-activity study. *Nanomedicine, 4*, 283–294.

Amedei, A., Prisco, D., & D'Elios, M. M. (2012). Multiple sclerosis: The role of cytokines in pathogenesis and in therapies. *International Journal of Molecular Sciences, 13*, 13438–13460.

An, Y., Tang, L., Jiang, X., Chen, H., Yang, M., Jin, L., Zhang, S., Wang, C., & Zhang, W. (2010). A photoelectrochemical immunosensor based on Au-doped TiO2 nanotube arrays for the detection of alpha-synuclein. *Chemistry, 16*, 14439–14446.

Androdias, G., Reynolds, R., Chanal, M., Ritleng, C., Confavreux, C., & Nataf, S. (2010). Meningeal T cells associate with diffuse axonal loss in multiple sclerosis spinal cords. *Annals of Neurology, 68*, 465–476.

Balkundi, S., Nowacek, A. S., Roy, U., Martinez-Skinner, A., McMillan, J., & Gendelman, H. E. (2010). Methods development for blood borne macrophage carriage of nanoformulated antiretroviral drugs. *Journal of Visualized Experiments* Dec 9; (46).

Balkundi, S., Nowacek, A. S., Veerubhotla, R. S., Chen, H., Martinez-Skinner, A., Roy, U., Mosley, R. L., Kanmogne, G., Liu, X., Kabanov, A. V., Bronich, T., McMillan, J., & Gendelman, H. E. (2011). Comparative manufacture and cell-based delivery of antiretroviral nanoformulations. *International Journal of Nanomedicine, 6*, 3393–3404.

Basso, A. S., Frenkel, D., Quintana, F. J., Costa-Pinto, F. A., Petrovic-Stojkovic, S., Puckett, L., Monsonego, A., Bar-Shir, A., Engel, Y., Gozin, M., & Weiner, H. L. (2008). Reversal of axonal loss and disability in a mouse model of progressive multiple sclerosis. *Journal of Clinical Investigation, 118*, 1532–1543.

Bataveljic, D., Stamenkovic, S., Bacic, G., & Andjus, P. R. (2011). Imaging cellular markers of neuroinflammation in the brain of the rat model of amyotrophic lateral sclerosis. *Acta Physiologica Hungarica, 98*, 27–31.

Batrakova, E. V., Li, S., Reynolds, A. D., Mosley, R. L., Bronich, T. K., Kabanov, A. V., & Gendelman, H. E. (2007). A macrophage-nanozyme delivery system for Parkinson's disease. *Bioconjugate Chemistry, 18*, 1498–1506.

Batrakova, E. V., Gendelman, H. E., & Kabanov, A. V. (2011). Cell-mediated drug delivery. *Expert Opinion on Drug Delivery, 8*, 415–433.

Belletti, D., Tosi, G., Forni, F., Gamberini, M. C., Baraldi, C., Vandelli, M. A., & Ruozi, B. (2012). Chemico-physical investigation of tenofovir loaded polymeric nanoparticles. *International Journal of Pharmaceutics, 436*, 753–763.

Benoit, M., Desnues, B., & Mege, J. L. (2008). Macrophage polarization in bacterial infections. *Journal of Immunology, 181*, 3733–3739.

Bernardi, A., Braganhol, E., Jager, E., Figueiro, F., Edelweiss, M. I., Pohlmann, A. R., Guterres, S. S., & Battastini, A. M. (2009). Indomethacin-loaded nanocapsules treatment reduces in vivo glioblastoma growth in a rat glioma model. *Cancer Letters, 281*, 53–63.

Beyerle, A., Merkel, O., Stoeger, T., & Kissel, T. (2010). PEGylation affects cytotoxicity and cell-compatibility of poly(ethylene imine) for lung application: Structure-function relationships. *Toxicology and Applied Pharmacology, 242*, 146–154.

Bhaskar, S., Tian, F., Stoeger, T., Kreyling, W., de la Fuente, J. M., Grazu, V., Borm, P., Estrada, G., Ntziachristos, V., & Razansky, D. (2010). Multifunctional nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: Perspectives on tracking and neuroimaging. *Particle and Fibre Toxicology, 7*, 3.

Bo, L., Vedeler, C. A., Nyland, H. I., Trapp, B. D., & Mork, S. J. (2003). Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *Journal of Neuropathology and Experimental Neurology, 62*, 723–732.

Boas, U., & Heegaard, P. M. (2004). Dendrimers in drug research. *Chemical Society Reviews, 33*, 43–63.

Bondi, M. L., Craparo, E. F., Giammona, G., & Drago, F. (2010). Brain-targeted solid lipid nanoparticles containing riluzole: Preparation, characterization and biodistribution. *Nanomedicine (London, England), 5*, 25–32.

Borchorst, S., & Moller, K. (2012). The role of dexamethasone in the treatment of bacterial meningitis – A systematic review. *Acta Anaesthesiologica Scandinavica, 56*, 1210–1221.

Brabers, N. A., & Nottet, H. S. (2006). Role of the pro-inflammatory cytokines TNF-alpha and IL-1beta in HIV-associated dementia. *European Journal of Clinical Investigation, 36*, 447–458.

Bronska, E., Kalmusova, J., Dzupova, O., Maresova, V., Kriz, P., & Benes, J. (2006). Dynamics of PCR-based diagnosis in patients with invasive meningococcal disease. *Clinical Microbiology and Infection, 12*, 137–141.

Brown, R. C., Lockwood, A. H., & Sonawane, B. R. (2005). Neurodegenerative diseases: An overview of environmental risk factors. *Environmental Health Perspectives, 113*, 1250–1256.

Brynskikh, A. M., Zhao, Y., Mosley, R. L., Li, S., Boska, M. D., Klyachko, N. L., Kabanov, A. V., Gendelman, H. E., & Batrakova, E. V. (2010). Macrophage delivery of therapeutic nanozymes in a murine model of Parkinson's disease. *Nanomedicine (London, England), 5*, 379–396.

Buckner, J. H. (2010). Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. *Nature Reviews Immunology, 10*, 849–859.

Byrd, D., Murray, J., Safdieh, G., & Morgello, S. (2012). Impact of opiate addiction on neuroinflammation in HIV. *Journal of Neurovirology, 18*, 364–373.

Chakraborty, S. P., Sahu, S. K., Pramanik, P., & Roy, S. (2012). In vitro antimicrobial activity of nanoconjugated vancomycin against drug resistant *Staphylococcus aureus*. *International Journal of Pharmaceutics, 436*, 659–676.

Checkoway, H., Lundin, J. I,. Kelada, S. N. (2011). Neurodegenerative diseases. *IARC Scientific Publications*, (163), 407–419.

Chen, X., & Schluesener, H. J. (2008). Nanosilver: A nanoproduct in medical application. *Toxicology Letters, 176*, 1–12.

Chen, R. Y., Westfall, A. O., Raper, J. L., Cloud, G. A., Chatham, A. K., Acosta, E. P., Pham, S. V., Tolson, J. M., Heudebert, G. R., & Saag, M. S. (2002). Immunologic and virologic consequences of temporary antiretroviral treatment interruption in clinical practice. *AIDS Research and Human Retroviruses, 18*, 909–916.

Chiappetta, D. A., Hocht, C., Opezzo, J. A., & Sosnik, A. (2013). Intranasal administration of antiretroviral-loaded micelles for anatomical targeting to the brain in HIV. *Nanomedicine (London, England), 8*, 223–237.

Chu, D., Tian, J., Liu, W., Li, Z., & Li, Y. (2007). Poly(lactic-co-glycolic acid) microspheres for the controlled release of huperzine A: In vitro and in vivo studies and the application in the treatment of the impaired memory of mice. *Chemical & Pharmaceutical Bulletin (Tokyo), 55*, 625–628.

Chulamokha, L., DeSimone, J. A., & Pomerantz, R. J. (2005). Antiretroviral therapy in the developing world. *Journal of Neurovirology, 11*(Suppl 1), 76–80.

Cohen, M. E., Muja, N., Fainstein, N., Bulte, J. W., & Ben-Hur, T. (2010). Conserved fate and function of ferumoxides-labeled neural precursor cells in vitro and in vivo. *Journal of Neuroscience Research, 88*, 936–944.

Cui, Z., Lockman, P. R., Atwood, C. S., Hsu, C. H., Gupte, A., Allen, D. D., & Mumper, R. J. (2005). Novel D-penicillamine carrying nanoparticles for metal chelation therapy in Alzheimer's and other CNS diseases. *European Journal of Pharmaceutics and Biopharmaceutics, 59*, 263–272.

Dash, P. K., Gendelman, H. E., Roy, U., Balkundi, S., Alnouti, Y., Mosley, R. L., Gelbard, H. A., McMillan, J., Gorantla, S., & Poluektova, L. Y. (2012). Long-acting nanoformulated

antiretroviral therapy elicits potent antiretroviral and neuroprotective responses in HIV-1-infected humanized mice. *AIDS, 26*, 2135–2144.

de Carvalho, M., Dengler, R., Eisen, A., England, J. D., Kaji, R., Kimura, J., Mills, K., Mitsumoto, H., Nodera, H., Shefner, J., & Swash, M. (2008). Electrodiagnostic criteria for diagnosis of ALS. *Clinical Neurophysiology, 119*, 497–503.

de Vries, H. E., Kuiper, J., de Boer, A. G., Van Berkel, T. J., & Breimer, D. D. (1997). The blood-brain barrier in neuroinflammatory diseases. *Pharmacological Reviews, 49*, 143–155.

de Vries, H. E., Kooij, G., Frenkel, D., Georgopoulos, S., Monsonego, A., & Janigro, D. (2012). Inflammatory events at blood-brain barrier in neuroinflammatory and neurodegenerative disorders: Implications for clinical disease. *Epilepsia, 53*(Suppl 6), 45–52.

Devonshire, V. A., & Verdun di Cantogno, E. (2011). Review of subcutaneous interferon beta-1a, delivered via the electronic self-injection device RebiSmart, for the treatment of multiple sclerosis. *Therapeutic Delivery, 2*, 1455–1465.

Dharamkar, A. K. (2008). Nanodelivery of myelin: A therapeutic option for multiple sclerosis. *Medical Hypotheses, 71*, 318–319.

Dou, H., Grotepas, C. B., McMillan, J. M., Destache, C. J., Chaubal, M., Werling, J., Kipp, J., Rabinow, B., & Gendelman, H. E. (2009). Macrophage delivery of nanoformulated antiretroviral drug to the brain in a murine model of neuro AIDS. *Journal of Immunology, 183*, 661–669.

Ellis-Behnke, R. (2007). Nano neurology and the four P's of central nervous system regeneration: Preserve, permit, promote, plasticity. *The Medical Clinics of North America, 91*, 937–962.

Elman, L. B., Houghton, D. J., Wu, G. F., Hurtig, H. I., Markowitz, C. E., & McCluskey, L. (2007). Palliative care in amyotrophic lateral sclerosis, Parkinson's disease, and multiple sclerosis. *Journal of Palliative Medicine, 10*, 433–457.

Esposito, E., Fantin, M., Marti, M., Drechsler, M., Paccamiccio, L., Mariani, P., Sivieri, E., Lain, F., Menegatti, E., Morari, M., & Cortesi, R. (2008). Solid lipid nanoparticles as delivery systems for bromocriptine. *Pharmaceutical Research, 25*, 1521–1530.

Fellay, J., Boubaker, K., Ledergerber, B., Bernasconi, E., Furrer, H., Battegay, M., Hirschel, B., Vernazza, P., Francioli, P., Greub, G., Flepp, M., & Telenti, A. (2001). Prevalence of adverse events associated with potent antiretroviral treatment: Swiss HIV Cohort Study. *Lancet, 358*, 1322–1327.

Fernandes, C., Soni, U., & Patravale, V. (2010). Nano-interventions for neurodegenerative disorders. *Pharmacological Research, 62*, 166–178.

Frank-Cannon, T. C., Alto, L. T., McAlpine, F. E., & Tansey, M. G. (2009). Does neuroinflammation fan the flame in neurodegenerative diseases? *Molecular Neurodegeneration, 4*, 47.

Gasperini, C., & Ruggieri, S. (2012). Development of oral agent in the treatment of multiple sclerosis: How the first available oral therapy, fingolimod will change therapeutic paradigm approach. *Drug Design, Development and Therapy, 6*, 175–186.

Gaucher, G., Satturwar, P., Jones, M. C., Furtos, A., & Leroux, J. C. (2010). Polymeric micelles for oral drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics, 76*, 147–158.

Gautam, N., Roy, U., Balkundi, S., Puligujja, P., Guo, D., Smith, N., Liu, X. M., Lamberty, B., Morsey, B., Fox, H. S., McMillan, J., Gendelman, H. E., & Alnouti, Y. (2013). Preclinical pharmacokinetics and tissue distribution of long-acting nanoformulated antiretroviral therapy. *Antimicrob Agents Chemother, 57*, 3110–3120.

Gelman, B. B., Spencer, J. A., Holzer, C. E., 3rd, & Soukup, V. M. (2006). Abnormal striatal dopaminergic synapses in National Neuro AIDS Tissue Consortium subjects with HIV encephalitis. *Journal of Neuroimmune Pharmacology, 1*, 410–420.

Gelman, B. B., Lisinicchia, J. G., Chen, T., Johnson, K. M., Jennings, K., Freeman, D. H., Jr., & Soukup, V. M. (2012). Prefrontal dopaminergic and enkephalinergic synaptic accommodation in HIV-associated neurocognitive disorders and encephalitis. *Journal of Neuroimmune Pharmacology, 7*, 686–700.

Georganopoulou, D. G., Chang, L., Nam, J. M., Thaxton, C. S., Mufson, E. J., Klein, W. L., & Mirkin, C. A. (2005). Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 2273–2276.

Goldenberg, M. M. (2012). Multiple sclerosis review. *PT, 37*, 175–184.

Graves, D., & Vernino, S. (2012). Immunotherapies in neurologic disorders. *The Medical Clinics of North America, 96*, 497–523, x.

Guo, D., Li, T., McMillan, J., Sajja, B. S., Puligujja, P., Boska, M. D., Gendelman, H. E., & Liu, X. (2013). Small magnetite antiretroviral therapeutic "SMART" nanoparticle probes for magnetic resonance imaging of drug biodistribution. *Nanomedicine* (In Press). PMID: 23905578.

Gupta, U., & Jain, N. K. (2010). Non-polymeric nano-carriers in HIV/AIDS drug delivery and targeting. *Advanced Drug Delivery Reviews, 62*, 478–490.

Harding, C. V., & Song, R. (1994). Phagocytic processing of exogenous particulate antigens by macrophages for presentation by class I MHC molecules. *Journal of Immunology, 153*, 4925–4933.

Hawkins, T. (2006). Appearance-related side effects of HIV-1 treatment. *AIDS Patient Care and STDs, 20*, 6–18.

Hu, W., Metselaar, J., Ben, L. H., Cravens, P. D., Singh, M. P., Frohman, E. M., Eagar, T. N., Racke, M. K., Kieseier, B. C., & Stuve, O. (2009). PEG minocycline-liposomes ameliorate CNS autoimmune disease. *PLoS One, 4*, e4151.

Jack, C. R., Jr., Albert, M. S., Knopman, D. S., McKhann, G. M., Sperling, R. A., Carrillo, M. C., Thies, B., & Phelps, C. H. (2011). Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia, 7*, 257–262.

Kadiu, I., & Gendelman, H. E. (2011). Human immunodeficiency virus type 1 endocytic trafficking through macrophage bridging conduits facilitates spread of infection. *Journal of Neuroimmune Pharmacology, 6*, 658–675.

Kadiu, I., Nowacek, A., McMillan, J., & Gendelman, H. E. (2011). Macrophage endocytic trafficking of antiretroviral nanoparticles. *Nanomedicine (London, England), 6*, 975–994.

Kanwar, J. R., Sriramoju, B., & Kanwar, R. K. (2012a). Neurological disorders and therapeutics targeted to surmount the blood-brain barrier. *International Journal of Nanomedicine, 7*, 3259–3278.

Kanwar, J. R., Sun, X., Punj, V., Sriramoju, B., Mohan, R. R., Zhou, S. F., Chauhan, A., & Kanwar, R. K. (2012b). Nanoparticles in the treatment and diagnosis of neurological disorders: Untamed dragon with fire power to heal. *Nanomedicine, 8*, 399–414.

Karussis, D., Karageorgiou, C., Vaknin-Dembinsky, A., Gowda-Kurkalli, B., Gomori, J. M., Kassis, I., Bulte, J. W., Petrou, P., Ben-Hur, T., Abramsky, O., & Slavin, S. (2010). Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. *Archives of Neurology, 67*, 1187–1194.

Kaul, M. (2009). HIV-1 associated dementia: Update on pathological mechanisms and therapeutic approaches. *Current Opinion in Neurology, 22*, 315–320.

Kedar, U., Phutane, P., Shidhaye, S., & Kadam, V. (2010). Advances in polymeric micelles for drug delivery and tumor targeting. *Nanomedicine, 6*, 714–729.

Khalil, N. M., Carraro, E., Cotica, L. F., & Mainardes, R. M. (2011). Potential of polymeric nanoparticles in AIDS treatment and prevention. *Expert Opinion on Drug Delivery, 8*, 95–112.

Koo, O. M., Rubinstein, I., & Onyuksel, H. (2005). Role of nanotechnology in targeted drug delivery and imaging: A concise review. *Nanomedicine, 1*, 193–212.

Kutzelnigg, A., Lucchinetti, C. F., Stadelmann, C., Bruck, W., Rauschka, H., Bergmann, M., Schmidbauer, M., Parisi, J. E., & Lassmann, H. (2005). Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain, 128*, 2705–2712.

Lassmann, H., & van Horssen, J. (2011). The molecular basis of neurodegeneration in multiple sclerosis. *FEBS Letters, 585*, 3715–3723.

Li, T. M., Swash, M., Alberman, E., & Day, S. J. (1991). Diagnosis of motor neuron disease by neurologists: A study in three countries. *Journal of Neurology, Neurosurgery, and Psychiatry, 54*, 980–983.

Linker, R. A., Weller, C., Luhder, F., Mohr, A., Schmidt, J., Knauth, M., Metselaar, J. M., & Gold, R. (2008). Liposomal glucocorticosteroids in treatment of chronic autoimmune demyelination: Long-term protective effects and enhanced efficacy of methylprednisolone formulations. *Experimental Neurology, 211*, 397–406.

Liu, G., Men, P., Kudo, W., Perry, G., & Smith, M. A. (2009a). Nanoparticle-chelator conjugates as inhibitors of amyloid-beta aggregation and neurotoxicity: A novel therapeutic approach for Alzheimer disease. *Neuroscience Letters, 455*, 187–190.

Liu, G., Men, P., Perry, G., & Smith, M. A. (2009b). Chapter 5 – Development of iron chelator-nanoparticle conjugates as potential therapeutic agents for Alzheimer disease. *Progress in Brain Research, 180*, 97–108.

Liu, L., Xu, K., Wang, H., Tan, P. K., Fan, W., Venkatraman, S. S., Li, L., & Yang, Y. Y. (2009c). Self-assembled cationic peptide nanoparticles as an efficient antimicrobial agent. *Nature Nanotechnology, 4*, 457–463.

Liu, Y., Pan, J., & Feng, S. S. (2010). Nanoparticles of lipid monolayer shell and biodegradable polymer core for controlled release of paclitaxel: Effects of surfactants on particles size, characteristics and in vitro performance. *International Journal of Pharmaceutics, 395*, 243–250.

Liu, Y., Tan, J., Thomas, A., Ou-Yang, D., & Muzykantov, V. R. (2012). The shape of things to come: Importance of design in nanotechnology for drug delivery. *Therapeutic Delivery, 3*, 181–194.

Lyck, R., & Engelhardt, B. (2012). Going against the tide–how encephalitogenic T cells breach the blood-brain barrier. *Journal of Vascular Research, 49*, 497–509.

Machtoub, L., Bataveljic, D., & Andjus, P. R. (2011). Molecular imaging of brain lipid environment of lymphocytes in amyotrophic lateral sclerosis using magnetic resonance imaging and SECARS microscopy. *Physiological Research, 60*(Suppl 1), S121–S127.

Mahajan, S. D., Aalinkeel, R., Law, W. C., Reynolds, J. L., Nair, B. B., Sykes, D. E., Yong, K. T., Roy, I., Prasad, P. N., & Schwartz, S. A. (2012). Anti-HIV-1 nanotherapeutics: Promises and challenges for the future. *International Journal of Nanomedicine, 7*, 5301–5314.

Mallipeddi, R., & Rohan, L. C. (2010). Progress in antiretroviral drug delivery using nanotechnology. *International Journal of Nanomedicine, 5*, 533–547.

Manickam, D. S., Brynskikh, A. M., Kopanic, J. L., Sorgen, P. L., Klyachko, N. L., Batrakova, E. V., Bronich, T. K., & Kabanov, A. V. (2012). Well-defined cross-linked antioxidant nanozymes for treatment of ischemic brain injury. *Journal of Controlled Release, 162*, 636–645.

Manjunath, K., & Venkateswarlu, V. (2006). Pharmacokinetics, tissue distribution and bioavailability of nitrendipine solid lipid nanoparticles after intravenous and intraduodenal administration. *Journal of Drug Targeting, 14*, 632–645.

Martinez-Serrano, A., & Bjorklund, A. (1998). Ex vivo nerve growth factor gene transfer to the basal forebrain in presymptomatic middle-aged rats prevents the development of cholinergic neuron atrophy and cognitive impairment during aging. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 1858–1863.

Martinez-Serrano, A., Hantzopoulos, P. A., & Bjorklund, A. (1996). Ex vivo gene transfer of brain-derived neurotrophic factor to the intact rat forebrain: Neurotrophic effects on cholinergic neurons. *European Journal of Neuroscience, 8*, 727–735.

Mayeux, R. (2003). Epidemiology of neurodegeneration. *Annual Review of Neuroscience, 26*, 81–104.

McIntyre, P. B., O'Brien, K. L., Greenwood, B., & van de Beek, D. (2012). Effect of vaccines on bacterial meningitis worldwide. *Lancet, 380*, 1703–1711.

McMillan, J., Batrakova, E., & Gendelman, H. E. (2011). Cell delivery of therapeutic nanoparticles. *Progress in Molecular Biology and Translational Science, 104*, 563–601.

Metcalfe, S. M., & Fahmy, T. M. (2012). Targeted nanotherapy for induction of therapeutic immune responses. *Trends in Molecular Medicine, 18*, 72–80.

Micheli, M. R., Bova, R., Magini, A., Polidoro, M., & Emiliani, C. (2012). Lipid-based nanocarriers for CNS-targeted drug delivery. *Recent Patents on CNS Drug Discovery, 7*, 71–86.

Mulik, R. S., Monkkonen, J., Juvonen, R. O., Mahadik, K. R., & Paradkar, A. R. (2012). ApoE3 mediated polymeric nanoparticles containing curcumin: Apoptosis induced in vitro anticancer activity against neuroblastoma cells. *International Journal of Pharmaceutics, 437*, 29–41.

Muller, F. J., Snyder, E. Y., & Loring, J. F. (2006). Gene therapy: Can neural stem cells deliver? *Nature Reviews Neuroscience, 7*, 75–84.

Neely, A., Perry, C., Varisli, B., Singh, A. K., Arbneshi, T., Senapati, D., Kalluri, J. R., & Ray, P. C. (2009). Ultrasensitive and highly selective detection of Alzheimer's disease biomarker using two-photon Rayleigh scattering properties of gold nanoparticle. *ACS Nano, 3*, 2834–2840.

Nel, A., Xia, T., Madler, L., & Li, N. (2006). Toxic potential of materials at the nanolevel. *Science, 311*, 622–627.

Nel, A. E., Madler, L., Velegol, D., Xia, T., Hoek, E. M., Somasundaran, P., Klaessig, F., Castranova, V., & Thompson, M. (2009). Understanding biophysicochemical interactions at the nano-bio interface. *Nature Materials, 8*, 543–557.

Noorbakhsh, F., Vergnolle, N., McArthur, J. C., Silva, C., Vodjgani, M., Andrade-Gordon, P., Hollenberg, M. D., & Power, C. (2005). Proteinase-activated receptor-2 induction by neuroinflammation prevents neuronal death during HIV infection. *Journal of Immunology, 174*, 7320–7329.

Nowacek, A., & Gendelman, H. E. (2009). NanoART, neuroAIDS and CNS drug delivery. *Nanomedicine (London, England), 4*, 557–574.

Nowacek, A., Kosloski, L. M., & Gendelman, H. E. (2009a). Neurodegenerative disorders and nanoformulated drug development. *Nanomedicine (London, England), 4*, 541–555.

Nowacek, A. S., Miller, R. L., McMillan, J., Kanmogne, G., Kanmogne, M., Mosley, R. L., Ma, Z., Graham, S., Chaubal, M., Werling, J., Rabinow, B., Dou, H., & Gendelman, H. E. (2009b). NanoART synthesis, characterization, uptake, release and toxicology for human monocyte-macrophage drug delivery. *Nanomedicine (London, England), 4*, 903–917.

Nowacek, A. S., McMillan, J., Miller, R., Anderson, A., Rabinow, B., & Gendelman, H. E. (2010). Nanoformulated antiretroviral drug combinations extend drug release and antiretroviral responses in HIV-1-infected macrophages: Implications for neuroAIDS therapeutics. *Journal of Neuroimmune Pharmacology, 5*, 592–601.

Nowacek, A. S., Balkundi, S., McMillan, J., Roy, U., Martinez-Skinner, A., Mosley, R. L., Kanmogne, G., Kabanov, A. V., Bronich, T., & Gendelman, H. E. (2011). Analyses of nanoformulated antiretroviral drug charge, size, shape and content for uptake, drug release and antiviral activities in human monocyte-derived macrophages. *Journal of Controlled Release, 150*, 204–211.

Nylander, A., & Hafler, D. A. (2012). Multiple sclerosis. *Journal of Clinical Investigation, 122*, 1180–1188.

O'Callaghan, J. P., Sriram, K., & Miller, D. B. (2008). Defining "neuroinflammation". *Annals of the New York Academy of Sciences, 1139*, 318–330.

Pacifici, M., Delbue, S., Ferrante, P., Jeansonne, D., Kadri, F., Nelson, S., Velasco-Gonzalez, C., Zabaleta, J., & Peruzzi, F. (2013). Cerebrospinal fluid miRNA profile in HIV-encephalitis. *Journal of Cellular Physiology, 228*, 1070–1075.

Paulo, C. S., Pires das Neves, R., & Ferreira, L. S. (2011). Nanoparticles for intracellular-targeted drug delivery. *Nanotechnology, 22*, 494002.

Persidsky, Y., Ramirez, S. H., Haorah, J., & Kanmogne, G. D. (2006). Blood-brain barrier: Structural components and function under physiologic and pathologic conditions. *Journal of Neuroimmune Pharmacology, 1*, 223–236.

Pichai, M. V., & Ferguson, L. R. (2012). Potential prospects of nanomedicine for targeted therapeutics in inflammatory bowel diseases. *World Journal of Gastroenterology, 18*, 2895–2901.

Popescu, B. F., Bunyan, R. F., Parisi, J. E., Ransohoff, R. M., & Lucchinetti, C. F. (2011). A case of multiple sclerosis presenting with inflammatory cortical demyelination. *Neurology, 76*, 1705–1710.

Puligujja, P., McMillan, J., Kendrick, L., Li, T., Balkundi, S., Smith, N., Veerubhotla, R. S., Edagwa, B. J., Kabanov, A. V., Bronich, T., Gendelman, H. E., & Liu, X. M. (2013). Macrophage folate receptor-targeted antiretroviral therapy facilitates drug entry, retention, antiretroviral activities and biodistribution for reduction of human immunodeficiency virus infections. *Nanomedicine, 9*(8), 1263–1273. doi: 10.1016/j.nano.2013.05.003.

Ransohoff, R. M., & Engelhardt, B. (2012). The anatomical and cellular basis of immune surveillance in the central nervous system. *Nature Reviews Immunology, 12*, 623–635.

Rao, S. S., Hofmann, L. A., & Shakil, A. (2006). Parkinson's disease: Diagnosis and treatment. *American Family Physician, 74*, 2046–2054.

Re, F., Gregori, M., & Masserini, M. (2012a). Nanotechnology for neurodegenerative disorders. *Maturitas, 73*, 45–51.

Re, F., Gregori, M., & Masserini, M. (2012b). Nanotechnology for neurodegenerative disorders. *Nanomedicine, 8*(Suppl 1), S51–S58.

Reddy, M. K., Wu, L., Kou, W., Ghorpade, A., & Labhasetwar, V. (2008). Superoxide dismutase-loaded PLGA nanoparticles protect cultured human neurons under oxidative stress. *Applied Biochemistry and Biotechnology, 151*, 565–577.

Reddy, S. B., Mainwaring, D. E., Kobaisi, M. A., Zeephongsekul, P., & Fecondo, J. V. (2012). Acoustic wave immunosensing of a meningococcal antigen using gold nanoparticle-enhanced mass sensitivity. *Biosensors and Bioelectronics, 31*, 382–387.

Rosenberg, G. A. (1997). Neuroinflammatory disease. IBC meeting on neuroinflammatory disease: Research and treatment strategies. London, UK, 17 and 18 September 1996. *Molecular Medicine Today, 3*, 12–13.

Rosenstein, N. E., Perkins, B. A., Stephens, D. S., Popovic, T., & Hughes, J. M. (2001). Meningococcal disease. *New England Journal of Medicine, 344*, 1378–1388.

Rovira, A., & Leon, A. (2008). MR in the diagnosis and monitoring of multiple sclerosis: An overview. *European Journal of Radiology, 67*, 409–414.

Roy, U., McMillan, J., Alnouti, Y., Gautum, N., Smith, N., Balkundi, S., Dash, P., Gorantla, S., Martinez-Skinner, A., Meza, J., Kanmogne, G., Swindells, S., Cohen, S. M., Mosley, R. L., Poluektova, L., & Gendelman, H. E. (2012). Pharmacodynamic and antiretroviral activities of combination nanoformulated antiretrovirals in HIV-1-infected human peripheral blood lymphocyte-reconstituted mice. *Journal of Infectious Diseases, 206*, 1577–1588.

Schmidt, J., Metselaar, J. M., & Gold, R. (2003a). Intravenous liposomal prednisolone downregulates in situ TNF-alpha production by T-cells in experimental autoimmune encephalomyelitis. *Journal of Histochemistry and Cytochemistry, 51*, 1241–1244.

Schmidt, J., Metselaar, J. M., Wauben, M. H., Toyka, K. V., Storm, G., & Gold, R. (2003b). Drug targeting by long-circulating liposomal glucocorticosteroids increases therapeutic efficacy in a model of multiple sclerosis. *Brain, 126*, 1895–1904.

Schuchat, A., Robinson, K., Wenger, J. D., Harrison, L. H., Farley, M., Reingold, A. L., Lefkowitz, L., & Perkins, B. A. (1997). Bacterial meningitis in the United States in 1995. Active surveillance team. *New England Journal of Medicine, 337*, 970–976.

Seremeta, K. P., Chiappetta, D. A., & Sosnik, A. (2013). Poly(epsilon-caprolactone), Eudragit(R) RS 100 and poly(epsilon-caprolactone)/Eudragit(R) RS 100 blend submicron particles for the sustained release of the antiretroviral efavirenz. *Colloids and Surfaces. B, Biointerfaces, 102*, 441–449.

Shapshak, P., Duncan, R., Kangueane, P., Somboonwit, C., Sinnott, J., Commins, D., Singer, E., & Levine, A. (2011). HIV associated dementia and HIV encephalitis II: Genes on chromosome

22 expressed in individually microdissected globus pallidus neurons (preliminary analysis). *Bioinformation, 6*, 183–186.

Sharma, A. K., Zhang, L., Li, S., Kelly, D. L., Alakhov, V. Y., Batrakova, E. V., & Kabanov, A. V. (2008). Prevention of MDR development in leukemia cells by micelle-forming polymeric surfactant. *Journal of Controlled Release, 131*, 220–227.

Shehu-Xhilaga, M., Tachedjian, G., Crowe, S. M., & Kedzierska, K. (2005). Antiretroviral compounds: Mechanisms underlying failure of HAART to eradicate HIV-1. *Current Medicinal Chemistry, 12*, 1705–1719.

Shibata, A., McMullen, E., Pham, A., Belshan, M., Sanford, B., Zhou, Y., Goede, M., Date, A. A., & Destache, C. J. (2013). Polymeric nanoparticles containing combination antiretroviral drugs for HIV type 1 treatment. *AIDS Research and Human Retroviruses, 29*, 746–754.

Silva, G. A. (2005). Nanotechnology approaches for the regeneration and neuroprotection of the central nervous system. *Surgical Neurology, 63*, 301–306.

Silva, J., Polesskaya, O., Knight, W., Zheng, J. T., Granger, M., Lopez, T., Ontiveros, F., Feng, C., Yan, C., Kasischke, K. A., & Dewhurst, S. (2012). Transient hypercapnia reveals an underlying cerebrovascular pathology in a murine model for HIV-1 associated neuroinflammation: Role of NO-cGMP signaling and normalization by inhibition of cyclic nucleotide phosphodiesterase-5. *Journal of Neuroinflammation, 9*, 253.

Stahl, J. P., Mailles, A., Dacheux, L., & Morand, P. (2011a). Epidemiology of viral encephalitis in 2011. *Médecine et Maladies Infectieuses, 41*, 453–464.

Stahl, J. P., Mailles, A., & Morand, P. (2011b). Viral encephalitis in 2010: From epidemiology to treatment. *Infect Disord Drug Targets, 140*, 372–381.

Stoeger, T., Reinhard, C., Takenaka, S., Schroeppel, A., Karg, E., Ritter, B., Heyder, J., & Schulz, H. (2006). Instillation of six different ultrafine carbon particles indicates a surface area threshold dose for acute lung inflammation in mice. *Environmental Health Perspectives, 114*, 328–333.

Stoeger, T., Takenaka, S., Frankenberger, B., Ritter, B., Karg, E., Maier, K., Schulz, H., & Schmid, O. (2009). Deducing in vivo toxicity of combustion-derived nanoparticles from a cell-free oxidative potency assay and metabolic activation of organic compounds. *Environmental Health Perspectives, 117*, 54–60.

Stoll, G., & Bendszus, M. (2010). New approaches to neuroimaging of central nervous system inflammation. *Current Opinion in Neurology, 23*, 282–286.

Tian, F., Cui, D., Schwarz, H., Estrada, G. G., & Kobayashi, H. (2006). Cytotoxicity of single-wall carbon nanotubes on human fibroblasts. *Toxicology In Vitro, 20*, 1202–1212.

Trapani, A., De Giglio, E., Cafagna, D., Denora, N., Agrimi, G., Cassano, T., Gaetani, S., Cuomo, V., & Trapani, G. (2011). Characterization and evaluation of chitosan nanoparticles for dopamine brain delivery. *International Journal of Pharmaceutics, 419*, 296–307.

Tsao, N., Kanakamma, P. P., Luh, T. Y., Chou, C. K., & Lei, H. Y. (1999). Inhibition of *Escherichia coli*-induced meningitis by carboxyfullerence. *Antimicrobial Agents and Chemotherapy, 43*, 2273–2277.

Tubaro, A., Puccini, F., De Nunzio, C., Digesu, G. A., Elneil, S., Gobbi, C., & Khullar, V. (2012). The treatment of lower urinary tract symptoms in patients with multiple sclerosis: A systematic review. *Current Urology Reports, 13*, 335–342.

Tunkel, A. R., Hartman, B. J., Kaplan, S. L., Kaufman, B. A., Roos, K. L., Scheld, W. M., & Whitley, R. J. (2004). Practice guidelines for the management of bacterial meningitis. *Clinical Infectious Diseases, 39*, 1267–1284.

Ulbrich, W., & Lamprecht, A. (2010). Targeted drug-delivery approaches by nanoparticulate carriers in the therapy of inflammatory diseases. *Journal of the Royal Society, Interface, 7*(Suppl 1), S55–S66.

Valcour, V., Sithinamsuwan, P., Letendre, S., & Ances, B. (2011). Pathogenesis of HIV in the central nervous system. *Current HIV/AIDS Reports, 8*, 54–61.

van de Beek, D., Brouwer, M. C., Thwaites, G. E., & Tunkel, A. R. (2012). Advances in treatment of bacterial meningitis. *Lancet, 380*, 1693–1702.

Wang, C., Zhang, T., Ma, H., Liu, J., Fu, F., & Liu, K. (2007). Prolonged effects of poly(lactic-co-glycolic acid) microsphere-containing huperzine A on mouse memory dysfunction induced by scopolamine. *Basic & Clinical Pharmacology & Toxicology, 100*, 190–195.

Wang, H., Xu, K., Liu, L., Tan, J. P., Chen, Y., Li, Y., Fan, W., Wei, Z., Sheng, J., Yang, Y. Y., & Li, L. (2010). The efficacy of self-assembled cationic antimicrobial peptide nanoparticles against *Cryptococcus neoformans* for the treatment of meningitis. *Biomaterials, 31*, 2874–2881.

Wang, J. X., Sun, X., & Zhang, Z. R. (2002). Enhanced brain targeting by synthesis of 3′,5′-dioctanoyl-5-fluoro-2′-deoxyuridine and incorporation into solid lipid nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics, 54*, 285–290.

Wilson, B., Samanta, M. K., Santhi, K., Kumar, K. P., Paramakrishnan, N., & Suresh, B. (2008). Targeted delivery of tacrine into the brain with polysorbate 80-coated poly(n-butylcyanoacrylate) nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics, 70*, 75–84.

Wing, K., & Sakaguchi, S. (2010). Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nature Immunology, 11*, 7–13.

Wong, H. L., Wu, X. Y., & Bendayan, R. (2012). Nanotechnological advances for the delivery of CNS therapeutics. *Advanced Drug Delivery Reviews, 64*, 686–700.

Yang, J., Wadghiri, Y. Z., Hoang, D. M., Tsui, W., Sun, Y., Chung, E., Li, Y., Wang, A., de Leon, M., & Wisniewski, T. (2011). Detection of amyloid plaques targeted by USPIO-Abeta1-42 in Alzheimer's disease transgenic mice using magnetic resonance microimaging. *NeuroImage, 55*, 1600–1609.

Yeste, A., Nadeau, M., Burns, E. J., Weiner, H. L., & Quintana, F. J. (2012). Nanoparticle-mediated codelivery of myelin antigen and a tolerogenic small molecule suppresses experimental autoimmune encephalomyelitis. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 11270–11275.

Yew, K. S., & Cheng, E. (2009). Acute stroke diagnosis. *American Family Physician, 80*, 33–40.

Yogev, R., & Tan, T. (2011). Meningococcal disease: The advances and challenges of meningococcal disease prevention. *Human Vaccines, 7*, 828–837.

Yu, J., & Lyubchenko, Y. L. (2009). Early stages for Parkinson's development: Alpha-synuclein misfolding and aggregation. *Journal of Neuroimmune Pharmacology, 4*, 10–16.

Zhao, Y., Haney, M. J., Klyachko, N. L., Li, S., Booth, S. L., Higginbotham, S. M., Jones, J., Zimmerman, M. C., Mosley, R. L., Kabanov, A. V., Gendelman, H. E., & Batrakova, E. V. (2011). Polyelectrolyte complex optimization for macrophage delivery of redox enzyme nanoparticles. *Nanomedicine (London, England), 6*, 25–42.

Neurodegenerative Aspects of Multiple System Atrophy

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Contents

Abstract

Multiple system atrophy (MSA) closely resembles Parkinson's disease clinically but with a range of autonomic signs in addition to motor symptoms, resulting in its designation as a Parkinson's-plus disease/atypical parkinsonism. However,

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unlike Parkinson's disease that displays primarily neuronal pathology, the neurodegenerative aspects of MSA reflect widespread glial cell dysfunction and the occurrence of intracytoplasmic inclusion bodies predominantly in oligodendrocytes. Alpha-synuclein, a key protein component of the glial cytoplasmic inclusions, is thought to mediate cytotoxicity via a range of abnormal modified and aggregated soluble molecular species that may even propagate from cell to cell as cargo in exosomes. Moreover, cellular model studies suggest that the sequestration of abnormal alpha-synuclein within microscopically visible intracellular inclusion bodies occurs as a defensive response that utilizes the aggresome machinery. Although aggregation of alpha-synuclein plays a central role in the disease pathology, it is becoming clear that there is a complex interplay of factors, including heat shock proteins, inflammation, calcium homeostasis, the ubiquitin proteasome system, autophagy, and oxidative stress. Recent studies have highlighted the involvement of metallothioneins, small ubiquitin-like modifier (SUMO), and p25α, thus providing several new potential targets for therapeutic intervention. Inflammatory pathways may also be exploited in new treatment approaches, although it remains to be determined whether the astrocyte and microglial activation present is secondary to alpha-synuclein aggregation or primary to the disease pathogenesis, and may even have some beneficial consequences.

Keywords
Aggresome • Alpha-synuclein • Autophagy • Calcium • Chaperones • Metallothionein • Multiple system atrophy • Neuroinflammation • SUMO

1 Introduction

Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder clinically characterized by autonomic failure with motor syndrome presenting as atypical parkinsonism and/or ataxia. The prevalence of MSA is estimated to be 4.6 cases per 100,000 people (Marpillat 2013; Schrag et al. 1999). The prognosis of MSA is poor with death occurring within 8 years after onset (Wenning and Stefanova 2009). MSA is a heterogeneous group of neurological diseases, often misdiagnosed clinically as Parkinson's disease, which have as a common neuropathological hallmark the occurrence of filamentous, intracytoplasmic inclusion bodies in oligodendrocytes throughout the brain (Graham and Oppenheimer 1969; Papp et al. 1989; Gilman et al. 2008). Previously known as three separate clinical conditions, namely, olivopontocerebellar atrophy, striatonigral degeneration, and Shy-Drager syndrome, the signs and symptoms of MSA include autonomic malfunction, highlighted by postural hypotension, urinary complications (e.g., inability to drain bladder, urge incontinence), and persistent constipation and motor complications, such as parkinsonism, cerebellar ataxia (lack of muscle coordination), and pyramidal symptoms (Wenning et al. 2004, 2008; Wenning and Jellinger 2005; Stefanova et al. 2009). There are two subtypes of MSA as determined by the motor complication: MSA-P refers to parkinsonism, and MSA-C refers to cerebellar ataxia.

In the western world, MSA-P has been shown to be the more common of the two at around 80 % prevalence (Wenning et al. 2008), although autonomic failure is seen in both subtypes (Wenning and Stefanova 2009). The macroscopic changes in brain anatomy associated with MSA have been reviewed recently (Ahmed et al. 2012). To summarize, the clinical signs, including ataxia and bradykinesia, correspond to the loss of neurons in multiple brain regions, including the striatum, substantia nigra pars compacta, locus coeruleus, cerebellum, pontine nuclei, inferior olives, and intermediolateral columns (Wenning et al. 1997; Jellinger 2003; Ozawa et al. 2004; Stefanova et al. 2009), and the severity of disease symptoms correlates well with the frequency of inclusion body-bearing cells (Papp and Lantos 1994; Jellinger and Lantos 2010). Another important aspect of the pathology of MSA is widespread gliosis associated with neuronal loss (Ishizawa et al. 2004; Stefanova et al. 2007; Song et al. 2009; Fellner et al. 2011) and the accompanying upregulation of neurotrophic factors, such as BDNF, GDNF, and IGF-I (Kawamoto et al. 1999, 2000; Pellecchia et al. 2010). The focus of this chapter is the molecular pathology of MSA, with the aim of highlighting key molecular components and their potential roles in the progression of the disease.

1.1 Glial Cytoplasmic Inclusions and the Aggresome Model

Many neurodegenerative diseases are characterized by microscopically visible intranuclear or intracytoplasmic protein inclusion bodies (IB), within various disease-specific neural cell populations. In MSA, the pathological inclusion bodies occur predominantly in the oligodendrocyte cells leading to MSA being referred to as a glial α-synucleinopathy, with the characteristic glial cytoplasmic inclusions (GCIs) composed largely of aggregated filamentous α-synuclein (Gai et al. 1998; Wenning et al. 2008), although a subpopulation of GCIs were also immunopositive for the tau protein associated with Alzheimer's disease (Cairns et al. 1997). Figure 1a represents a typical α-synuclein-positive GCI in an oligodendrocyte in MSA tissue (visual cortex). Although the inclusion bodies are seen mostly in oligodendrocytes, cytoplasmic and intranuclear neuronal inclusions and astrocyte inclusions are also seen on occasion (Nishie et al. 2004). The GCIs are often sickle shaped and found in the cytoplasm adjacent to the nucleus (Arima et al. 1992), with the inclusion body adjacent to and/or wrapped around the nucleus, and recent studies have shown that GCIs occur in mature oligodendrocytes and not in oligodendrocyte precursor cells (Ahmed et al. 2013).

In general, pathological inclusion bodies, and by extension GCIs, are believed to be formed actively in a defensive response to soluble cytotoxic protein aggregates, resembling aggresomes (Kopito 2000; Olzmann et al. 2008; Chin et al. 2010). An aggresome is formed when misfolded proteins are either not refolded with the aid of chaperones or not degraded by the normal proteolytic mechanisms (such as lysosomal autophagy and the ubiquitin-proteosome system (UPS)) (Wong and Cuervo 2010; Johnston et al. 1998). Protein misfolding can be caused by genetic or environmental factors, such as oxidative stress and metal binding (reviewed in Jomova et al. 2010). Perinuclear aggresomes are rich in aggregation-prone protein, such as α-synuclein and

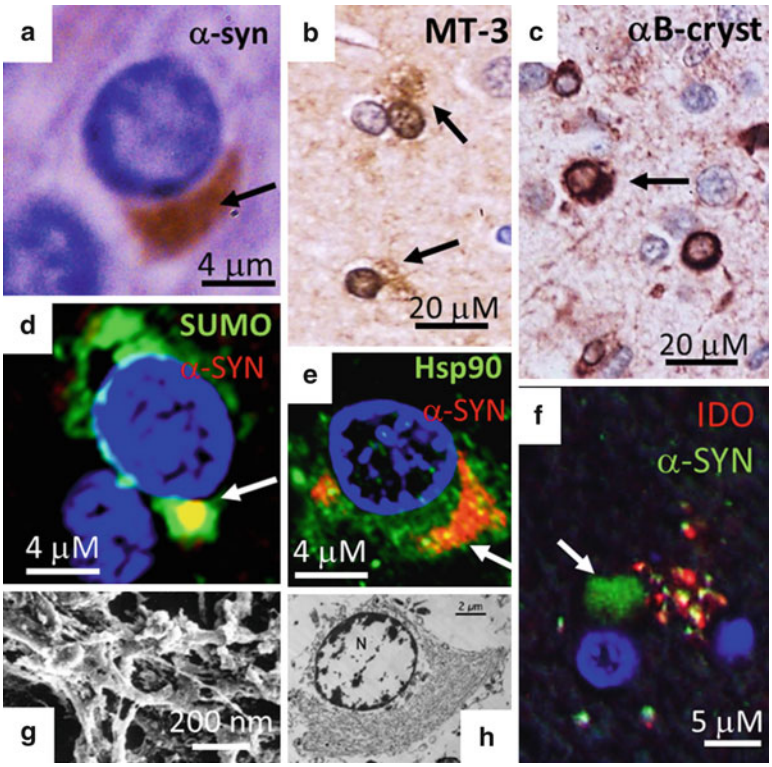

Fig. 1 *Glial cytoplasmic inclusion bodies (GCIs) and gliosis are the primary pathological hallmarks of MSA.* Although composed largely of filamentous α-synuclein aggregates, GCIs are immunopositive for ubiquitin and a diverse range of other components linked to cellular systems, such as autophagy, the proteasome pathway, inflammation, and the cytoskeleton. Illustrative micrographs of MSA brain tissue sections are presented showing (**a**) α-synuclein, (**b**) metallothionein-III, and (**c**) αB-crystallin immunostaining (visual cortex). Confocal immunofluorescence (**d–f**) shows the relationships between α-synuclein and (**d**) SUMO-1, (**e**) Hsp90, and (**f**) indoleamine-2,3-dioxygenase (IDO). Panels (**g–h**) represent the ultrastructural features of GCIs. (**g**) Example scanning electron micrograph of isolated GCI surface showing ~10 nm single filaments and ~30–50 nm bundles of filaments. (**h**) Transmission electron micrograph of typical perinuclear GCI, showing the partial alignment of filaments towards the poles of the crescent-shaped inclusion body

ubiquitin, and cytoskeletal proteins, such as vimentin, that form a "cage" around the developing aggresome. Protein aggregates are actively transported to the microtubule-organizing center (MTOC) along microtubules by the dynein motor proteins, along with other components to form a microscopically visible aggregate, or aggresome (Iwata et al. 2005; Olzmann et al. 2007). p97/VCP protein escorts ubiquitinated proteins to the aggresome via interactions with other ubiquitin-binding proteins, such as rad23 or HDAC6, respectively (Ju et al. 2008). This process can also be mediated by Hsp90-dependent trafficking and is inhibited by the overexpression of dynamitin that dissociates dynein from its cargo and by inhibition of tubulin re-polymerization by

nocodazole (Taylor et al. 2003; Thomas et al. 2006). Recent studies have found that the parkin ubiquitin-protein isopeptide ligase (or E3) and lysine residue 63 (K63)-linked polyubiquitination are vital for dynein transport and therefore aggresome formation (Olzmann et al. 2007; Chin and Olzmann 2010). Under proteasomal impairment, parkin cooperates with the E2 enzyme Ubc13/Uev1a and PACRG to mediate Lys63-linked polyubiquitination of misfolded proteins (Taylor et al. 2012). Lys63-linked polyubiquitin chains promote binding to HDAC6 adaptor protein and thereby link the misfolded proteins to the dynein motor complex for retrograde transport towards the MTOC to form the aggresome. Binding is strengthened by editing of the K63-linked ubiquitin chains by ataxin-3 that creates free ubiquitin C-termini with increased affinity for HDAC6 (Ouyang et al. 2012). Recruitment of polyubiquitinated proteins to the aggresome is promoted by the TRIM50 protein. Lys63-linked polyubiquitination may also promote binding to the p62 protein and thereby facilitate the recruitment of autophagic membrane to the aggresome via interaction with LC3 to form an autophagosome (Fusco et al. 2012). Fusion of the autophagosome with the lysosome may allow degradation of aggregated proteins by lysosomal proteases, a process that may be overwhelmed in protein misfolding disease.

GCIs, in common with other IBs, often contain chaperone proteins, including Hsp90, and ubiquitin, which has been shown to be intimately linked to aggresome biogenesis (Olzmann et al. 2007). The GCIs are comprised largely of 9–10 nm filaments of the α-synuclein protein, which is also found in the neuronal inclusion bodies (Lewy bodies) associated with Parkinson's disease and Lewy body dementia (Gai et al. 1998; Spillantini and Goedert 2000; Pountney et al. 2005a), leading to the grouping together of these diseases as α-synucleinopathies. Some studies implicate the dysregulation of metals, such as copper, iron, calcium, and zinc, which are able to interact with α-synuclein, inducing aggregation, oligomerization, and conformational changes in the protein (Breydo et al. 2012). Other IB components that have been identified besides α-synuclein aggregates are microtubule proteins, αB-crystallin, neurofilament proteins, lipids, organelles (lysosomes and mitochondria), microtubule-associated protein 1B, and parts of the UPS (Gai et al. 1999; Ruipérez et al. 2010). Figure 1b–e illustrates the typical pattern of immunoreactivity of several GCI components in MSA tissue. Isolated GCIs can be resolved to reveal many distinct protein species (Fig. 2). An extensive list of GCI components that have been identified has been reported in the recent review by Jellinger and Lantos (2010). In the remainder of this chapter, several key factors linked to cellular systems will be highlighted that may be important to disease pathogenesis.

2 α-Synuclein

The main component of GCIs in MSA is aggregated filamentous α-synuclein, leading to the classification of the disease as an α-synucleinopathy (Gai et al. 1998; Spillantini and Goedert 2000). Indeed, GCIs containing α-synuclein are required to be present upon autopsy in order for the diagnosis of MSA to be confirmed (Gilman et al. 2008). Although the origin of α-synuclein in oligodendrocytes in MSA is unclear, the

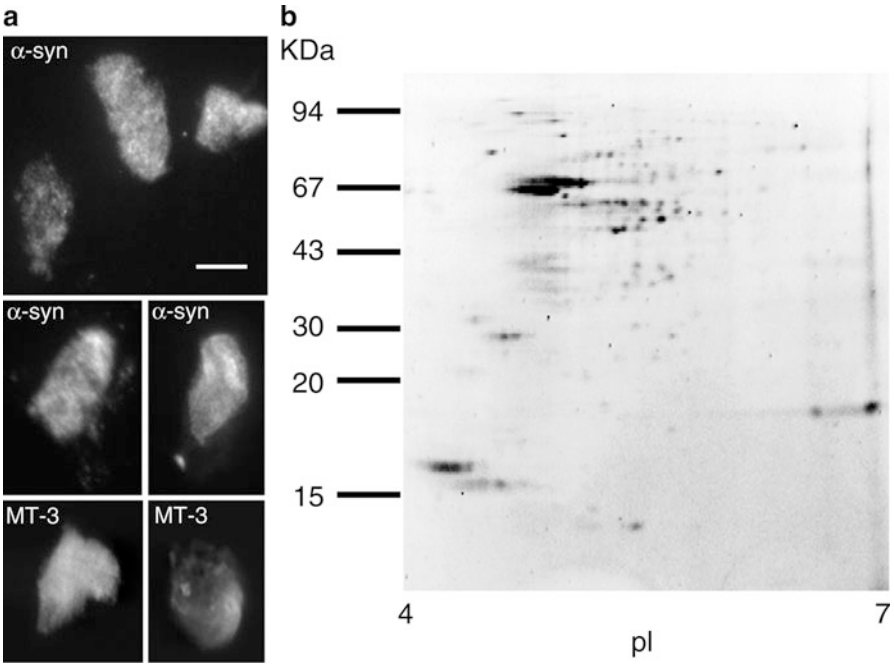

Fig. 2 *Isolated GCIs show differential distributions of inclusion body components and resolve by electrophoresis to reveal abundant distinct protein species*. (**a**) Immunofluorescence of GCIs immunoisolated from fresh, frozen MSA brain tissue using magnetic beads (Pountney et al. 2011). α-Synuclein and metallothionein-III show different patterns of immunoreactivity. Scale bar, 2 μm. (**b**) Two-dimensional gel electrophoresis of purified GCIs (SYPRO Ruby stain, inverted) illustrates the huge diversity of distinct protein species present in the inclusion bodies (for conditions, see Pountney et al. 2005b)

accumulation of α-synuclein-positive GCIs in white matter in MSA brain tissue has been shown to correlate well with the severity of tissue injury (Ishizawa et al. 2008). The α-synuclein protein is encoded by the SNCA gene and comes from a family of synuclein proteins. This group encompasses three other proteins: β-syn, γ-syn, and synoretin, all of which are expressed in neuronal cells (Lavedan 1998). A wide range of in vitro and in vivo data strongly suggests a central role of α-synuclein molecular interactions in the pathogenesis of α-synucleinopathies (Polymeropoulos et al. 1997; Lesage and Brice 2009). A53T, G51D, E46K, and A30P mutations linked to familial PD promote the formation of soluble α-synuclein protofibrils, although only the A53T and E46K mutants accelerate filament formation (Conway et al. 2000; Fredenburg et al. 2007; Kiely et al. 2013). Unlike other α-synucleinopathies, such as Parkinson's disease and dementia with Lewy bodies, there have been no familial forms of MSA reported that are linked to α-synuclein gene mutations, although GCI pathology has recently been found in Parkinson's disease with the G51D mutation (Kiely et al. 2013). Indeed, no clear genetic links to MSA have so far been elucidated (as reviewed by Stemberger et al. 2011; Ahmed et al. 2012), with the exception of recent studies

associating MSA cases with the H1 haplotype of MAPT, commonly associated with tauopathies, such as progressive supranuclear palsy (Vilariño-Güell et al. 2011) and *COQ2* mutations linked to coenzyme Q_{10} deficiency (Tsuji et al. 2013).

α-Synuclein is a protein composed of 140 amino acids, with three distinct domains: a hydrophobic central domain, a negatively charged carboxyl terminus capable of calcium binding, and an amino terminal lipid-binding domain (Ruipérez et al. 2010; Pountney et al. 2005a; Nielsen et al. 2001). Currently, the role of α-syn is unknown. It is expressed in copious amounts in neurons of the central nervous system and is concentrated at presynaptic terminals near synaptic vesicles (Cookson 2009; Leong et al. 2009). In animal models, overexpression of α-synuclein has been attributed to the formation of aggregates and cytotoxicity (Fernagut and Chesselet 2004; Feany and Bender 2000), and cells overexpressing α-synuclein (wild-type or mutants) form α-synuclein-positive cytoplasmic aggregates (Bodner et al. 2006). Indeed, oligodendrocyte expression of α-synuclein in transgenic mice in conjunction with oxidative stress could replicate MSA pathology (Stefanova et al. 2005). Although, the normal cellular function of α-synuclein is still not fully understood, it has been implicated in neurotransmitter vesicle binding and recycling (Nemani et al. 2010). While the free α-synuclein monomer is a natively unfolded protein in dilute solution (Uversky 2009), interaction with lipid vesicles results in folding, yielding a more ordered structure (Uversky and Eliezer 2009; Perlmutter et al. 2009; Drescher et al. 2010). Many different exogenous factors, including oxidation, metal ions, and lipids, have been shown to induce the formation of potentially cytotoxic soluble α-synuclein oligomers (Breydo et al. 2012).

There is no endogenous expression of α-synuclein in oligodendrocytes in either MSA or normal human brain (Miller et al. 2005). Moreover, the expression of α-synuclein in oligodendrocytes in rat has been found to be developmentally regulated, with no expression in mature cells (Richter-Landsberg et al. 2000). Thus, the origin of α-synuclein in GCIs is likely ectopic and may result from uptake of extracellular protein. Indeed, extracellular α-synuclein has recently been identified as a potential therapeutic target in α-synucleinopathies (Vekrellis and Stefanis 2012). Neuronal secretion of α-synuclein can occur via exosomes (Marques and Outeiro 2012), and oligodendrocyte uptake of extracellular α-synuclein is mediated by endocytosis (Konno et al. 2012). There has also been much recent interest in blood and CSF α-synuclein and the possibility that circulating forms of the protein could be analyzed to provide diagnostic information for α-synucleinopathy diseases, including MSA (Eller and Williams 2011).

2.1 Alpha-Synuclein Aggregation and p25α

The native structure of α-synuclein is a soluble unfolded or disordered conformation that can form insoluble fibrils (Vilar et al. 2008). However, the N-terminus can bind to lipid membranes where it becomes α-helical in its secondary structure (Perlmutter et al. 2009; Sulzer et al. 2010). This has led to the implication that the protein may function in controlling synaptic transmission, through binding to

synaptic vesicles and controlling neurotransmitter release and vesicle salvage (Nemani et al. 2010). However, studies with knockout α-synuclein mice showed no detrimental signs, suggesting there are alternate methods in the control of synaptic transmission available and that α-synuclein is not critical to neural development (Fernagut and Tison 2012). In vitro studies have shown that α-synuclein readily forms aggregates, including both filamentous/fibrillar and oligomeric forms. Soluble oligomers of α-synuclein have been shown to have a number of toxic effects, such as inhibiting the UPS, forming pores in lipid membranes, and disturbing intracellular transport (Pountney et al. 2005a; Schmidt et al. 2011). The protein C-terminus has also been shown to have a low affinity for and is required for interaction with calcium ions (Lowe et al. 2004; Nath et al. 2011), and calcium (II) binding to α-synuclein has recently been shown to induce the formation of annular oligomers (Lowe et al. 2004; Nath et al. 2011).

In GCIs, ultrastructural studies have found that α-synuclein is predominantly fibrillar, comprising single ~10 nm amyloid-like filaments as well as ~30 nm bundles of filaments (Gai et al. 2003), although there is also some interstitial, amorphous α-synuclein aggregate. Figure 1g shows a scanning electron micrograph of the surface features of a typical GCI isolated from MSA brain tissue homogenate (Pountney et al. 2003). Furthermore, transmission electron microscopy has revealed that α-synuclein filaments often align within GCIs, especially near the poles of the most common type of crescent-shaped inclusion bodies (Fig. 1h), and has also provided evidence of entrapped vesicular structures, such as mitochondria (Gai et al. 2003; Arima et al. 1992). By treating isolated GCIs with mild detergents, it was possible to release stable annular α-synuclein oligomeric species (Pountney et al. 2004) that closely resemble annular α-synuclein species formed in vitro (Lowe et al. 2004; Nath et al. 2011) and bacterial pore-forming toxins, such as hemolysin.

Various factors, including calcium and other metal ions (Lowe et al. 2004; Nath et al. 2011; Uversky 2009; Uversky and Eliezer 2009; Follett et al. 2012), lipids (Riedel et al. 2011), and oxidative stress (Riedel et al. 2010; Goodwin et al. 2012), have been implicated in promoting α-synuclein aggregation, and recent studies have also implicated the p25α protein (also known as tubulin polymerization-promoting protein, TPPP) that is differentially expressed in MSA brain tissue and itself a GCI component, in promoting the formation of cytotoxic α-synuclein aggregates (Lindersson et al. 2005; Song et al. 2007; Hasegawa et al. 2010; Kragh et al. 2013).

2.2 Posttranslational Modifications of Alpha-Synuclein

Several modified forms of α-synuclein have been reported in MSA and α-synucleinopathy pathology, including Ser129-phosphorylation (Fujiwara et al. 2002; Braithwaite et al. 2012; Anderson et al. 2006; Kragh et al. 2009), glycosylation (Shimura et al. 2001), nitrotyrosine (Beyer and Ariza 2013), and various truncated and cross-linked forms of the protein (Anderson et al. 2006; Pountney et al. 2004). Recently, SUMOylation of α-synuclein has been shown to inhibit filament formation and reduce neuronal toxicity in animal models (Krumova et al. 2011).

The relative importance of these different species in relation to the disease progression cannot currently be estimated; however, many recent studies have focussed on the abundant phosphorylated (phospho-Ser129) α-synuclein species that can be produced by leucine-rich repeat kinase 2 (LRRK2; Qing et al. 2009), also a GCI component (Huang et al. 2008). Earlier studies have linked the glycosylated 22KDa α-synuclein form to targeting of the protein to the ubiquitin proteasome pathway (Shimura et al. 2001). The nitrotyrosine modification in α-synuclein has been proposed as a specific molecular marker indicating oxidative stress (Schildknecht et al. 2013). Oxidative dityrosine cross-linking has also been implicated in stabilizing α-synuclein oligomers (Pountney et al. 2004). The various low-molecular weight truncated forms of α-synuclein, especially the C-terminal truncations that are more prone to aggregation, have received considerable recent attention in proteomics analyses (Anderson et al. 2006), whereas the nature of the wide array of high-molecular weight, stable and cross-linked oligomeric α-synuclein species is still relatively unclear (Deleersnijder et al. 2013).

3 Heat Shock Proteins

3.1 αB-Crystallin and Small Heat Shock Proteins

Heat shock proteins (Hsp), including members of the small heat shock family, have been linked to the formation of aggresomes, cytoplasmic accumulations of aggregated proteins at the microtubule-organizing center (MTOC), in response to unfolded protein stress (Berke and Paulson 2003; Bolhuis and Richter-Landsberg 2010). The chaperone activity of αB-crystallin and several other small chaperone proteins, such as HSP27, is influenced by a wide range of posttranslational modifications, including phosphorylation (Aquilina et al. 2004; Kostenko and Moens 2009). αB-Crystallin is upregulated in neurodegenerative diseases (Muchowski and Wacker 2005) and αB-crystallin has been shown to be present in GCIs (Gai et al. 1999). The interaction between αB-crystallin and α-synuclein can result in inhibition of chaperone activity (Rekas et al. 2004), and recent studies have shown that αB-crystallin can influence the α-synuclein fibrillation pathway (Waudby et al. 2010) and have implicated hyperphosphorylated αB-crystallin forms in MSA (Pountney et al. 2005b).

3.2 Heat Shock Protein 90

Hsp90 is a multifunctional protective chaperone protein, often working in concert with Hsp70 that is involved in the folding, refolding, and disaggregation of a diverse range of proteins. Other important functions of Hsp90 include the maintenance of tertiary structure and ATPase activity of proteasomes, activation of protein kinases, and activation of heat shock factor-1 (HSF-1). This functional diversity and specificity of Hsp90 is made possible by its conformational flexibility and dynamic association with various co-chaperone complexes. The association of Hsp70 and Hsc70 with

GCIs has been reported by Kawamoto and co-workers (2007), and the association of Hsp90 with GCIs has been reported by Trojanowksi and co-workers (Uryu et al. 2006), showing granular cytoplasmic Hsp90 staining of GCI-bearing cells. Recent studies indicate that pharmacological inhibition of Hsp90 can induce Hsp70 and promote clearance and detoxification of α-synuclein aggregates (Putcha et al. 2010). Hsp90 has also been linked to one form of chaperone-mediated autophagy (Kaushik et al. 2011), and the endoplasmic reticulum analogue of Hsp90 participates in the sorting of cargo proteins in autophagic clearance (Suntharalingam et al. 2012). Other recent studies have shown that Hsp90 associates with lysosomes and SUMO-1 in GCI-bearing cells in MSA (Wong et al. 2013).

4 Inflammation and Inflammatory Markers

Severe neuronal loss in MSA is seen in the substantia nigra, cerebellum, putamen, and locus coeruleus (Wenning et al. 1997), with neuronal loss in the brainstem and hypothalamus correlated with the autonomic dysfunction typical of MSA cases (Ozawa 2006). Considerable neuronal loss is also apparent in cortical areas, especially the motor cortex (Spargo et al. 1996; Su et al. 2001). Accompanying the neuronal loss, there is also commonly widespread demyelination observed in the white matter tracts (Papp et al. 1989). Astrogliosis and activated microglia are also found in those brain regions affected by neuronal loss and where there is a high GCI burden (Ozawa et al. 2004; Ishizawa et al. 2008), suggesting that inflammatory pathways are of major importance in MSA pathogenesis.

4.1 Microglia and Astroglia

The appearance of highly stellate, swollen, strongly GFAP-positive astrocytes is one of the most striking pathological features of MSA brain tissue, with this type of activated astrocyte morphology most often occurring in affected brain regions in cells neighboring GCI-positive oligodendrocytes. Figure 3 illustrates the juxtaposition of factors influencing oligodendrocyte GCI formation and astrocyte activation in MSA, with inset the typical morphology of an activated, GFAP-stained astrocyte and nearby GCI-positive oligodendrocyte observed in MSA brain tissue. The rounded morphology of activated microglia is also seen to accompany the more obvious astrocytic changes. Microglia have also been found to be more abundant in degraded regions of white matter in MSA (Ishizawa et al. 2008). The morphological indications of glial involvement in MSA is also reflected in the expression of markers for activated glia, such as increased astrocytic expression of metallothionein-III (Pountney et al. 2011) and indoleamine-2,3-dioxygenase (IDO; Radford et al. (unpublished data); Fig. 1f). Mouse models of MSA have also consistently implicated gliosis, especially microgliosis (Stefanova et al. 2005, 2007), and the interaction between α-synuclein and glial cell activation has recently been linked to the TLR4 receptor (Fellner et al. 2013).

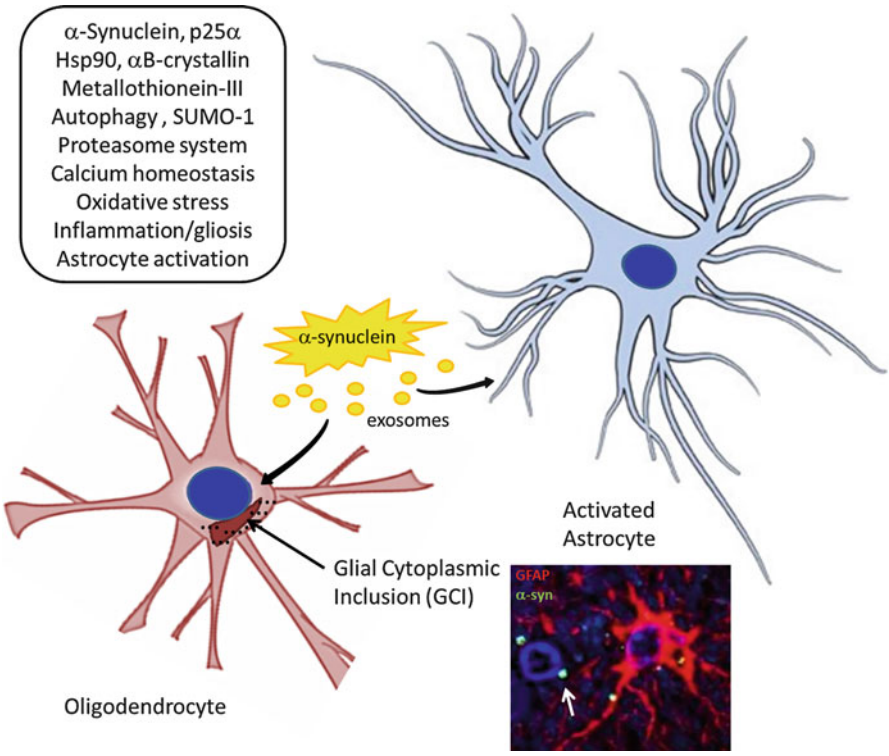

Fig. 3 *Diverse factors implicated in MSA pathogenesis result in oligodendroglial cytoplasmic inclusion body (GCI) formation and astrocyte and microglial activation*. GCI formation and oligodendrocyte dysfunction together with the activation of neighboring astrocytes and microglia are the primary features characterizing MSA, leading to the secondary neuronal degeneration. α-Synuclein secreted via the exosome pathway is taken up by oligodendrocytes and may act on astrocytes; however, the relationship between GCI formation and astrocyte activation remains unclear. Inset shows immunofluorescence of MSA tissue (visual cortex) stained for the astrocyte marker, GFAP, and α-synuclein, illustrating a GCI-bearing oligodendrocyte (*arrow*) and the characteristic morphology of a nearby activated astrocyte

4.2 Metallothionein

The metallothioneins (MTs) are small (6–7 KDa) proteins, the expression of which is induced by a wide range of factors, including heavy metals, glucocorticoids, cytokines, and oxidative stress, that current evidence suggest may have a number of specific functions in the brain (Chung et al. 2008; West et al. 2008; Vasák 2005). MTs bind zinc and copper, as well as toxic heavy metals, and have also been shown to efficiently scavenge reactive oxygen species, such as nitric oxide, thereby playing a protective role against oxidative cell damage. Mammalian MT isoforms are grouped according to their charge. MT-I/II isoforms are expressed in all mammalian tissues, whereas MT-III is expressed primarily in brain and MT-IV in

squamous endothelial tissues. Recently, a significant increase was found in the number of MT-III-positive cells, especially astrocytes, in MSA brain, compared to normal brain tissue (Pountney et al. 2011). MT-III immunoreactivity was also detected in GCIs, whereas the MT-I/II isoforms were not detected, indicating a specific association between MT-III and MSA. Recent evidence shows that MT-I/II is released from astrocytes during brain injury and interacts with a neuronal cell-surface receptor to promote neuronal survival, although much less is known about the function of other isoforms. MT-III (or neuronal growth inhibitory factor, GIF) can inhibit neuritic sprouting of cultured neurons and has been reported to be downregulated in Alzheimer's disease neurons (Uchida et al. 1991; Su et al. 2001) yet is unchanged in Alzheimer's mouse models (Carrasco et al. 2006). MT-III may be linked to the cellular response to mild CNS insult, such as neurotrauma, kainic acid-induced seizures, and autoimmune responses, being coordinated with the action of various neurotrophic factors. The origin of metallothionein-III in MSA may relate to the known association of these proteins with neuroinflammation and oxidative stress (Manso et al. 2011) or may indicate a disturbance of metal ion homeostasis. MT-III has also been linked to lysosome function in astrocytes (Lee and Koh 2010). Interestingly, recent studies have shown that astrocyte expression of MT-III can promote actin polymerization (Lee et al. 2011), and so it may be that increased expression of this protein occurs as a consequence of the cytoskeletal changes during astrocyte activation.

5 Calcium Homeostasis

It has been speculated that calcium may play a crucial role in the pathogenesis of neurodegenerative diseases, such as α-synucleinopathies (Mattson 2007). In PD pathology, it has been noted previously that neurons with higher levels of the calcium buffer, calbindin/D28K, are relatively spared (Yamada et al. 1990) and there may be an interplay between calcium homoeostasis and dopamine in mediating neuronal cell death (Mosharov et al. 2009). Indeed, α-synuclein has been found to perturb cellular calcium homoeostasis (Hettiarachchi et al. 2009; Danzer et al. 2007). Recently, it was demonstrated that increased intracellular free calcium causes the formation of microscopically visible α-synuclein aggregates in the cytoplasm of α-syn-eGFP-transfected human glioma cells (Nath et al. 2011) and more recently that oxidative stress and raised calcium can act synergistically to cause α-synuclein aggregation (Goodwin et al. 2012).

6 Ubiquitin and Ubiquitin Homologues

Ubiquitin, a small protein that attaches covalently to other proteins to target them to cellular pathways, such as proteasomal degradation, has long been known as

a major component of GCIs, and its presence has been linked both to the ubiquitin proteasome system, autophagy, and aggresome biogenesis. Recent studies indicate that polyubiquitin chains can be linked one ubiquitin to the next via many different ubiquitin functional groups, with each different type of ubiquitin chain conferring a different function on the modified protein carrying it, with the lysine-63-linked polyubiquitin targeting proteins to the aggresome and K48-linked ubiquitin chains targeting the proteasome (Kulathu and Komander 2012; Trempe 2011). Deubiquitinated α-synuclein is targeted for autophagic degradation, while monoubiquitination preferentially targets α-synuclein to the proteasome (Rott et al. 2011). In MSA, ubiquitin modifications of various proteins have been reported, including parkin and α-synuclein, and compromise of the ubiquitin proteasome system has also been inferred based on reduced 20S proteasome subunit expression (Bukhatwa et al. 2010). Soluble aggregated forms of α-synuclein have been shown to bind to the 19S cap of the 26S proteasome and inhibit proteasome-mediated protein degradation (Snyder et al. 2003; Lindersson et al. 2004). In a recent study using a transgenic mouse model of MSA expressing α-synuclein under an oligodendrocyte-specific promoter, Stefanova and co-workers (2012) have shown that proteasome inhibition caused by administration of a chemical inhibitor led to an MSA phenotype with pathological features, including fibrillar glial α-synuclein aggregates, demyelination, and axonal disturbances, that closely mirror those of MSA.

6.1 SUMO-1

In 2005, it was reported that minute subdomains exist in GCIs that are marked by small ubiquitin-like modifier, SUMO-1 (Pountney et al. 2005c). SUMO (small ubiquitin-like modifier) is similar to ubiquitin in structure but with more diverse physiological functions. SUMOylation requires the coordinated actions of E1 (SUMO activating), E2 (SUMO-specific conjugating, Ubc9) and, in most cases, E3 (SUMO ligase, e.g., PIAS3) enzymes, and despite being a covalent protein modification, SUMOylation is readily reversed by SUMO-specific deSUMOylation enzymes, SENPs. Thus, the balance between Ubc9-mediated conjugation and SENP-mediated deconjugation determines the SUMOylation state (Martin et al. 2007). SUMOylation can inhibit protein binding or, alternatively, SUMOylation of target proteins can promote interactions with other proteins via SUMO interaction motifs (SIMs). SUMOylation functions to regulate nucleocytoplasmic transport by modification of RanGAP1 and subsequent association with the nuclear pore complex and also has roles in mitochondrial fission. SUMOylation modifies the mitotic spindle and, by mediating non-covalent interactions with dynamin, influences endocytosis. SUMO-1 has also been linked to a range of neuropathological protein species; indeed, both α-synuclein and tau have been shown to be capable of SUMO-1 modification (Krumova and Weishaupt 2012). SUMO-1 was first identified in pathological inclusion bodies in neuronal intranuclear inclusions in neuronal

intranuclear inclusion disease, spinocerebellar ataxia type III, and Huntington's disease (Pountney et al. 2003). Recent studies have shown that neuron-specific expression of the SUMO-2 isoform can result in some SUMOylation of α-synuclein and that the SUMOylated protein is less prone to aggregation (Krumova et al. 2011). However, the significance of this finding to glial cells is unclear. Several target proteins of SUMO-1 have been determined in intranuclear inclusions that could be associated with lysosomal or secretory pathways (Hsp90, Munc18, NSF, and dynamin-1) (Pountney et al. 2008), and it has been shown that SUMO-1 marks a subset of lysosomes in MSA and in glial cell culture models of proteolytic stress and protein aggregation. This tagging of lysosomes by SUMO-1 is correlated with the presence of cytoplasmic inclusion bodies and associated with Hsp90, suggesting SUMO-dependent modulation of the autophagy pathway in MSA (Wong et al. 2013).

7 Autophagy

Autophagy is an evolutionarily conserved bulk degradation process that involves lysosomes. There are three types of autophagy: microautophagy which is the direct uptake of cytosolic proteins or organelles into the lysosome, chaperone-mediated autophagy which involves the chaperone conjugate Hsc70 directing protein substrates to the lysosome, and also macroautophagy (autophagy) which involves the formation of autophagosomes that then fuse with endosomes or lysosomes (Klionsky et al. 2011). Significant progress has been made in delineating the molecular mechanisms of chaperone-mediated autophagy that includes targeting of proteins via EF1α and interaction with GFAP at the lysosomal membrane (Kaushik et al. 2011). Recent studies have linked hsc70 also to a selective variant of autophagy that involves translocation of proteins via the multivesicular body (Sahu et al. 2011). Experiments have shown that autophagy is highly important in neural cells and disruption of the process can lead to aggregate formation and neurodegeneration, further highlighting its importance in neural tissue. The autophagy pathway has been implicated in neurodegenerative disease, and evidence of the pathways' involvement in MSA is seen in the presence of LC3 and lysosome components in glial inclusion bodies (Schwarz et al. 2012). Autophagy has been shown to contribute to aggresome clearance in neurodegenerative models and is believed to be an important cellular response to increased aggregating protein stress. Mouse knockouts of autophagy genes show progressive neurodegeneration, and disease-linked α-synuclein forms have been shown to inhibit chaperone-mediated autophagy (Rami 2009). Macroautophagy can enable the clearance of α-synuclein aggregates, and disruption of the process can lead to aggregate formation and neurodegeneration, further highlighting its importance in neural tissue (Crews et al. 2010). All of these studies indicate a neuroprotective role for the autophagy pathway in neurodegenerative disease and raise the possibility of modulating this pathway as a therapeutic treatment.

8 Oxidative Stress

Oxidative stress arises from an imbalance between the cellular processes that can generate powerful oxidants, such as superoxide and peroxynitrite, and the cellular defenses, such as glutathione, that normally combat them. Oxidative stress of neural cells has been implicated in the pathology of many neurodegenerative diseases, including MSA, and may play an important role in the development of MSA pathology, with the occurrence in GCIs of oxidized components, such as nitrated α-synuclein and protein-bound 4-hydroxy-2-nonenal (Gómez-Tortosa et al. 2002; Norris and Giasson 2005; Shibata et al. 2010). Oxidative nucleic acid damage has also been reported in MSA (Kikuchi et al. 2002). Furthermore, oxidation is among the many different exogenous factors, including metal ions and lipids, that have been shown to induce the formation of potentially cytotoxic soluble α-synuclein oligomers in cell culture and animal models of MSA (Breydo et al. 2012; Stefanova et al. 2005, 2007; Riedel et al. 2007; Ubhi et al. 2009). Recently, the interaction between oxidation/oxidative stress and raised calcium was investigated, and it was found that these two factors act synergistically to induce α-synuclein aggregates in vitro and in glial cells (Goodwin et al. 2012).

9 Conclusion

In conclusion, there is a strong burden of evidence pointing to a pivotal role of α-synuclein aggregation in MSA development. Although it is likely that aggregated α-synuclein forms occur at an early stage in the disease progression, there are many factors that have been identified, including oxidative stress and calcium dysregulation, that may participate in triggering the production of the pathogenic forms of the protein. The role of α-synuclein posttranslational modifications, such as Ser-129 phosphorylation, is also unclear, especially with respect to the timing of the modifications either as a precursor to or a consequence of aggregate formation. Accumulation in GCIs of α-synuclein and another small, acidic protein, metallothionein-III, even though mature oligodendrocytes do not normally express either protein, suggests that abnormal uptake of proteins by oligodendrocytes may be a feature of the etiology of MSA. Indeed, extracellular roles for both α-synuclein and MT-III have recently been reported. Future studies will need to address the causes of (and potential remedies for) α-synuclein aggregation in MSA as well as the origin of the protein in glial cells.

Another important aspect of MSA pathogenesis that will need to be probed further is the relationship between the formation of GCIs primarily in oligodendrocyte cells and the activation of neighboring astrocytes and microglia. Although it is clear that neuronal degeneration in the disease occurs as a downstream consequence of the glial cell disturbances, the relative importance of oligodendroglial dysfunction and demyelination compared to the influence of the inflammatory cytokines and astrocyte and microglial activation is unknown. Indeed, recent studies by Sofroniew and

co-workers suggest that reactive astrogliosis may even have some neuroprotective as well as deleterious effects on neuronal survival in injured brain tissue (Sofroniew 2009; Verkhratsky et al. 2012).

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References

Ahmed, Z., Asi, Y. T., Sailer, A., Lees, A. J., Houlden, H., Revesz, T., & Holton, J. L. (2012). The neuropathology, pathophysiology and genetics of multiple system atrophy. *Neuropathology and Applied Neurobiology, 38*, 4–24.

Ahmed, Z., Asi, Y. T., Lees, A. J., Revesz, T., & Holton, J. L. (2013). Identification and quantification of oligodendrocyte precursor cells in multiple system atrophy progressive supranuclear palsy and Parkinson's disease. *Brain Pathology, 23*(3), 263–273. doi: 10.1111/j.1750-3639.2012.00637.x.

Anderson, J. P., Walker, D. E., Goldstein, J. M., De Laat, R., Banducci, K., Caccavello, R. J., Barbour, R., Huang, J., Kling, K., Lee, M., Diep, L., Keim, P. S., Shen, X., Chataway, T., Schlossmacher, M. G., Seubert, P., Schenk, D., Sinha, S., Gai, W. P., & Chilcote, T. J. (2006). Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *The Journal of Biological Chemistry, 281*, 29739–29752.

Aquilina, J. A., Benesch, J. L., Ding, L. L., Yaron, O., Horwitz, J., & Robinson, C. V. (2004). Phosphorylation of alphaB-crystallin alters chaperone function through loss of dimeric substructure. *Journal of Biological Chemistry, 279*, 28675–28680.

Arima, K., Murayama, S., Mukoyama, M., & Inose, T. (1992). Immunocytochemical and ultrastructural studies of neuronal and oligodendroglial cytoplasmic inclusions in multiple system atrophy. 1. Neuronal cytoplasmic inclusions. *Acta Neuropathologica, 83*, 453–460.

Berke, S. J., & Paulson, H. L. (2003). Protein aggregation and the ubiquitin proteasome pathway: Gaining the UPPer hand on neurodegeneration. *Current Opinion in Genetics and Development, 13*, 253–261.

Beyer, K., & Ariza, A. (2013). Alpha-synuclein posttranslational modification and alternative splicing as a trigger for neurodegeneration. *Molecular Neurobiology, 47*, 509–524.

Bodner, R. A., Outeiro, T. F., Altmann, S., Maxwell, M. M., Cho, S. H., Hyman, B. T., McLean, P. J., Young, A. B., Housman, D. E., Kazantsev, A. G. (2006). Pharmacological promotion of inclusion formation: A therapeutic approach for Huntington's and Parkinson's diseases. *Proceedings of the National Academy of Sciences USA*, *103*, 4246–4251.

Bolhuis, S., & Richter-Landsberg, C. (2010). Effect of proteasome inhibition by MG-132 on HSP27 oligomerization, phosphorylation, and aggresome formation in the OLN-93 oligodendroglia cell line. *Journal of Neurochemistry, 114*, 960–971.

Braithwaite, S. P., Stock, J. B., & Mouradian, M. M. (2012). α-Synuclein phosphorylation as a therapeutic target in Parkinson's disease. *Reviews in Neuroscience, 23*, 191–198.

Breydo, L., Wu, J. W., & Uversky, V. N. (2012). Alpha-synuclein misfolding and Parkinson's disease. *Biochimica et Biophysica Acta, 1822*, 261–285.

Bukhatwa, S., Zeng, B. Y., Rose, S., & Jenner, P. (2010). A comparison of changes in proteasomal subunit expression in the substantia nigra in Parkinson's disease, multiple system atrophy and progressive supranuclear palsy. *Brain Research, 1326*, 174–183.

Cairns, N. J., Atkinson, P. F., Hanger, D. P., Anderton, B. H., Daniel, S. E., & Lantos, P. L. (1997). Tau protein in the glial cytoplasmic inclusions of multiple system atrophy can be distinguished from abnormal tau in Alzheimer's disease. *Neuroscience Letters, 230*, 49–52.

Carrasco, J., Adlard, P., Coman, D., Quintana, A., Penkowa, M., Xu, F., van Nostrand, W. E., & Hidalgo, J. (2006). Metallothionein-I and -III expression in animal models of Alzheimer's disease. *Neuroscience, 143*, 911–922.

Chin, L. S., Olzmann, J. A., & Li, L. (2010). Parkin-mediated ubiquitin signalling in aggresome formation and autophagy. *Biochemical Society Transactions, 38*, 144–149.

Chung, R. S., Hidalgo, J., & West, A. K. (2008). New insight into the molecular pathways of metallothionein-mediated neuroprotection and regeneration. *Journal of Neurochemistry, 104*, 14–20.

Conway, K. A., Lee, S. J., Rochet, J. C., Ding, T. T., Williamson, R. E., & Lansbury, P. T. Jr. (2000). Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. *Proceedings of the National Academy of Sciences USA*, *97*, 571–576.

Cookson, M. R. (2009). α-Synuclein and neuronal cell death. *Molecular Neurodegeneration, 4*, 9.

Crews, L., Spencer, B., Desplats, P., Patrick, C., Paulino, A., Rockenstein, E., Hansen, L., Adame, A., Galasko, D., & Masliah, E. (2010). Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of alpha-synucleinopathy. *PLoS One, 5*, e9313.

Danzer, K. M., Haasen, D., Karow, A. R., Moussaud, S., Habeck, M., Giese, A., Kretzschmar, H., Hengerer, B., & Kostka, M. (2007). Different species of α-synuclein oligomers induce calcium influx and seeding. *Journal of Neuroscience, 27*, 9220–9232.

Deleersnijder, A., Gerard, M., Debyser, Z., & Baekelandt, V. (2013). The remarkable conformational plasticity of alpha-synuclein: Blessing or curse? *Trends in Molecular Medicine, 19*, 368–377.

Drescher, M., van Rooijen, B. D., Veldhuis, G., Subramaniam, V., & Huber, M. (2010). A stable lipid-induced aggregate of alpha-synuclein. *Journal of the American Chemical Society, 132*, 4080–4082.

Eller, M., & Williams, D. R. (2011). α-Synuclein in Parkinson disease and other neurodegenerative disorders. *Clinical Chemistry and Laboratory Medicine, 49*, 403–408.

Feany, M. B., & Bender, W. W. (2000). A Drosophila model of Parkinson's disease. *Nature, 404*, 394–398.

Fellner, L., Jellinger, K. A., Wenning, G. K., & Stefanova, N. (2011). Glial dysfunction in the pathogenesis of α-synucleinopathies: Emerging concepts. *Acta Neuropathologica, 121*, 675–693.

Fellner, L., Irschick, R., Schanda, K., Reindl, M., Klimaschewski, L., Poewe, W., Wenning, G. K., & Stefanova, N. (2013). Toll-like receptor 4 is required for α-synuclein dependent activation of microglia and astroglia. *Glia, 61*, 349–360.

Fernagut, P. O., & Chesselet, M. F. (2004). Alpha-synuclein and transgenic mouse models. *Neurobiology of Disease, 17*, 123–130.

Fernagut, P. O., & Tison, F. (2012). Animal models of multiple system atrophy. *Neuroscience, 211*, 77–82.

Follett, J., Darlow, B., Wong, M. B., Goodwin, J., & Pountney, D. L. (2012). Potassium depolarization and raised calcium induces α-synuclein aggregates. *Neurotoxicity Research, 23*(4), 378–392.

Fredenburg, R. A., Rospigliosi, C., Meray, R. K., Kessler, J. C., Lashuel, H. A., Eliezer, D., & Lansbury, P. T., Jr. (2007). The impact of the E46K mutation on the properties of alpha-synuclein in its monomeric and oligomeric states. *Biochemistry, 46*, 7107–7118.

Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M. S., Shen, J., Takio, K., & Iwatsubo, T. (2002). Alpha-synuclein is phosphorylated in synucleinopathy lesions. *Nature Cell Biology, 4*, 160–164.

Fusco, C., Micale, L., Egorov, M., Monti, M., D'addetta, E. V., Augello, B., Cozzolino, F., Calcagnì, A., Fontana, A., Polishchuk, R. S., Didelot, G., Reymond, A., Pucci, P., & Merla, G. (2012). The E3-ubiquitin ligase TRIM50 interacts with HDAC6 and p62, and promotes the sequestration and clearance of ubiquitinated proteins into the aggresome. *PLoS One, 7*, e40440.

Gai, W. P., Power, J. H., Blumbergs, P. C., & Blessing, W. W. (1998). Multiple-system atrophy: A new alpha-synuclein disease? *Lancet, 352*(9127), 547–548.

Gai, W. P., Power, J. H., Blumbergs, P. C., Culvenor, J. G., & Jensen, P. H. (1999). Alpha-synuclein immunoisolation of glial inclusions from multiple system atrophy brain tissue reveals multiprotein components. *Journal of Neurochemistry, 73*, 2093–2100.

Gai, W. P., Pountney, D. L., Power, J. H. T., Blumbergs, P. C., Li, Q.-X., Culvenor, J. G., Jensen, P. H. (2003) Alpha-synuclein fibrils constitute the central core of oligodenroglial inclusion filaments in multiple system atrophy. *Experimental Neurology, 181*, 68–78.

Gilman, S., Wenning, G. K., Low, P. A., Brooks, D. J., Mathias, C. J., Trojanowski, J. Q., Wood, N. W., Colosimo, C., Durr, A., Fowler, C. J., Kaufmann, H., Klockgether, T., Lees, A., Poewe, W., Quinn, N., Revesz, T., Robertson, D., Sandroni, P., Seppi, K., & Vidailhet, M. (2008). Second consensus statement on the diagnosis of multiple system atrophy. *Neurology, 71*, 670–676.

Gómez-Tortosa, E., Gonzalo, I., Newell, K., García Yébenes, J., Vonsattel, P., & Hyman, B. T. (2002). Patterns of protein nitration in dementia with Lewy bodies and striatonigral degeneration. *Acta Neuropathologica, 103*, 495–500.

Goodwin, J., Nath, S., Engelborghs, Y., & Pountney, D. L. (2012). Raised calcium and oxidative stress cooperatively promote alpha-synuclein aggregate formation. *Neurochemistry International, 62*(5), 703–711. doi:pii:%20S0197-0186(12)00356-7.%2010.1016/j.neuint.2012.11.004.

Graham, J. G., & Oppenheimer, D. R. (1969). Orthostatic hypotension and nicotine sensitivity in a case of multiple system atrophy. *Journal of Neurology, Neurosurgery, and Psychiatry, 32*, 28–34.

Hasegawa, T., Baba, T., Kobayashi, M., Konno, M., Sugeno, N., Kikuchi, A., Itoyama, Y., & Takeda, A. (2010). Role of TPPP/p25 on α-synuclein-mediated oligodendroglial degeneration and the protective effect of SIRT2 inhibition in a cellular model of multiple system atrophy. *Neurochemistry International, 57*, 857–866.

Hettiarachchi, N. T., Parker, A., Dallas, M. L., Pennington, K., Hung, C. C., Pearson, H. A., Boyle, J. P., Robinson, P., & Peers, C. (2009). α-Synuclein modulation of Ca^{2+} signaling in human neuroblastoma (SH-SY5Y) cells. *Journal of Neurochemistry, 111*, 1192–1201.

Huang, Y., Song, Y. J., Murphy, K., Holton, J. L., Lashley, T., Revesz, T., Gai, W. P., & Halliday, G. M. (2008). LRRK2 and parkin immunoreactivity in multiple system atrophy inclusions. *Acta Neuropathologica, 116*, 639–646.

Ishizawa, K., Komori, T., Sasaki, S., Arai, N., Mizutani, T., & Hirose, T. J. (2004). Microglial activation parallels system degeneration in multiple system atrophy. *Journal of Neuropathology and Experimental Neurology, 63*, 43–52.

Ishizawa, K., Komori, T., Arai, N., Mizutani, T., & Hirose, T. (2008). Glial cytoplasmic inclusions and tissue injury in multiple system atrophy: A quantitative study in white matter (olivopontocerebellar system) and gray matter (nigrostriatal system). *Neuropathology, 28*, 249–257.

Iwata, A., Riley, B. E., Johnston, J. A., & Kopito, R. R. (2005). HDAC6 and microtubules are required for autophagic degradation of aggregated huntingtin. *The Journal of Biological Chemistry, 280*, 40282–40292.

Jellinger, K. A. (2003). Neuropathological spectrum of synucleinopathies. *Movement Disorders: Official Journal of the Movement Disorder Society, 18*(Suppl 6), S2–S12.

Jellinger, K. A., & Lantos, P. L. (2010). Papp-Lantos inclusions and the pathogenesis of multiple system atrophy: An update. *Acta Neuropathologica, 119*, 657–667.

Johnston, J. A., Ward, C. L., & Kopito, R. R. (1998). Aggresomes: A cellular response to misfolded proteins. *The Journal of Cell Biology, 143*, 1883–1898.

Jomova, K., Vondrakova, D., Lawson, M., & Valko, M. (2010). Metals, oxidative stress and neurodegenerative disorders. *Molecular and Cellular Biochemistry, 345*, 91–104.

Ju, J. A., Miller, S. E., Hanson, P. I., & Weihl, C. C. (2008). Impaired protein aggregate handling and clearance underlie the pathogenesis of p97/VCP-associated disease. *The Journal of Biological Chemistry, 283*, 30289–30299.

Kaushik, S., Bandyopadhyay, U., Sridhar, S., Kiffin, R., Martinez-Vicente, M., Kon, M., Orenstein, S. J., Wong, E., & Cuervo, A. M. (2011). Chaperone-mediated autophagy at a glance. *Journal of Cell Science, 124*, 495–499.

Kawamoto, Y., Nakamura, S., Akiguchi, I., & Kimura, J. (1999). Increased brain-derived neurotrophic factor-containing axons in the basal ganglia of patients with multiple system atrophy. *Journal of Neuropathology and Experimental Neurology, 58*, 765–772.

Kawamoto, Y., Nakamura, S., Matsuo, A., & Akiguchi, I. (2000). Glial cell line-derived neurotrophic factor-like immunoreactivity in the cerebella of normal subjects and patients with multiple system atrophy. *Acta Neuropathologica, 100*, 131–137.

Kawamoto, Y., Akiguchi, I., Shirakashi, Y., Honjo, Y., Tomimoto, H., Takahashi, R., & Budka, H. (2007). Accumulation of Hsc70 and Hsp70 in glial cytoplasmic inclusions in patients with multiple system atrophy. *Brain Research, 1136*, 219–227.

Kiely, A. P., Asi, Y. T., Kara, E., Limousin, P., Ling, H., Lewis, P., Proukakis, C., Quinn, N., Lees, A. J., Hardy, J., Revesz, T., Houlden, H., & Holton, J. L. (2013). α-synucleinopathy associated with G51D SNCA mutation: A link between Parkinson's disease and multiple system atrophy? *Acta Neuropathologica, 125*, 753–769.

Kikuchi, A., Takeda, A., Onodera, H., Kimpara, T., Hisanaga, K., Sato, N., Nunomura, A., Castellani, R. J., Perry, G., Smith, M. A., & Itoyama, Y. (2002). Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiology of Disease, 9*, 244–248.

Klionsky, D. J., Baehrecke, E. H., Brumell, J. H., Chu, C. T., Codogno, P., Cuervo, A. M., Debnath, J., Deretic, V., Elazar, Z., Eskelinen, E. L., Finkbeiner, S., Fueyo-Margareto, J., Gewirtz, D., Jäättelä, M., Kroemer, G., Levine, B., Melia, T. J., Mizushima, N., Rubinsztein, D. C., Simonsen, A., Thorburn, A., Thumm, M., & Tooze, S. A. (2011). *Autophagy, 7*, 1273–1294.

Konno, M., Hasegawa, T., Baba, T., Miura, E., Sugeno, N., Kikuchi, A., Fiesel, F. C., Sasaki, T., Aoki, M., Itoyama, Y., & Takeda, A. (2012). Suppression of dynamin GTPase decreases alpha-synuclein uptake by neuronal and oligodendroglial cells: A potent therapeutic target for synucleinopathy. *Molecular Neurodegeneration, 71*, 38.

Kopito, R. R. (2000). Aggresomes, inclusion bodies and protein aggregation. *Trends in Cell Biology, 10*, 524–530.

Kostenko, S., & Moens, U. (2009). Heat shock protein 27 phosphorylation: Kinases, phosphatases, functions and pathology. *Cellular and Molecular Life Science, 66*, 3289–3307.

Kragh, C. L., Lund, L. B., Febbraro, F., Hansen, H. D., Gai, W. P., El-Agnaf, O., Richter-Landsberg, C., & Jensen, P. H. (2009). Alpha-synuclein aggregation and Ser-129 phosphorylation-dependent cell death in oligodendroglial cells. *The Journal of Biological Chemistry, 284*, 10211–10222.

Kragh, C. L., Fillon, G., Gysbers, A., Hansen, H. D., Neumann, M., Richter-Landsberg, C., Haass, C., Zalc, B., Lubetzki, C., Gai, W. P., Halliday, G. M., Kahle, P. J., & Jensen, P. H. (2013). FAS-dependent cell death in α-synuclein transgenic oligodendrocyte models of multiple system atrophy. *PLoS One, 8*(1), e55243.

Krumova, P., Meulmeester, E., Garrido, M., Tirard, M., Hsiao, H. H., Bossis, G., Urlaub, H., Zweckstetter, M., Kügler, S., Melchior, F., Bähr, M., & Weishaupt, J. H. (2011). Sumoylation inhibits alpha-synuclein aggregation and toxicity. *Journal of Cell Biology, 194*, 49–60.

Krumova, P., Weishaupt, J.H. (2012). Sumoylation in neurodegenerative diseases. *Cellular and Molecular Life Sciences, 70*, 2123–2138.

Kulathu, Y., & Komander, D. (2012). Atypical ubiquitylation – The unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nature Reviews. Molecular Cell Biology, 13*, 508–523.

Lavedan, C. (1998). The synuclein family. *Genome Research, 8*, 871–880.

Lee, J. A. (2009). Autophagy in neurodegeneration: Two sides of the same coin. *BMB Report, 42*, 324–330.

Lee, S. J., & Koh, J. Y. (2010). Roles of zinc and metallothionein-3 in oxidative stress-induced lysosomal dysfunction, cell death, and autophagy in neurons and astrocytes. *Molecular Brain, 3*(1), 30.

Lee, S. J., Cho, K. S., Kim, H. N., Kim, H. J., & Koh, J. Y. (2011). Role of zinc metallothionein-3 (ZnMt3) in epidermal growth factor (EGF)-induced c-Abl protein activation and actin polymerization in cultured astrocytes. *The Journal of Biological Chemistry, 286*, 40847–40856.

Leong, S. L., Cappai, R., Barnham, K. J., & Pham, C. L. (2009). Modulation of alpha-synuclein aggregation by dopamine: A review. *Neurochemistry Research, 34*, 1838–1846.

Lesage, S., & Brice, A. (2009). Parkinson's disease: From monogenic forms to genetic susceptibility factors. *Human Molecular Genetics, 18*(R1), R48–R59.

Lindersson, E., Beedholm, R., Højrup, P., Moos, T., Gai, W., Hendil, K. B., & Jensen, P. H. (2004). Proteasomal inhibition by alpha-synuclein filaments and oligomers. *The Journal of Biological Chemistry, 279*, 12924–12934.

Lindersson, E., Lundvig, D., Petersen, C., Madsen, P., Nyengaard, J. R., Højrup, P., Moos, T., Otzen, D., Gai, W. P., Blumbergs, P. C., & Jensen, P. H. (2005). p25alpha stimulates alpha-synuclein aggregation and is co-localized with aggregated alpha-synuclein in alpha-synucleinopathies. *The Journal of Biological Chemistry, 280*, 5703–5715.

Lowe, R., Pountney, D. L., Jensen, P. H., Gai, W. P., & Voelcker, N. H. (2004). Calcium(II) selectively induces alpha-synuclein annular oligomers via interaction with the C-terminal domain. *Protein Science, 13*, 3245–3252.

Manso, Y., Adlard, P. A., Carrasco, J., Vašák, M., & Hidalgo, J. (2011). Metallothionein and brain inflammation. *Journal of Biological Inorganic Chemistry, 16*, 1103–1113.

Marpillat, N. (2013). Prevalence of rare diseases: Bibliographic data, Orphanet Report Series, http://www.orpha.net/orphacom/cahiers/docs/GB/Prevalence_of_rare_diseases_by_alphabetical_list.pdf.

Marques, O., & Outeiro, T. F. (2012). Alpha-synuclein: From secretion to dysfunction and death. *Cell Death and Disease, 3*, e350. doi:"10.1038".

Martin, S., Wilkinson, K. A., Nishimune, A., & Henley, J. M. (2007). Emerging extranuclear roles of protein SUMOylation in neuronal function and dysfunction. *Nature Reviews. Neuroscience, 8*, 948–959.

Mattson, M. P. (2007). Calcium and neurodegeneration. *Aging Cell, 6*, 337–350.

Miller, D. W., Johnson, J. M., Solano, S. M., Hollingsworth, Z. R., Standaert, D. G., & Young, A. B. (2005). Absence of alpha-synuclein mRNA expression in normal and multiple system atrophy oligodendroglia. *Journal of Neural Transmission, 112*, 1613–1624.

Mosharov, E. V., Larsen, K. E., Kanter, E., Phillips, K. A., Wilson, K., Schmitz, Y., Krantz, D. E., Kobayashi, K., Edwards, R. H., & Sulzer, D. (2009). Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. *Neuron, 62*, 218–229.

Muchowski, P. J., & Wacker, J. L. (2005). Modulation of neurodegeneration by molecular chaperones. *Nature Reviews in Neuroscience, 6*, 11–22.

Nath, S., Goodwin, J., Engelborghs, Y., & Pountney, D. L. (2011). Raised calcium promotes α-synuclein aggregate formation. *Molecular and Cellular Neuroscience, 46*, 516–526.

Nemani, V. M., Lu, W., Berge, V., Nakamura, K., Onoa, B., Lee, M. K., Chaudhry, F. A., Nicoll, R. A., & Edwards, R. H. (2010). Increased expression of α-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron, 65*, 66–79.

Nielsen, M. S., Vorum, H., Lindersson, E., & Jensen, P. H. (2001). Ca^{2+} binding to α-synuclein regulates ligand binding and oligomerization. *The Journal of Biological Chemistry, 276*, 22680–22684.

Nishie, M., Mori, F., Yoshimoto, M., Takahashi, H., & Wakabayashi, K. (2004). A quantitative investigation of neuronal cytoplasmic and intranuclear inclusions in the pontine and inferior olivary nuclei in multiple system atrophy. *Neuropathology and Applied Neurobiology, 30*, 546–554.

Norris, E. H., & Giasson, B. I. (2005). Role of oxidative damage in protein aggregation associated with Parkinson's disease and related disorders. *Antioxidants and Redox Signalling, 7*, 672–684.

Olzmann, J. A., Li, L., Chudaev, M. V., Chen, J., Perez, F. A., Palmiter, R. D., & Chin, L. S. (2007). Parkin-mediated K63-linked polyubiquitination targets misfolded DJ-1 to aggresomes via binding to HDAC6. *The Journal of Cell Biology, 178*, 1025–1038.

Olzmann, J. A., Li, L., & Chin, L. S. (2008). Aggresome formation and neurodegenerative diseases: Therapeutic implications. *Current Medicinal Chemistry, 15*, 47–60.

Ouyang, H., Ali, Y. O., Ravichandran, M., Dong, A., Qiu, W., Mackenzie, F., Dhe-Paganon, S., Arrowsmith, C. H., & Zhai, R. G. (2012). Protein aggregates are recruited to aggresome by histone deacetylase 6 via unanchored ubiquitin C termini. *The Journal of Biological Chemistry, 287*, 2317–2327.

Ozawa, T. (2006). Pathology and genetics of multiple system atrophy: an approach to determining genetic susceptibility spectrum. *Acta Neuropathologica, 112*, 531–538.

Ozawa, T., Paviour, D., Quinn, N. P., Josephs, K. A., Sangha, H., Kilford, L., Healy, D. G., Wood, N. W., Lees, A. J., Holton, J. L., & Revesz, T. (2004). The spectrum of pathological involvement of the striatonigral and olivopontocerebellar systems in multiple system atrophy: Clinicopathological correlations. *Brain, 127*, 2657–2671.

Papp, M. I., & Lantos, P. L. (1994). The distribution of oligodendroglial inclusions in multiple system atrophy and its relevance to clinical symptomatology. *Brain, 117*, 235–243.

Papp, M. I., Kahn, J. E., & Lantos, P. L. (1989). Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). *Journal of Neurological Sciences, 94*(1–3), 79–100.

Pellecchia, M. T., Pivonello, R., Longo, K., Manfredi, M., Tessitore, A., Amboni, M., Pivonello, C., Rocco, M., Cozzolino, A., Colao, A., & Barone, P. (2010). Multiple system atrophy is associated with changes in peripheral insulin-like growth factor system. *Movement Disorders: Official Journal of the Movement Disorder Society, 25*, 2621–2626.

Perlmutter, J. D., Braun, A. R., & Sachs, J. N. (2009). Curvature dynamics of alpha-synuclein familial Parkinson disease mutants: Molecular simulations of the micelle- and bilayer-bound forms. *Journal of Biological Chemistry, 284*, 7177–7189.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., & Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science, 276*, 2045–2047.

Pountney, D. L., Huang, Y., Burns, R. J., Haan, E., Thompson, P. D., Blumbergs, P. C., & Gai, W. P. (2003). SUMO-1 marks the nuclear inclusions in familial neuronal intranuclear inclusion disease. *Experimental Neurology, 184*, 436–446.

Pountney, D. L., Lowe, R., Quilty, M., Vickers, J. C., Voelcker, N. H., & Gai, W. P. (2004). Annular alpha-synuclein species from purified multiple system atrophy inclusions. *Journal of Neurochemistry, 90*, 502–512.

Pountney, D. L., Voelcker, N. H., & Gai, W. P. (2005a). Annular α-synuclein oligomers are potentially toxic agents in α-synucleinopathy. *Neurotoxicity Research, 7*, 59–67.

Pountney, D. L., Treweek, T. M., Chataway, T., Huang, Y., Chegini, F., Blumbergs, P. C., Raftery, M. J., & Gai, W. P. (2005b). Alpha B-crystallin is a major component of glial cytoplasmic inclusions in multiple system atrophy. *Neurotoxicity Research, 7*(1–2), 77–85.

Pountney, D. L., Chegini, F., Shen, X., Blumbergs, P. C., & Gai, W. P. (2005c). SUMO-1 marks subdomains within glial cytoplasmic inclusions of multiple system atrophy. *Neuroscience Letters, 381*, 74–79.

Pountney, D. L., Raftery, M. J., Chegini, F., Blumbergs, P. C., & Gai, W. P. (2008). NSF, Unc-18-1, dynamin-1 and HSP90 are inclusion body components in neuronal intranuclear inclusion disease identified by anti-SUMO-1-immunocapture. *Acta Neuropathologica, 116*, 603–614.

Pountney, D. L., Dickson, T. C., Power, J. H., Vickers, J. C., West, A. K., & Gai, W. P. (2011). Association of metallothionein-III with oligodendroglial cytoplasmic inclusions in multiple system atrophy. *Neurotoxicity Research, 19*, 115–122.

Putcha, P., Danzer, K. M., Kranich, L. R., Scott, A., Silinski, M., Mabbett, S., Hicks, C. D., Veal, J. M., Steed, P. M., Hyman, B. T., & Mclean, P. J. (2010). Brain-permeable small-molecule inhibitors of Hsp90 prevent α-synuclein oligomer formation and rescue α-synuclein-induced toxicity. *The Journal of Pharmacology and Experimental Therapeutics, 332*, 849–857.

Qing, H., Wong, W., McGeer, E. G., & McGeer, P. L. (2009). Lrrk2 Phosphorylates alpha synuclein at serine 129: Parkinson disease implications. *Biochemical Biophysical Research Communications, 387*, 149–152.

Radford, R., Guillemin, G., & Pountney, D. L. (unpublished data).

Rami, A. (2009). Review: Autophagy in neurodegeneration: Firefighter and/or incendiarist? *Neuropathology and Applied Neurobiology, 35*, 449–461.

Rekas, A., Adda, C. G., Aquilina, J. A., Barnham, K. J., Sunde, M., Galatis, D., Williamson, N. A., Masters, C. L., Anders, R. F., Robinson, C. V., Cappai, R., & Carver, J. A. (2004). Interaction of the molecular chaperone alphaB-crystallin with alpha-synuclein: Effects on amyloid fibril formation and chaperone activity. *Journal of Molecular Biology, 340*, 1167–1183.

Richter-Landsberg, C., Gorath, M., Trojanowski, J. Q., & Lee, V. M. (2000). α-synuclein is developmentally expressed in cultured rat brain oligodendrocytes. *Journal of Neuroscience Research, 62*, 9–14.

Rott, R., Szargel, R., Haskin, J., Bandopadhyay, R., Lees, A. J., Shani, V., & Engelender, S. (2011). α-Synuclein fate is determined by USP9X-regulated monoubiquitination. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 18666–18671.

Ruipérez, V., Darios, F., & Davletov, B. (2010). Alpha-synuclein, lipids and Parkinson's disease. *Progress in Lipid Research, 49*, 420–428.

Sahu, R., Kaushik, S., Clement, C. C., Cannizzo, E. S., Scharf, B., Follenzi, A., Potolicchio, I., Nieves, E., Cuervo, A. M., & Santambrogio, L. (2011). Microautophagy of cytosolic proteins by late endosomes. *Developmental Cell, 20*, 131–139.

Schildknecht, S., Gerding, H. R., Karreman, C., Drescher, M., Lashuel, H. A., Outeiro, T. F., Di Monte, D. A., & Leist, M. (2013). Oxidative and nitrative alpha-synuclein modifications and proteostatic stress: Implications for disease mechanisms and interventions in synucleinopathies. *Journal of Neurochemistry, 125*, 491–511.

Schmidt, F., Levin, J., Kamp, F., Kretzschmar, H., Giese, A., & Botzel, K. (2012). Single-channel electrophysiology reveals a distinct and uniform pore complex formed by α-synuclein oligomers in lipid membranes. *PLoS One, 7*(8), e42545.

Schrag, A., Ben-Shlomo, Y., & Quinn, N. P. (1999). Prevalence of progressive supranuclear palsy and multiple system atrophy: A cross-sectional study. *Lancet, 354*, 1771–1775.

Schwarz, L., Goldbaum, O., Bergmann, M., Probst-Cousin, S., & Richter-Landsberg, C. (2012). Involvement of macroautophagy in multiple system atrophy and protein aggregate formation in oligodendrocytes. *Journal of Molecular Neuroscience, 47*, 256–266.

Shibata, N., Inose, Y., Toi, S., Hiroi, A., Yamamoto, T., & Kobayashi, M. (2010). Involvement of 4-hydroxy-2-nonenal accumulation in multiple system atrophy. *Acta Histochemica Cytochemica, 43*, 69–75.

Shimura, H., Schlossmacher, M. G., Hattori, N., Frosch, M. P., Trockenbacher, A., Schneider, R., Mizuno, Y., Kosik, K. S., & Selkoe, D. J. (2001). Ubiquitination of a new form of alpha-synuclein by parkin from human brain: Implications for Parkinson's disease. *Science, 293*, 263–269.

Snyder, H., Mensah, K., Theisler, C., Lee, J., Matouschek, A., & Wolozin, B. (2003). Aggregated and monomeric alpha-synuclein bind to the S6' proteasomal protein and inhibit proteasomal function. *The Journal of Biological Chemistry, 278*, 11753–11759.

Sofroniew, M. V. (2009). Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in Neurosciences, 32*, 638–647.

Song, Y. J., Lundvig, D. M., Huang, Y., Gai, W. P., Blumbergs, P. C., Højrup, P., Otzen, D., Halliday, G. M., & Jensen, P. H. (2007). p25alpha relocalizes in oligodendroglia from myelin to cytoplasmic inclusions in multiple system atrophy. *The American Journal of Pathology, 171*, 1291–1303.

Song, Y. J., Halliday, G. M., Holton, J. L., Lashley, T., O'Sullivan, S. S., McCann, H., Lees, A. J., Ozawa, T., Williams, D. R., Lockhart, P. J., & Revesz, T. R. (2009). Degeneration in different parkinsonian syndromes relates to astrocyte type and astrocyte protein expression. *Journal of Neuropathology and Experimental Neurology, 68*, 1073–1083.

Spillantini, M. G., & Goedert, M. (2000). The alpha-synucleinopathies: Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. *Annals of the New York Academy of Sciences, 920*, 16–27.

Stefanova, N., Reindl, M., Neumann, M., Haass, C., Poewe, W., Kahle, P. J., & Wenning, G. K. (2005). Oxidative stress in transgenic mice with oligodendroglial alpha-synuclein overexpression replicates the characteristic neuropathology of multiple system atrophy. *The American Journal of Pathology, 166*, 869–876.

Stefanova, N., Reindl, M., Neumann, M., Kahle, P. J., Poewe, W., & Wenning, G. K. (2007). Microglial activation mediates neurodegeneration related to oligodendroglial alpha-synucleinopathy: Implications for multiple system atrophy. *Movement Disorders, 22*, 2196–2203.

Stefanova, N., Bücke, P., Duerr, S., & Wenning, G. K. (2009). Multiple system atrophy: An update. *Lancet Neurology, 8*, 1172–1178.

Stefanova, N., Kaufmann, W. A., Humpel, C., Poewe, W., & Wenning, G. K. (2012). Systemic proteasome inhibition triggers neurodegeneration in a transgenic mouse model expressing human α-synuclein under oligodendrocyte promoter: Implications for multiple system atrophy. *Acta Neuropathologica, 124*, 51–65.

Stemberger, S., Scholz, S. W., Singleton, A. B., & Wenning, G. K. (2011) Genetic players in multiple system atrophy: Unfolding the nature of the beast. *Neurobiology of Aging, 32*, 1924.e5–14.

Su, M., Yoshida, Y., Hirata, Y., Watahiki, Y., & Nagata, K. (2001). Primary involvement of the motor area in association with the nigrostriatal pathway in multiple system atrophy: Neuropathological and morphometric evaluations. *Acta Neuropathologica, 101*, 57–64.

Su, W. H., Lukiw, W. J., Bergeron, C., Niznik, H. B., & Fraser, P. E. (2001). Metallothionein III is reduced in Alzheimer's disease. *Brain Research, 894*, 37–45.

Suntharalingam, A., Abisambra, J. F., O'leary, J. C., 3rd, Koren, J., 3rd, Zhang, B., Joe, M. K., Blair, L. J., Hill, S. E., Jinwal, U. K., Cockman, M., Duerfeldt, A. S., Tomarev, S., Blagg, B. S., Lieberman, R. L., & Dickey, C. A. (2012). Grp94 triage of mutant myocilin through ERAD subverts a more efficient autophagic clearance mechanism. *The Journal of Biological Chemistry, 287*(48), 40661–40669.

Sulzer, D. (2010). Clues to how alpha-synuclein damages neurons in Parkinson's disease. *Movement Disorders, 25*(Suppl 1), S27–S31.

Taylor, J. P., Tanaka, F., Robitschek, J., Sandoval, C. M., Taye, A., Markovic-Plese, S., & Fischbeck, K. H. (2003). Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. *Human Molecular Genetics, 12*, 749–757.

Taylor, J. M., Brody, K. M., & Lockhart, P. J. (2012). Parkin Co-Regulated Gene is involved in aggresome formation and autophagy in response to proteasomal impairment. *Experimental Cell Research, 318*, 2059–2070.

Thomas, M., Harrell, J. M., Morishima, Y., Peng, H. M., Pratt, W. B., & Lieberman, A. P. (2006). Pharmacologic and genetic inhibition of hsp90-dependent trafficking reduces aggregation and promotes degradation of the expanded glutamine androgen receptor without stress protein induction. *Human Molecular Genetics, 15*, 1876–1883.

Trempe, J. F. (2011). Reading the ubiquitin postal code. *Current Opinion in Structural Biology, 21*, 792–801.

Tsuji, S., & Members of the Multiple-System Atrophy Research Collaboration. (2013). Mutations in *COQ2* in familial and sporadic multiple-system atrophy. *New England Journal of Medicine*. doi:10.1056/NEJMoa1212115.

Ubhi, K., Lee, P. H., Adame, A., Inglis, C., Mante, M., Rockenstein, E., Stefanova, N., Wenning, G. K., & Masliah, E. (2009). Mitochondrial inhibitor 3-nitroproprionic acid enhances oxidative modification of alpha-synuclein in a transgenic mouse model of multiple system atrophy. *Journal of Neuroscience Research, 87*, 2728–2739.

Uchida, Y., Takio, K., Titani, K., Ihara, Y., & Tomonaga, M. (1991). The growth inhibitory factor that is deficient in the Alzheimer's disease brain is a 68 amino acid metallothionein-like protein. *Neuron, 7*, 337–347.

Uryu, K., Richter-Landsberg, C., Welch, W., Sun, E., Goldbaum, O., Norris, E. H., Pham, C. T., Yazawa, I., Hilburger, K., Micsenyi, M., Giasson, B. I., Bonini, N. M., Lee, V. M., & Trojanowski, J. Q. (2006). Convergence of heat shock protein 90 with ubiquitin in filamentous alpha-synuclein inclusions of alpha-synucleinopathies. *The American Journal of Pathology, 168*, 947–961.

Uversky, V. N. (2009). Intrinsic disorder in proteins associated with neurodegenerative diseases. *Frontiers in Bioscience, 14*, 5188–5238.

Uversky, V. N., & Eliezer, D. (2009). Biophysics of Parkinson's disease: Structure and aggregation of α-synuclein. *Current Protein and Peptide Science, 10*, 483–499.

Vasák, M. (2005). Advances in metallothionein structure and functions. *Journal of Trace Elements in Medicine and Biology, 19*, 13–17.

Vekrellis, K., & Stefanis, L. (2012). Targeting intracellular and extracellular alpha-synuclein as a therapeutic strategy in Parkinson's disease and other synucleinopathies. *Expert Opinion on Therapeutic Targets, 16*, 421–432.

Verkhratsky, A., Sofroniew, M. V., Messing, A., Delanerolle, N. C., Rempe, D., Rodríguez, J. J., & Nedergaard, M. (2012). Neurological diseases as primary gliopathies: A reassessment of neurocentrism. *ASN Neuro, 4*(3), 131–149. doi:"10.1042/AN20120010".

Vilar, M., Chou, H. T., Lührs, T., Maji, S. K., Riek-Loher, D., Verel, R., Manning, G., Stahlberg, H., & Riek, R. (2008) The fold of alpha-synuclein fibrils. *Proceedings of the National Academy of Sciences USA*, *105*, 8637–8642.

Vilariño-Güell, C., Soto-Ortolaza, A. I., Rajput, A., Mash, D. C., Papapetropoulos, S., Pahwa, R., Lyons, K. E., Uitti, R. J., Wszolek, Z. K., Dickson, D. W., Farrer, M. J., & Ross, O. A. (2011). MAPT H1 haplotype is a risk factor for essential tremor and multiple system atrophy. *Neurology, 76*, 670–672.

Waudby, C. A., Knowles, T. P., Devlin, G. L., Skepper, J. N., Ecroyd, H., Carver, J. A., Welland, M. E., Christodoulou, J., Dobson, C. M., & Meehan, S. (2010). The interaction of alphaB-crystallin with mature alpha-synuclein amyloid fibrils inhibits their elongation. *Biophysical Journal, 98*, 843–851.

Wenning, G. K., & Jellinger, K. A. (2005). The role of alpha-synuclein in the pathogenesis of multiple system atrophy. *Acta Neuropathologica, 109*, 129–140.

Wenning, G. K., & Stefanova, N. (2009). Recent developments in multiple system atrophy. *Journal of Neurology, 256*, 1791–1808.

Wenning, G. K., Tison, F., Ben Shlomo, Y., Daniel, S. E., & Quinn, N. P. (1997). Multiple system atrophy: A review of 203 pathologically proven cases. *Movement Disorders: Official Journal of the Movement Disorder Society, 12*, 133–147.

Wenning, G. K., Colosimo, C., Geser, F., & Poewe, W. (2004). Multiple system atrophy. *Lancet Neurology, 3*, 93–103.

Wenning, G. K., Stefanova, N., Jellinger, K. A., Poewe, W., & Schlossmacher, M. G. (2008). Multiple system atrophy: A primary oligodendrogliopathy. *Annals of Neurology, 64*, 239–246.

West, A. K., Hidalgo, J., Eddins, D., Levin, E. D., & Aschner, M. (2008). Metallothionein in the central nervous system: Roles in protection, regeneration and cognition. *NeuroToxicology, 29*, 489–503.

Wong, E., & Cuervo, A. M. (2010). Autophagy gone awry in neurodegenerative diseases. *Nature Neuroscience, 13*, 805–811.

Wong, M. B., Goodwin, J., Norazit, A., Meedeniya, A. C. B., Richter-Landsberg, C., Gai, W. P., & Pountney, D. L. (2013). SUMO-1 is associated with a subset of lysosomes in glial protein aggregate diseases. *Neurotoxicity Research, 23*, 1–21.

Yamada, T., McGeer, P. L., Baimbridge, K. G., & McGeer, E. G. (1990). Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D28K. *Brain Research, 526*, 303–307.

Neurotoxic Vulnerability Underlying Schizophrenia Spectrum Disorders

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Contents

Abstract

Neurotoxic vulnerability that putatively contributes to the etiopathogenesis of schizophrenia spectrum disorders encompasses perinatal adversity, genetic linkage, epigenetic disadvantage, and neurodegenerative propensities that affect both symptom domains, positive, negative, and cognitive, and biomarkers of the disorder. Molecular and cellular apoptosis/excitotoxicity that culminates in regional brain loss, reductions in reelin expression, trophic disruption, perinatal adversity, glycogen synthase kinase-3 dysregulation, and various instances of

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oxidative stress all influence the final end point disorder. The existence of prodromal psychotic phases, structural–functional aspects of regional neuroimaging, dopamine signal overexpression, and psychosis propensity provide substance for neurodegenerative influences. The pathophysiology of schizophrenia spectrum disorder encompasses the destruction of normal functioning of the neurotrophins, in particular brain-derived neurotrophic factor (BDNF), dyskinesia of necessary movements, and metabolic–metabolomic and proteomic markers. Neurotoxic accidents combined with genetic susceptibility appear to play a role in interfering with normal neurodevelopment or in tissue-destructive neurodegeneration or both, thereby elevating the eventual risk for disorder tendencies and eventual expression.

Keywords
GFK • Imaging • Infection • MAM • Perinatal • Reelin • Trophic factors • Vulnerability

1 Introduction

The contributions of neurotoxic circumstances to the development, predisposition, precipitation, and eventual expression of schizophrenia spectrum disorders may be sought at different levels of etiopathophysiogenesis. Several lines of evidence imply that these disorders originate from the abnormal development of the central nervous system (CNS) peculiar to the human condition, as exemplified by the entorhinal cortex that is involved in widespread cognitive networks (Akil and Lewis 1997; Arnold et al. 1991, 1997; Falkai et al. 2003). These may be illustrated in several respects. Both structural and functional abnormalities are associated with development of schizophrenia spectrum disorders: for example, lateral ventricular and third and fourth ventricular enlargements accompanied by reduced prefrontal, thalamic, and hippocampal volume have been observed consistently in the brain of patients afflicted by the disorder (Welch et al. 2011). These deficiencies include smaller-sized hippocampus and entorhinal cortex both associated with reduced neuronal body sizes (Baiano et al. 2008; Jonsson et al. 1999; Turetsky et al. 2003; Zaidel et al. 1997). Alexander-Bloch et al. (2010) have observed disrupted modularity and local connectivity of brain functional networks in childhood-onset schizophrenia. It has been shown too that both neuropsychologically near normal and neuropsychologically impaired patients had markedly smaller gray matter and larger third ventricle volumes than healthy comparison subjects. However, only neuropsychologically impaired patients had significantly less white matter and larger lateral ventricle volumes than healthy comparison subjects (Wexler et al. 2009). Thoma et al. (2009) showed that neuropsychological-functioning schizophrenic patients were impaired overall, and anterior hippocampus volume was smaller in the patient group. In healthy controls, the hippocampus–memory relationships involved the anterior and posterior hippocampus wherein correlations were significant for verbal memory measures. Among the schizophrenia patients,

positive correlations were constrained to the posterior hippocampus. Negative correlations emerged between anterior hippocampus and verbal and visual memory measures. For both groups, cortical volume negatively correlated with age, but a negative correlation between age and hippocampus volume was found only among the schizophrenia patients. In their sample of adults with schizophrenia, atypical relationships between regional hippocampus volumes and episodic memory ability were found, as was an atypical negative association between hippocampus volume and age. Taken together, these diverse observations on the abnormal structural–functional relationships in schizophrenia spectrum disorders prompt notions of neurodegenerative disruptions during the early and later life cycles of brain development.

Imaging studies (positron emission tomography [PET]) or single photon emission tomography (SPET) and magnetic resonance imaging (MRI) attest to regional glutamatergic abnormalities in psychosis and are beginning to describe both the neurodevelopment of these abnormalities over the course of the illness and their response to therapeutic intervention (Egerton and Stone 2012). Fitzgerald (2012) discussed an N-methyl-D-aspartate Receptor (NMDAR) "suppression" hypothesis that focuses upon several aspects of evidence for NMDA influence in disorder: (1) epilepsy studies, (2) neurotoxicity studies, (3) mouse knockout studies of particular receptor subunits, (4) electrophysiological studies, (5) 2-deoxyglucose studies, and (6) functional brain imaging studies. Further, the activation of glycogen synthase kinase-3 (GSK-3) has been implicated in the cellular neurotoxicity of NMDAR antagonists (Large et al. 2011), with GSK-3 also implicated in schizophrenia (Brambrink et al. 2012; Pollard et al. 2012). Chan et al. (2012) have shown the critical role of glycogen synthase kinase-3 (GSK-3) in the expression of neurobehavioral aberrations associated with NMDAR hypofunction. They have suggested that GSK-3 inhibitors may ameliorate certain behavioral and cognitive dysfunctions in patients presenting schizophrenia. Neurotoxicity may be initiated by inflammatory agents: as described by Burd et al. (2012), infection-induced maternal immune activation leads to a fetal inflammatory response mediated through cytokines and are implicated in the pathophysiology of not only periventricular leukomalacia and cerebral palsy but also a spectrum of neurodevelopmental disorders, including attention-deficit hyperactivity disorder (ADHD), autism, and schizophrenia. Alternatively, Marona-Lewicka et al. (2011) have proposed an LSD model of schizophrenia whereby the chronic treatment of rats with low doses of LSD can serve as a new animal model of psychosis that may mimic the development and progression of the disorder.

Neurotoxic influences in schizophrenia spectrum disorders, to a large extent, would appear to be based upon circumstantial evidence rather than hard neurodegenerative loss of essential tissue, thereby rendering the associations somewhat tenuous. Notwithstanding these obvious constraints, there exists a sufficiency of observations from laboratory animal model studies, neuroimaging studies, and postmortem assays together with a neurodevelopmental viewpoint of disorder etiopathogenesis, suggesting that neurodegenerative processes contribute to heightened predisposition and susceptibility to the precipitation of symptom domains through a staging notion (Archer et al. 2010a, b). Genetic risk factors may impact

upon or disrupt developmental trajectories (Dauvermann et al. 2012; Sullivan et al. 2003). Indeed, genetic predispositions for neurodegenerative influences may be quite likely, since despite the absence of astrogliosis, impaired expression of astrocyte- and oligodendrocyte-related genes was observed in patients with major psychiatric disorders, including schizophrenia and mood disorders (Steiner et al. 2011). There is much support for the notion that schizophrenia spectrum disorders have their origin during gestation and/or in early infancy (Graff et al. 2011; Sawa and Snyder 2002). Insults during the early infancy or perinatal period, or even during the course of early-to-late childhood and adolescence, are implicated in the etiopathogenesis of the marked structural and functional abnormalities that distinguish the symptoms of disorder (Cannon et al. 2002a, b; Fontes et al. 2011; Geddes et al. 1999). The eventual contribution of neurodegenerative processes affecting the disorder must take into account the plethora of interactive factors and agents that include inherited and acquired attributes (Elia et al. 2011; Jablonka and Raz 2009). The disorder pathophysiology may involve the influences of several contributory processes, including cellular apoptosis/excitotoxicity, viral and bacterial infections, anoxic birth injury, maternal starvation, and other conditions exerting detrimental effects on neurogenesis. Both neurogenetic and epigenetic forces arising from gene-environment interactions affecting developmental trajectories impart levels of predisposing vulnerability that may or may not lead to the manifestation of schizophrenic symptoms (Gratacos et al. 2007; Hoenicka et al. 2010) or those of related conditions (Bergman et al. 2011; Jacob et al. 2010). Whether or not the neurodegenerative aspect of the disorder may be neurogenetic or epigenetic, there does exist compelling evidence for expressions of the disruptive influence affecting normal development.

2 Regional Loss

At first sight, the accumulated evidence for progressive volumetric loss, e.g., in cortical volume/thickness (Honea et al. 2008; Goldman et al. 2009) and loss of functional connectivity (Meyer-Lindenberg et al. 2005; Yu et al. 2011), argues for the aspect of neurodegeneration in schizophrenia. Yet the possible impact of peristaltic and environmental agents must be understood prior to deriving a reliable conclusion (Meyer-Lindenberg 2011). For example, Goto et al. (2010) compared 18 patients (9 males, 9 females; age range: 13–52 years) with 18 healthy volunteers (9 males, 9 females; age range: 15–49 years) with no current or past psychiatric history, using magnetic resonance spectroscopy (MRS). They found that levels of *N*-acetylaspartate/Cr in the left basal ganglia and parieto-occipital lobe, but not in the frontal lobe, were significantly lower in patients with first-episode schizophrenia psychosis than in control subjects. No differences were observed between the serum brain-derived neurotrophic factor levels of patients with first-episode schizophrenia psychosis and the healthy control subjects. The plasma levels of catecholamine metabolites, plasma 3-methoxy-4-hydroxyphenylglycol (MHPG), but not homovanillic acid (HVA), were

significantly lower in the patients with first-episode psychosis than in control subjects. In addition, a significantly positive correlation was observed between the levels of *N*-acetylaspartate/Cr of the left basal ganglia and plasma MHPG in all subjects. The authors concluded that brain *N*-acetylaspartate levels in the left basal ganglia and plasma MHPG levels were significantly reduced at the first episode of schizophrenia psychosis, indicating that neurodegeneration via noradrenergic neurons might be associated with the initial progression of the disease.

Regional loss of glutamate and glutamine in schizophrenic patients (Tayoshi et al. 2009) may offer evidence of an excitotoxic process in the disorder pathophysiology. The loss of parvalbumin-containing cells, an observation that has been reported consistently in postmortem analysis of the brains of schizophrenic patients following NMDAR blockade, offers support for this notion (Adell et al. 2012; Dean et al. 2011). Aoyama et al. (2011) have demonstrated that the loss of thalamic glutamate in the brain of schizophrenia patients correlated significantly with loss of gray matter in the middle and inferior frontal gyrus and temporal pole. Thalamic gray matter and glutamate losses were observed in individuals at risk for developing schizophrenia (Stone et al. 2009). Glutamate-glutamine ratios in the frontal cortices of adolescents at risk for the disorder are correlated, as well as performance scores on the Global Assessment of Functioning Scale (Tibbo et al. 2004). It has been suggested that the elevated glutamine levels in never-treated patients followed by the decreased level of thalamic glutamine and gray matter loss in connected regions may be indicative of either neurodegeneration or a plastic response to reduced subcortical activity (Theberge et al. 2007). There appears to be a consensus of sorts for an excitotoxic contribution primarily in early stage schizophrenia (Bustillo et al. 2010). Glutamatergic alterations and loss of cortical gray matter originating from basal ganglia-corticothalamic circuits may be the expressions of excitotoxicity through these regions. First-episode schizophrenic patients have exhibited increased membrane breakdown in magnetic resonance spectroscopy applying labeled phosphorous (Miller et al. 2009).

3 Perinatal Adversity

Several perinatal insults have been shown to induce schizophrenia-like symptoms in a laboratory setting (Archer 2010). Bilateral excitotoxin-induced lesions, administering ibotenic acid to the ventral hippocampus typically to 7-day-old rat pups, induces disruptions of prepulse inhibition (PPI) to the acoustic startle response, a model of sensory gating in rodents, hyper-responsiveness to stressful stimuli, supersensitivity to dopamine (DA) agonists, and NMDAR antagonists (Lipska et al. 1992, 1995, 2001; Tseng et al. 2007, 2009). Additionally, persistent deficits in working memory and spatial navigational learning (O'Donnell et al. 2002), together with hyper-responsiveness to stimulation of the ventral tegmental area (Lillrank et al. 1999; Lipska and Weinberger 1994), have all been observed in neonatal ventral hippocampus-lesioned rats. Prenatal administration of methylazoxymethanol (MAM) disrupts early brain development with structural and functional

abnormalities in the cortex and hippocampus, many of which bear similarities to schizophrenia, expressed in the adult rats (Flagstad et al. 2005; Lodge and Grace 2008; Mohammed et al. 1986a, b; Moore et al. 2006). MAM induces programmed cell death in neuroepithelial-proliferating cells (Cattabeni and Di Luca 1997), without causing any teratogenic effects in any other organs (Balduini et al. 1991). It is generally administered on embryonic, gestation, day 17 (E17) as this is when neurogenesis peaks in the hippocampal formation (Bayer and Altman 2004), and the time when projections between the hippocampus and entorhinal cortex are established (Ceranik et al. 1999). Hippocampal formation disruption is central to schizophrenia pathophysiology (Harrison 2004). The utility of prenatal MAM as an animal model of the disorder highlights the neurodegenerative aspect (Chen and Hillman 1986; Ferguson and Holson 1997; Ferguson et al. 1996; Virgili et al. 1997), particularly with regard to the neurocognitive deficits (Fiore et al. 2002; Gourevitch et al. 2004; Lee and Rabe 1992; Leng et al. 2005). The model has been validated by several studies (cf. Gill et al. 2011; Hradetzky et al. 2011; Lodge and Grace 2012; Valenti et al. 2011). Neurophysiological studies confirm that MAM-treated rats show an augmented DA system function consistent with enhanced DA signaling underlying positive symptoms of disorder and defective hippocampal interneurons (Konradi et al. 2011; Lahti et al. 2006). Similar to patients presenting cognitive deficit profiles in schizophrenia spectrum disorder (Karilampi et al. 2007, 2011), MAM induces marked deficits on an attentional-shift task (analogous to the Wisconsin Card Sorting Task) and on a differential reinforcement of low rate of responding (DRL-20 performance, analogous to continuous performance) task (Featherstone et al. 2007). Prenatal MAM administration disrupts early cortical development causing deficits in medial prefrontal functions at adult ages (Goto and Grace 2006). Sanderson et al. (2012) obtained a reduction in synaptic innervation and synaptic transmission in the dorsal hippocampus of MAM (E17)-treated rats, accompanied by a pronounced increase in CA1 pyramidal neuron excitability. These effects of the prenatally administered neurotoxin interrupt normal neuronal development, stress and immune response systems, and signal transduction mechanisms (Ciani et al. 2003; Ferrer et al. 1997; Lafarga et al. 1997; Rice and Barone 2000), thereby reinforcing its utility as a model of schizophrenia (Gill et al. 2011; Le Pen et al. 2011) and implying the neurotoxic aspect of the disorder.

Traumatic/adverse experiences during infancy and early childhood compromise healthy brain development in several domains, including cognitive, emotional, and motor. Traumatic environmental confrontation during early neurodevelopment, such as parental loss (maternal separation), that induces a marked provocation of the hypothalamic–pituitary–adrenal (HPA) axis was found to cause schizophrenia-like deficits, e.g., impairments of sensory gating, symptoms in the rats as adults (Ellenbroek et al. 1998), as well as other expression of the disorder (Furukawa et al. 1998). Rosenberg et al. (2007) surveyed 569 adults presenting schizophrenia with regard to adverse childhood events (including physical abuse, sexual abuse, parental mental illnesses, loss of a parent, parental separation or divorce, witnessing domestic violence, and foster or kinship care), evaluating the relationships between cumulative exposure to these events and psychiatric, physical, and functional

outcomes. They found that increased exposure to adverse childhood events was strongly related to psychiatric problems in both cognitive and emotional domains (suicidal thinking, hospitalizations, distress, and posttraumatic stress disorder), substance abuse, physical health problems (HIV infection), medical service utilization (physician visits), and poor social and daily care functioning (homelessness or criminal justice involvement). Similarly, Morgan et al. (2007) observed that separation from, and/or the death of, one parent prior to the age of 16 years was strongly associated with a twofold to threefold increases in the risk of psychosis. It was shown that the strength of these associations was similar for White British and Black Caribbean (but not Black African) subjects, although separation from (but not death of) a parent was more common among Black Caribbean controls than White British controls. Finally, a 10-year cohort of high security hospital patients who had either a personality disorder or schizophrenia found a rate of child–parent separation of 178/289 (62 %) in the schizophrenia group compared with a rate of 119/147 (81 %) in the personality disorder group of patients that had been separated from one or both parents before the age of 16 (Pert et al. 2004). Several studies have described the extreme adverse effects of perinatal starvation/malnutrition involving severe deficits of necessary nutrients, including folate, essential fatty acids, retinoids, vitamin D, and iron, the "nutritional hypothesis of schizophrenia" (Brown and Susser 2008). Additionally, elevated prenatal homocysteine level provides a plausible risk factor for schizophrenia because of its partial antagonism of *N*-methyl-D-aspartate receptors under physiologic glycine concentrations and its association with abnormal placental function and pregnancy complications (Bleich et al. 2007; Brown and Susser 2005; Brown et al. 2007).

An abundance of clinical and preclinical (animal modeling) findings posit the notion that immune system dysfunction(s) – related to genetic, environmental, and neurobiological influences – may play a role in the etiology of schizophrenia in a subset of patients (Richard and Brahm 2012). Exposure to prenatal immune challenge induces early pre- and postnatal alterations in peripheral and central inflammatory response systems. Consequentially, these alterations may disrupt the normal development and maturation of neuronal systems from juvenile to adult stages of life, thereby affecting brain structure and function. Developmental neuroinflammatory processes of this type may adversely affect processes that are pivotal for normal brain maturation, including myelination, synaptic pruning, and neuronal remodeling, all of which occur to a great extent during postnatal brain maturation (Meyer 2011). Brown and co-workers (Brown and Derkits 2010; Brown and Patterson 2011; Brown et al. 2009, 2011) and others (Arias et al. 2012; Nielsen et al. 2011; Tedla et al. 2011) have conducted birth cohort studies to address whether in utero exposure to infectious agents, prospectively documented by biomarker assays of archived maternal sera and by detailed obstetric records, confers an increased risk of schizophrenia in adult offspring. Prenatal exposure to influenza, elevated toxoplasma antibody, rubella, genital/reproductive infections, and other infections have been associated with an increased risk of schizophrenia among offspring. Animal models have supported these epidemiologic findings by revealing that maternal immune activation causes phenotypes analogous to those

found in patients with schizophrenia (Brown 2011). In a population-based study, Benros et al. (2011) have shown that autoimmune disease conditions and the number of infections requiring hospitalization pose risk factors for schizophrenia. Increased risk for the disorder was compatible with an immunological hypothesis in subgroups of schizophrenia patients.

4 Reelin Reduction

Reelin, present throughout the brain, spinal cord, blood, and other organs and tissues, is a large secreted extracellular matrix glycoprotein that facilitates regulation of processes involving neuronal migration and positioning in the developing brain by controlling cell–cell interactions and maintains its role in the adult brain. Reelin modulates synaptic plasticity and stimulates dendrite and dendritic spine development and regulates the continuing migration of neuroblasts generated at sites of adult neurogenesis (Niu et al. 2008). Expression of the protein is found to be significantly lower in schizophrenia and psychotic bipolar disorder (Maloku et al. 2010; Pisanté et al. 2009; Suzuki et al. 2008). Several studies have shown that in schizophrenia spectrum disorders, reelin expression in several brain regions, such as the cerebral cortex and hippocampus, is reduced (Eastwood and Harrison 2006; Fatemi et al. 2000; Impagnatiello et al. 1998). Reelin expression is reduced in the prefrontal cortex (PFC), hippocampus, caudate nucleus, and cerebellum of patients presenting psychotic illness (Guidotti et al. 2000; Tueting et al. 2006); GABAergic interneurons express reelin in several brain regions (Miyata et al. 2010; Pascual et al. 2004; Pesold et al. 1998). In order to induce prenatal infection (gestation days 15 and 16) in rat offspring, Nouel et al. (2012) exposed them to lipopolysaccharide, a bacterial endotoxin, and then assessed the number of reelin-immunoreactive (reelin+) cells and glutamic acid decarboxylase 67 (GAD67) immunoreactive (GAD67+) cells that were present in the hippocampus at postnatal days 14 and 28. They observed decreases in both reelin+ and GAD67+ expression in the dentate gyrus of infected animals at postnatal day 14 and concluded that prenatal infection caused postnatal reductions in postnatal reelin and GAD67. Prenatal exposure to influenza, elevated toxoplasma antibody, genital/reproductive infections, rubella, and other pathogens have been shown to be associated with schizophrenia (Brown 2012; Ducharme et al. 2012; Kneeland and Fatemi 2012; Macêdo et al. 2012).

Reelin regulates radial neuronal migration in the embryonic brain, promotes dendrite outgrowth in the developing postnatal forebrain, and strengthens synaptic transmission in the adult brain. In contrast, reelin deficiency induces specific defects in the molecular composition of the synapses in the adult brain (Ventruti et al. 2011). The MAM E17 model of schizophrenia was observed to cause whole neuronal density changes from the PFC and parvalbumin-positive-cell density changes from the hippocampus, and similar deficits were seen in the PFC of schizophrenic patients (Lodge et al. 2009; Moore et al. 2006). Matricon et al. (2010) exposed rat fetuses to MAM during E17 and then studied behavioral parameters and biomarkers in the adult animal. They found that behavioral

characteristics and neuropathology pertaining to the PFC similar to clinical features of the disorder were in evidence. In comparison with untreated offspring, MAM-exposed offspring showed a reduction in the volume of entorhinal cortex, hippocampus, and mediodorsal thalamus that was associated with reduced neuronal soma. The entorhinal cortex also showed laminar disorganization and neuronal clusters. Reelin methylation in the hippocampus formation was decreased although reelin + neurons and reelin expression were unchanged. They concluded that MAM-treated animals have a diminished neuropil possibly arising from abnormal neurite formation. The deterioration of the neuropil in cortical regions is implicated in the neuropathology of schizophrenia (Somenarain and Jones 2010). Protein loss in the disorder induces dendrites and spines to deteriorate in the dorsolateral PFC that is involved in several key cognitive domains.

5 Trophic Disruption

Brain-derived neurotrophic factor (BDNF) is essential for the maintenance of functional neurons, regulating growth, differentiation, synaptic connectivity, neurorepair, and longevity (Altar et al. 1997; Niu and Yip 2011). BDNF, central for the survival and differentiation of midbrain dopaminergic neurons (Hyman et al. 1991) and phenotypic differentiation of locus coeruleus noradrenergic neurons (Traver et al. 2006), has emerged as an important biomarker for schizophrenia spectrum disorder (Pae et al. 2012; Favalli et al. 2012; Zhang et al. 2012b). Sixty-three patients presenting schizophrenia symptoms were compared with 52 age- and sex-matched healthy controls for performance on a battery of neuropsychological tests by Niitsu et al. (2011). They found that although there were no significant differences in serum BDNF levels between normal controls and schizophrenic patients, serum BDNF levels for normal controls, but not schizophrenic patients, showed negative correlations with verbal working memory. On the other hand, serum BDNF levels of schizophrenic patients indicated positive correlations with the scores of the Scale for the Assessment of Negative Symptoms (SANS) and the Information subtest scores of Wechsler Adult Intelligence Scale Revised (WAIS-R). Serum BDNF levels were related with the impairment of verbal working memory and negative symptoms in patients with schizophrenia. In 22 acute schizophrenic patients and 22 age-matched healthy volunteers, Lee et al. (2011) observed significantly reduced serum levels in the unmedicated schizophrenic patients ($n = 22$; 4.38 ± 2.1 ng/mL) compared to the age-matched healthy volunteers. The percentage change of BDNF (increase, 173 % $\pm$ 110) correlated negatively with the percentage change of PANSS score with BDNF increase during psychotic treatment, as shown by several others (e.g., Pedrini et al. 2011; Yoshimura et al. 2007, 2010). Reduced BDNF concentrations have been reported in chronic antipsychotic-treated patients (Grillo et al. 2007; Ikeda et al. 2008; Rizos et al. 2010), neuroleptic-free (Palomino et al. 2006) and neuroleptic-naïve (Chen and Huang 2011; Rizos et al. 2008) patients. Finally, the levels of peripheral BDNF seem to be altered in first-episode patients presenting psychosis as well as in chronic schizophrenic patients. Although a paucity

of studies have reported changes in peripheral BDNF levels following antipsychotic treatment, the role of Val66Met polymorphism in BDNF has been found to contribute to structural and functional plasticity in schizophrenia (Buckley et al. 2007, 2011).

Long-term administration of antipsychotic agents induces tardive dyskinesias (TDs), a syndrome composed of involuntary, hyperkinetic, and abnormal movements often expressed through excessive chewing or dancing/foot shuffling behaviors (Jafari et al. 2012; Correll and Schenk 2008). Lower serum concentrations of BDNF, inversely correlated with Abnormal Involuntary Movement Scale (AIMS) scores, have been observed in patients presenting TDs (Tan et al. 2005). Additionally, associations between TD and BDNF polymorphisms have accumulated (Park et al. 2009; Zai et al. 2009). Yang et al. (2011) compared serum BDNF levels in schizophrenic patients with ($n = 129$) and without ($n = 235$) TDs with healthy controls ($n = 323$), with concurrent assessment of AIMS and the Positive and Negative Symptoms Scale (PANSS). Schizophrenic patients presenting TDs showed lower BDNF concentrations, while schizophrenic patients lacking TDs had BDNF levels similar to that of healthy controls. Lower BDNF serum concentrations correlated with higher PANSS negative subscores, but not with AIMS scores. The authors concluded that reduced BDNF concentrations may be associated with greater TD pathophysiology and negative symptoms in schizophrenia. Zhang et al. (2010) have indicated that schizophrenic patients with TD presented higher serum levels of S100B, a calcium-binding protein, than normal, healthy controls and those patients without TD. The serum S100B levels were positively correlated with AIMS scores in patients with TD. The authors concluded from these results that increased S100B levels may be related to the neurodegenerative aspect of disorder, associated with TD pathophysiology. TDs are generally indicative of neurodegenerative pathologies (e.g., Miller and Chouinard 1993).

6 GSK Regulation

The glycogen synthase kinase-3 s (GSK, α, and β), involved in glycogen metabolism, are expressed ubiquitously over various tissues and are abundant in brain tissue (Jaworski et al. 2011; Perez-Costas et al. 2010). Kaidanovich-Beilin and Woodgett (2011) have discussed the fundamental roles for these protein kinases in memory, behavior, and neuronal fate determination. It has been argued that Schizophrenia-1 (DISC1) represents a candidate gene for neuropsychiatric disorders and an influential involvement during brain structural and functional development. Singh et al. (2011) have observed that DISC1 variants are implicated in the loss of function in Wnt/GSK-3β signaling and thereby interrupt the course of brain development. Common DISC1 polymorphisms (variants) are associated with neuropsychiatric phenotypes including altered cognition, brain structure, and function. The contribution of the DISC1 gene to neural development and the eventual neurodevelopment of schizophrenia risk has been studied (Wexler and Geschwind 2011). GSK-3, a regulator of a wide range of cellular processes, plays a key dual role in apoptosis with putative contribution to schizophrenia neuropathology

(Emamian et al. 2004) and other neurodegenerative disorders (Avila et al. 2004; Berger et al. 2005; Onishi et al. 2011). Its role in the apoptotic signaling underlying excessive cell death may imply a neurodegenerative involvement in schizophrenia spectrum disorders, at least during the early stages of development (Gomez-Sintes et al. 2011). Astrocytic glycogenolysis and glycogen mobilization are implicated in normal brain function (Brown and Ransom 2007; Brown et al. 2003; Swanson 1992) and neuronal energy requirements (Brown et al. 2004; Wender et al. 2000), and not least in the necessities of cognitive function (Gibbs et al. 2006; Hertz et al. 2003; Suzuki et al. 2011). The links between glycogen metabolism and the glutathione (GSH) system have been studied (Dringen and Hirrlinger 2003): Shinohara et al. (2010) have shown that activation of GSK-3β is a key mediator of the initial phase of acetaminophen-induced liver injury through modulating glutamate cysteine ligase (GCL) and myeloid cell leukemia sequence-1 degradation. GSH deficits occur both in neurodegenerative conditions and in schizophrenia (Ballatori et al. 2009; Do et al. 2009; Dringen et al. 1997), and a GCL gene polymorphism, affecting GSH synthesis, is associated with the disorder (Tosic et al. 2006). In this regard, Lavoie et al. (2011) have found that glucose metabolism and glycogen utilization are dysregulated in astrocytes showing chronic deficit in GSH, implying dysfunctional brain energy metabolism in schizophrenia.

7 Oxidative Stress

Dysregulation in reactive oxygen species (ROS) and reactive nitrogen species (RNS) evidenced by lipid peroxidation in plasma, red blood cells, blood platelets, and cerebrospinal fluid have been observed in schizophrenic patients (Dietrich-Muszalska et al. 2005; Li et al. 2006; Yao et al. 1998, 2001). For example, Dietrich-Muszalska et al. (2005) found that oxidative stress occurred as reflected by superoxide dismutase (SOD) in platelets from schizophrenic patients; suppressed SOD activity was associated with enhanced ROS generation and lipid peroxidation as compared with healthy volunteers. In the acute phase of psychosis in schizophrenic patients, levels of isoprostanes were remarkably high compared with the healthy control group (Dietrich-Muszalska and Olas 2007). The isoprostanes are prostaglandin-like compounds formed in vivo from the free radical-catalyzed peroxidation of essential fatty acids (primarily arachidonic acid) without the direct action of cyclooxygenase (COX) enzyme, and increased levels are associated with oxidative stress and oxidative damage of lipids. During the same acute phase of psychosis, biomarker concentrations of oxidation/nitration proteins in plasma were markedly higher in patients, as too was the homocysteine (Hcys) level (Dietrich-Muszalska et al. 2009): Hcys may function as an oxidant both in vitro and in vivo (Carluccio et al. 2007; Olas et al. 2008) since there is a reduced plasma status of antioxidants in schizophrenic patients (Dietrich-Muszalska and Kontek 2010). Significant positive relationships between brain atrophy and plasma Hcys levels have been obtained (Bleich et al. 2002; Sachdev et al. 2002), with Hcys metabolism implicated in schizophrenia pathophysiology (Song et al. 2007).

Furthermore, increased third trimester Hcys concentrations could well enhance the risk for development of the disorder through structural and functional alterations (Brown and Ransom 2007). In this regard, Dietrich-Muszalska et al. (2012) investigated, in schizophrenic patients, the effect of elevated Hcys on parameters of oxidative stress, including thiobarbituric acid reactive substances (TBARS), levels of carbonyl groups in plasma proteins, and levels of 3-nitrotyrosine in plasma proteins. They observed that in these patients, plasma Hcys was greater than in controls. Also, the biomarkers of oxidative/nitrative stress, carbonyl groups and 3-nitrotyrosine, were enhanced significantly.

The pathophysiology of schizophrenia spectrum disorders may be the result of deregulation of synaptic plasticity, with downstream alterations of inflammatory immune processes regulated by cytokines, impaired antioxidant defense, and increased lipid peroxidation. Pedrini et al. (2012) examined serum oxidative stress markers and inflammatory cytokines in early and late phases of chronic SZ. They studied patients at early stage (within first 10 years of a psychotic episode), 39 at late stage (minimum 10 years after diagnosis of SZ), and their respective matched controls. From each patient/subject 5 mL blood samples were collected (venipuncture) for assay of TBARS, total reactive antioxidant potential (TRAP), protein carbonyl content (PCC), Interleukins 6 and 10 (IL-6, IL-10), and tumor necrosis factor alpha (TNF-alpha). TBARS, IL-6, and PCC levels were significantly higher in schizophrenic patients at early and late stages than in healthy controls. No differences for TRAP and TNF-alpha levels in patients with SZ at early and late stages than in controls were obtained. IL-10 levels were decreased in patients at late stage and a decrease trend in early stage was found. Their findings offer evidence consistent with comparable biological markers across the chronic disorder. Other results suggest that oxidative stress may be involved in the pathophysiology of the disorder and associated functioning, e.g., cognitive impairment: Zhang et al. (2012a) found that in schizophrenic patients' plasma, total antioxidant status was inversely associated with some domains of cognitive deficits in schizophrenia, such as attention and immediate memory. It is known too that adult neurogenesis transiently generates oxidative stress which may further contribute to the pathophysiology (Walton et al. 2012). Finally, Sullivan and O'Donnell (2012) have advanced the notion of an *excitation–inhibition imbalance* hypothesis of the neurodevelopment of symptom profiles in schizophrenia spectrum disorders; they have examined ongoing efforts that investigate the determination of whether or not cortical inhibitory interneurons are affected by oxidative stress during brain development.

8 Saitohin Gene Expression

Finally, Saitohin, an intronless gene nested within the human tau gene that contains a single nucleotide polymorphism (A/G), may be involved in the pathophysiology of neurodegenerative disorders (Combarros et al. 2003). The Saitohin gene is unique to humans and their closest relatives although function is not currently understood. It contains a single polymorphism (Q7R); the Q allele is human specific

and confers susceptibility to several neurodegenerative diseases (Gao et al. 2005; Wang et al. 2011). Certain neurodegenerative disorders, particularly Alzheimer's disease and progressive nuclear palsy, have been found to be associated with the presence of the Q allele of the Saitohin gene (Janković et al. 2008; Lorenzi et al. 2010; Schutte et al. 2011). In a sample of 48 schizophrenic patients and 47 healthy controls, Bosia et al. (2011) tested the role of saitohin polymorphism as a concurring factor of cognitive decline among these patients using the Wisconsin Card Sorting Test for executive functioning. They observed a significantly greater frequency of G allele among patients with frontotemporal dementia and schizophrenic patients presenting impaired Wisconsin Card Sorting Test performance. They concluded that the products expressed by the Saitohin gene affected core frontal executive function deterioration likely through mechanisms occurring through neurodevelopment with neurodegenerative outcome.

9 Conclusion

The myelin-centered model of the human brain's extensive and effective myelination implies the availability of higher metabolic resources underlying evolutionary adaptations resulting in the quadratic (inverted U) myelination trajectory that is peaking during the sixth decade of life (Bartzokis 2011). The notion proposes further that optimal brain function depends on exquisite action potential synchronization that myelin makes possible and that myelin's exceptional vulnerability to subtle metabolic/oxidative abnormalities may promote both developmental and degenerative diseases. It remains a possibility that in the interventions for schizophrenia spectrum disorders, the widespread use of psychotropic treatments may induce underappreciated CNS metabolic and neurotransmitter effects on myelination, its plasticity, and neuronal repair (Bartzokis 2011).

References

Adell, A., Jiménez-Sánchez, L., López-Gil, X., & Romón, T. (2012). Is the acute NMDA receptor hypofunction a valid model of schizophrenia? *Schizophrenia Bulletin, 38*, 9–14.

Akil, M., & Lewis, D. A. (1997). Cytoarchitecture of the entorhinal cortex in schizophrenia. *The American Journal of Psychiatry, 154*, 1010–1012.

Alexander-Bloch, A. F., Gogtay, N., Meunier, D., Birn, R., Clasen, L., Lalonde, F., Lenroot, R., Giedd, J., & Bullmore, E. T. (2010). Disrupted modularity and local connectivity of brain functional networks in childhood-onset schizophrenia. *Frontiers in Systems Neuroscience, 4*, 147.

Altar, C. A., Cai, N., Bliven, T., Juhasz, M., Conner, J. M., Acheson, A. L., Lindsay, R. M., & Wiegand, S. J. (1997). Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature, 389*, 856–860.

Aoyama, N., Théberge, J., Drost, D. J., Manchanda, R., Northcott, S., Neufeld, R. W., Menon, R. S., Rajakumar, N., Pavlosky, W. F., Densmore, M., Schaefer, B., & Williamson, P. C. (2011). Grey matter and social functioning correlates of glutamatergic metabolite loss in schizophrenia. *The British Journal of Psychiatry, 198*, 448–456.

Archer, T. (2010). Neurodegeneration in schizophrenia. *Expert Review of Neurotherapeutics, 10*, 1131–1141.

Archer, T., Kostrzewa, R. M., Beninger, R. J., & Palomo, T. (2010a). Staging perspectives in neurodevelopmental aspects of neuropsychiatry: Agents, phases and ages at expression. *Neurotoxicity Research, 18*, 287–305.

Archer, T., Kostrzewa, R. M., Palomo, T., & Beninger, R. J. (2010b). Clinical staging in the pathophysiology of psychotic and affective disorders: Facilitation of prognosis and treatment. *Neurotoxicity Research, 18*, 211–228.

Arias, I., Sorlozano, A., Villegas, E., de Dios, L. J., McKenney, K., Cervilla, J., Gutierrez, B., & Gutierrez, J. (2012). Infectious agents associated with schizophrenia: A meta-analysis. *Schizophrenia Research, 136*(1–3), 128–136.

Arnold, S. E., Hyman, B. T., Van Hoesen, G. W., & Damasio, A. R. (1991). Some cytoarchitectural abnormalities of the entorhinal cortex in schizophrenia. *Archives of General Psychiatry, 48*, 625–632.

Arnold, S. E., Ruscheinsky, D. D., & Han, L. Y. (1997). Further evidence of abnormal cytoarchitecture of the entorhinal cortex in schizophrenia using spatial point pattern analysis. *Biological Psychiatry, 42*, 639–647.

Avila, J., Lucas, J. J., Perez, M., & Hernandez, F. (2004). Role of tau protein in both physiological and pathological conditions. *Physiological Reviews, 84*, 361–384.

Baiano, M., Perlini, C., Rambaldelli, G., Cerini, R., Dusi, N., Bellani, M., Spezzapria, G., Versace, A., Balestrieri, M., Mucelli, R. P., Tansella, M., & Brambilla, P. (2008). Decreased entorhinal cortex volumes in schizophrenia. *Schizophrenia Research, 102*, 171–180.

Balduini, W., Lombardelli, G., Peruzzi, G., & Cattabeni, F. (1991). Treatment with methylazoxymethanol at different gestational days: Physical, reflex development and spontaneous activity in the offspring. *Neurotoxicology, 12*, 179–188.

Ballatori, N., Krance, S. M., Notenboom, S., Shi, S., Tieu, K., & Hammond, C. L. (2009). Glutathione dysregulation and the etiology and progression of human diseases. *Biological Chemistry, 390*, 191–214.

Bartzokis, G. (2011). Neuroglialpharmacology: White matter pathophysiologies and psychiatric treatments. *Frontiers in Bioscience, 17*, 2695–2733.

Bayer, S. A., & Altman, J. (2004). Development of the telencephalon: Neural stem cells, neurogenesis, and neuronal migration. In G. Paxinos (Ed.), *The rat neural system* (pp. 27–73). San Diego: Elsevier.

Benros, M. E., Nielsen, P. R., Nordentoft, M., Eaton, W. W., Dalton, S. O., & Mortensen, P. B. (2011). Autoimmune diseases and severe infections as risk factors for schizophrenia: A 30-year population-based register study. *The American Journal of Psychiatry, 168*, 1303–1310.

Berger, Z., Ttofi, E. K., Michel, C. H., Pasco, M. Y., Tenant, S., Rubinsztein, D. C., & O'Kane, C. J. (2005). Lithium rescues toxicity of aggregate-prone proteins in *Drosophila* by perturbing Wnt pathway. *Human Molecular Genetics, 14*, 3003–3011.

Bergman, O., Westberg, L., Lichtenstein, P., Eriksson, E., & Larsson, H. (2011). Study on the possible association of brain-derived neurotrophic factor polymorphism with the developmental course of symptoms of attention deficit and hyperactivity. *The International Journal of Neuropsychopharmacology, 14*, 1367–1376.

Bleich, S., Frieling, H., & Hillemacher, T. (2007). Elevated prenatal homocysteine levels and the risk of schizophrenia. *Archives of General Psychiatry, 64*, 980–981.

Bleich, S., Junemann, A., Von Ahsen, N., Lausen, B., Ritter, K., Beck, G., Naumann, G. O., & Kamhuber, J. (2002). Homocysteine and risk of open-angle glaucoma. *Journal of Neural Transmission, 109*, 1499–1504.

Bosia, M., Buonocure, M., Guglielmino, C., Pirovana, A., Lorenzi, C., Marcone, A., Bramanti, P., Cappa, S. F., Aquglia, E., Smerladi, E., & Cavallaro, R. (2011). Saitohin polymorphism and executive dysfunction in schizophrenia. *Neurological Sciences, 33*(5), 1051–1056. doi:10.1007/s10072-0893-9.

Brambrink, A. M., Evers, A. S., Avidan, M. S., Farber, N. B., Smith, D. J., Martin, L. D., Dissen, G. A., Creeley, C. E., & Olney, J. W. (2012). Ketamine-induced neuroapoptosis in the fetal and neonatal rhesus macaque brain. *Anesthesiology, 116*(2), 372–384.

Brown, A. M., & Ransom, B. R. (2007). Astrocyte glycogen and brain energy metabolism. *Glia, 55*, 1263–1271.

Brown, A. M., Baltan Tekkok, S., & Ransom, B. R. (2003). Glycogen regulation and functional role in mouse white matter. *The Journal of Physiology, 549*, 501–512.

Brown, A. M., Baltan Tekkok, S., & Ransom, B. R. (2004). Energy transfer from astrocytes to axons: The role of CNS glycogen. *Neurochemistry International, 45*, 529–536.

Brown, A. S. (2011). Exposure to prenatal infection and risk of schizophrenia. *Frontiers in Psychiatry, 2*, 63.

Brown, A. S. (2012). Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism. *Developmental Neurobiology, 72*(10), 1272–1276. doi:10.1002/dneu.22024.

Brown, A. S., & Derkits, E. J. (2010). Prenatal infection and schizophrenia: A review of epidemiologic and translational studies. *The American Journal of Psychiatry, 167*, 261–280.

Brown, A. S., & Patterson, P. H. (2011). Maternal infection and schizophrenia: Implications for prevention. *Schizophrenia Bulletin, 37*, 284–290.

Brown, A. S., Bottiglieri, T., Schaefer, C. A., Quesenberry, C. P. J. R., Liu, L., Bresnahan, M., & Susser, E. S. (2007). Elevated prenatal homocysteine levels as a risk factor for schizophrenia. *Archives of General Psychiatry, 64*, 980–981.

Brown, A. S., & Susser, E. S. (2005). Homocysteine and schizophrenia: From prenatal to adult life. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29*, 1175–1180.

Brown, A. S., & Susser, E. S. (2008). Prenatal nutritional deficiency and risk of adult schizophrenia. *Schizophrenia Bulletin, 34*, 1054–1063.

Brown, A. S., Vinogradov, S., Kremen, W. S., Poole, J. H., Deicken, R. F., Penner, J. D., McKeague, I. W., Kochetkova, A., Kern, D., & Schaefer, C. A. (2009). Prenatal exposure to maternal infection and executive dysfunction in adult schizophrenia. *The American Journal of Psychiatry, 166*, 683–690.

Brown, A. S., Vinogradov, S., Kremen, W. S., Poole, J. H., Bao, Y., Kern, D., & McKeague, I. W. (2011). Association of maternal genital and reproductive infections with verbal memory and motor deficits in adult schizophrenia. *Psychiatry Research, 188*, 179–186.

Buckley, P. F., Pillai, A., Evans, D., Stirewalt, E., & Mahadik, S. (2007). Brain-derived neurotrophic factor in first-episode psychosis. *Schizophrenia Research, 91*, 1–5.

Buckley, P. F., Pillai, A., & Howell, K. R. (2011). Brain-derived neurotrophic factor: Findings in schizophrenia. *Current Opinion in Psychiatry, 24*(2), 122–127.

Burd, I., Balakrishnan, B., & Kannan, S. (2012). Models of fetal brain injury, intrauterine inflammation, and preterm birth. *American Journal of Reproductive Immunology, 67*, 287–294. doi:10.1111/j.1600-0897.2012.01110.x.

Bustillo, J. R., Rowland, L. M., Mullins, P., Jung, R., Chen, H., Qualls, C., Hammond, R., Brooks, W. M., & Lauriello, J. (2010). 1H-MRS at 4 tesla in minimally treated early schizophrenia. *Molecular Psychiatry, 15*, 629–636.

Cannon, M., Caspi, A., & Moffitt, T. E. (2002a). Evidence for early, specific, pandevelopment impairment in schizophreniform disorder: Results from a longitudinal birth cohort. *Archives of General Psychiatry, 59*, 449–456.

Cannon, M., Murray, R. M., & Jones, P. (2002b). Obstetric complications and schizophrenia: Past, present and future. *The American Journal of Psychiatry, 159*, 1080–1092.

Carluccio, M. A., Ancora, M. A., Massaro, M., Carluccio, M., Scoditti, E., Distante, A., Storelli, C., & De Caterina, R. (2007). Homocysteine induces VCAM-1 gene expression through NF-kappaB and NAD(P)H oxidase activation: Protective role of Mediterranean diet polyphenolic antioxidants. *American Journal of Physiology. Heart and Circulatory Physiology, 293*, H2344–H2354.

Cattabeni, F., & Di Luca, M. (1997). Developmental models of brain dysfunctions induced by targeted cellular ablations with methylazoxymethanol. *Physiological Reviews, 77*, 199–215.

Ceranik, K., Deng, J., Heimrich, B., Lübke, J., Zhao, S., Förster, E., & Frotscher, M. (1999). Hippocampal Cajal-Retzius cells project to the entorhinal cortex: Retrograde tracing and intracellular tracing studies. *The European Journal of Neuroscience, 11*, 4278–4290.

Chan, M. H., Chiu, P. H., Lin, C. Y., & Chen, H. H. (2012). Inhibition of glycogen synthase kinase-3 attenuates psychotomimetic effects of ketamine. *Schizophrenia Research, 136*, 96–103.

Chen, C. C., & Huang, T. L. (2011). Effects of antipsychotics on the serum BDNF levels in schizophrenia. *Psychiatry Research, 189*, 327–330.

Chen, S., & Hillman, D. E. (1986). Selective ablation of neurons by methylazoxymethanol during pre- and postnatal brain development. *Experimental Neurology, 94*, 103–119.

Ciani, E., Frenquelli, M., & Contestabile, A. (2003). Developmental expression of the cell cycle and apoptosis controlling gene, Lotl, in the rat cerebellum and in cerebellar granule cells. *Brain Research. Developmental Brain Research, 142*, 193–202.

Combarros, O., Rodero, L., Infante, J., Palacio, E., Llorca, J., Fernández-Viadero, C., Peña, N., & Berciano, J. (2003). Age-dependent association between the Q7R polymorphism in the Saitohin gene and sporadic Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders, 16*, 132–135.

Correll, C. U., & Schenk, E. M. (2008). Tardive dyskinesias and new antipsychotics. *Current Opinion in Psychiatry, 21*, 151–156.

Dauvermann, M. R., Mukherjee, P., Moorhead, W. T., Stanfield, A. C., Fusar-Poli, P., Lawrie, S. M., & Whalley, H. C. (2012). Relationship between gyrification and functional connectivity of the prefrontal cortex in subjects at high genetic risk of schizophrenia. *Current Pharmaceutical Design, 18*(4), 434–442. PMID: 22239574.

Dean, O. M., van den Buuse, M., Berk, M., Copolov, D. L., Mavros, C., & Bush, A. I. (2011). N-acetyl cysteine restores brain glutathione loss in combined 2-cyclohexene-1-one and D-amphetamine-treated rats: Relevance to schizophrenia and bipolar disorder. *Neuroscience Letters, 499*, 149–153.

Dietrich-Muszalska, A., & Kontek, B. (2010). Lipid peroxidation in patients with schizophrenia. *Psychiatry and Clinical Neuroscience*, *64*, 469–475.

Dietrich-Muszalska, A., & Olas, B. (2007). Isoprostanes as indicators of oxidative stress in schizophrenia. *The World Journal of Biological Psychiatry, 14*, 1–6.

Dietrich-Muszalska, A., Olas, B., & Rabe-Jablonska, J. (2005). Oxidative stress in blood platelets from schizophrenic patients. *Platelets, 16*, 386–391.

Dietrich-Muszalska, A., Olas, B., Glowacki, R., & Bald, E. (2009). Oxidative/nitrative modifications of plasma proteins and thiols from patients with schizophrenia. *Neuropsychobiology, 59*, 1–7.

Dietrich-Muszalska, A., Malinowska, J., Olas, B., Glowacki, R., Bald, E., Wachowicz, B., & Rabe-Jablonska, J. (2012). The oxidative stress may be induced by the elevated homocysteine in schizophrenia patients. *Neurochemical Research, 37*, 1057–1062.

Do, K. Q., Cabungal, J. H., Frank, A., Steullet, P., & Cuenod, M. (2009). Redox dysregulation, neurodevelopment and schizophrenia. *Current Opinion in Neurobiology, 19*, 220–230.

Dringen, R., & Hirrlinger, J. (2003). Glutathione pathways in the brain. *Biological Chemistry, 384*, 505–516.

Dringen, K. O., Löschmann, P. A., & Hamprecht, B. (1997). Use of dipeptides for the synthesis of glutathione by astroglia-rich primary cultures. *Journal of Neurochemistry, 69*, 868–874.

Ducharme, G., Lowe, G. C., Goutagny, R., & Williams, S. (2012). Early alterations in hippocampal circuitry and theta rhythm generation in a mouse model of prenatal infection: Implications for schizophrenia. *PloS One, 7*(1), e29754.

Eastwood, S. L., & Harrison, P. J. (2006). Cellular basis of reduced cortical reelin expression in schizophrenia. *The American Journal of Psychiatry, 163*, 540–542.

Egerton, A., & Stone, J. M. (2012). The glutamate hypothesis of schizophrenia: Neuroimaging and drug development. *Current Pharmaceutical Biotechnology, 13*(8), 1500–1512.

Elia, J., Laracy, S., Allen, J., Nissley-Tsiopinis, J., & Borgmann-Winter, K. (2011). Epigenetics: Genetics versus life experiences. *Current Topics in Behavioral Neuroscience, 9*, 317–340.

Ellenbroek, B. A., van den Kroonenberg, P. T., & Cools, A. R. (1998). The effects of an early stressful life event on sensorimotor gating in adult rats. *Schizophrenia Research, 30*, 251–260.

Emamian, E. S., Hall, D., Birnbaum, M. J., Karayiorgou, M., & Gogos, J. A. (2004). Convergent evidence for impaired AKT1-GSK3 signaling in schizophrenia. *Nature Genetics, 36*, 131–137.

Falkai, P., Kovalenko, S., Schneider-Axmann, T., Ovary, I., & Honer, W. G. (2003). Second replication of disturbed pre-alpha-cell migration in the entorhinal cortex of schizophrenic patients. *Schizophrenia Research, 60*, 71–74.

Fatemi, S. H., Earle, J. A., & McMenomy, T. (2000). Reduction in reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. *Molecular Psychiatry, 5*, 654–663.

Favalli, G., Li, J., Belmonte-de-Abreu, P., Wong, A. H., & Daskalakis, Z. J. (2012). The role of BDNF in the pathophysiology and treatment of schizophrenia. *Journal of Psychiatric Research, 46*, 1–11.

Featherstone, R. E., Rizos, Z., Nobrega, J. N., Kapur, S., & Fletcher, P. J. (2007). Gestational methylazoxymethanol acetate treatment impairs select cognitive functions: Parallels to schizophrenia. *Neuropsychopharmacology, 32*, 483–492.

Ferguson, S. A., Paule, M. G., & Holson, R. R. (1996). Functional effects of methylazoxymethanol-induced cerebellar hypoplasia in rats. *Neurotoxicology and Teratology*, *18*, 529–537.

Ferguson, S. A., & Holson, R. R. (1997). Methylazoxymethanol-induced micrencephaly in the brown Norway strain: Behavior and brain weight. *International Journal of Developmental Neuroscience, 15*, 75–86.

Ferrer, I., Pozas, E., Marti, M., Blanco, R., & Planas, A. M. (1997). Methylazoxymethanol-induced apoptosis in the external granule cell layer of the developing cerebellum of the rat is associated with strong c-jun expression and formation of high molecular weight c-jun complexes. *Journal of Neuropathology and Experimental Neurology, 56*, 1–9.

Fiore, M., Korf, J., Antonelli, A., Talamini, L., & Aloe, L. (2002). Long-lasting effects of prenatal MAM treatment on water maze performance in rats: Associations with altered brain development and neurotrophin levels. *Neurotoxicology and Teratology, 24*, 179–191.

Fitzgerald, P. J. (2012). The NMDA receptor may participate in widespread suppression of circuit level neural activity, in addition to a similarly prominent role in circuit level activation. *Behavioural Brain Research, 230*, 291–298.

Flagstad, P., Glenthoj, B. Y., & Didriksen, M. (2005). Cognitive deficits caused by late gestational disruption of neurogenesis in rats: A preclinical model of schizophrenia. *Neuropsychopharmacology, 30*, 250–260.

Fontes, M. A., Bolla, K. I., Cunha, P. J., Almeida, P. P., Jungerman, F., Laranjeira, R. R., Bressan, R. A., & Lacerda, A. L. (2011). Cannabis use before age 15 and subsequent executive functioning. *The British Journal of Psychiatry, 198*, 442–447.

Furukawa, T., Mizukawa, R., Hirai, T., Fujihara, S., Kitamura, T., & Takahashi, K. (1998). Childhood parental loss and schizophrenia: Evidence against pathogens but for some pathoplastic effects. *Psychiatry Research, 81*, 353–362.

Gao, L., Tse, S. W., Conrad, C., & Andreadis, A. (2005). Saitohin, which is nested in the tau locus and confers allele-specific susceptibility to several neurodegenerative diseases, interacts with peroxiredoxin 6. *The Journal of Biological Chemistry, 280*, 39268–39272.

Geddes, J. R., Verdoux, H., & Takei, N. (1999). Schizophrenia and complications of pregnancy and labour: An individual patient data meta-analysis. *Schizophrenia Bulletin, 25*, 413–423.

Gibbs, M. E., Anderson, D. G., & Hertz, L. (2006). Inhibition of glycogenolysis in astrocytes interrupts memory consolidation in young chickens. *Glia, 54*, 214–222.

Gill, K. M., Lodge, D. J., Cook, J. M., Aras, S., & Grace, A. A. (2011). A novel α5GABAAR-postive allosteric modulator reverses hyperactivation of the dopamine system in the MAM model of schizophrenia. *Neuropsychopharmacology, 36*, 1903–1911.

Goldman, A. L., Pezawas, L., Mattay, V. S., Fischl, B., Verchinski, B. A., Chen, Q., Weinberger, D. R., & Meyer-Lindenberg, A. (2009). Widespread reductions of cortical thickness in schizophrenia and spectrum disorders and evidence of heritability. *Archives of General Psychiatry, 66*, 467–477.

Gomez-Sintes, R., Hernandez, F., Lucas, J. J., & Avila, J. (2011). GSK-3 mouse models to study neuronal apoptosis and neurodegeneration. *Frontiers in Molecular Neuroscience, 4*, 1–11. doi:103389/fnmol.2011.00045.

Goto, Y., & Grace, A. A. (2006). Alterations in medial prefrontal cortical activity and plasticity in rats with disruption of cortical development. *Biological Psychiatry, 60*, 1259–1267.

Goto, N., Yoshimura, R., Kakeda, S., Moriya, J., Hayashi, K., Ikenouchi-Sugita, A., Umene-Nakano, W., Hori, H., Ueda, N., Korogi, Y., & Nakamura, J. (2010). Comparison of brain N-acetylaspartate levels and serum brain-derived neurotrophic factor (BDNF) levels between patients with first-episode schizophrenia psychosis and healthy controls. *European Psychiatry, 26*, 57–63.

Gourevitch, R., Rocher, C., Le Pen, G., Krebs, M. O., & Jay, T. M. (2004). Working memory deficits in adult rats after prenatal disruption of neurogenesis. *Behavioural Pharmacology, 15*, 287–292.

Graff, J., Dohoon, K., Dobbin, M. M., & Tsai, L. H. (2011). Epigenetic regulation of gene expression in physiological and pathological brain processes. *Physiological Reviews, 91*, 603–649.

Gratacos, M., Gonzalez, J. R., Mercader, J. M., de Cid, R., Urretavizcaya, M., & Estivill, X. (2007). Brain-derived neurotrophic factor Val66Met and psychiatric disorders: Meta-analysis of case–control studies confirm association to substance-related disorders, eating disorders and schizophrenia. *Biological Psychiatry, 61*, 911–922.

Grillo, R. W., Ottoni, G. L., Leke, R., Souza, D. O., Portela, L. V., & Lara, D. R. (2007). Reduced serum BDNF levels in schizophrenic patients on clozapine or typical antipsychotics. *Journal of Psychiatric Research, 41*, 31–35.

Guidotti, A., Auta, J., Davis, J. M., Di-Giorgi-Gerevini, V., Dwivedi, Y., Grayson, D. R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., & Costa, E. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: A postmortem brain study. *Archives of General Psychiatry, 57*, 1061–1069.

Harrison, P. J. (2004). The hippocampus in schizophrenia: A review of the neuropathological evidence and its pathophysiological implications. *Psychopharmacology, 174*, 151–162.

Hertz, L., O'Dowd, B. S., Ng, K. T., & Gibbs, M. E. (2003). Reciprocal changes in forebrain contents of glycogen and glutamate/glutamate during early memory consolidation in the day-old chick. *Brain Research, 994*, 226–233.

Hoenicka, J., Garrido, E., Martínez, I., Ponce, G., Aragüés, M., Rodríguez-Jiménez, R., España-Serrano, L., Alvira-Botero, X., Santos, J. L., Rubio, G., Jiménez-Arriero, M. A., Palomo, T., & PARGPARG. (2010). Gender-specific COMT Val158Met polymorphism association in Spanish schizophrenic patients. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 153B*, 79–85.

Honea, R. A., Meyer-Lindenberg, A., Hobbs, K. B., Pezawas, L., Mattay, V. S., Egan, M. F., Verchinski, B., Passingham, R. E., Weinberger, D. R., & Callicott, J. H. (2008). Is grey matter volume an intermediate phenotype for schizophrenia? A voxel-based morphometric study of patients with schizophrenia and their healthy siblings. *Biological Psychiatry, 63*, 465–474.

Hradetzky, S., Sanderson, T. M., Tsang, T. M., Sherwood, J. L., Fitzjohn, S. M., Lakics, V., Malik, N., Schoeffmann, S., O'Neill, M. J., Cheng, T. M., Harris, L. W., Rahmoune, H., Guest, P. C., Sher, E., Collingridge, G. L., Holmes, E., Tricklebank, M. D., & Bahn, S. (2011). The methylazoxymethanol acetate (MAM-E17) rat model: Molecular and functional effects in the hippocampus. *Neuropsychopharmacology, 37*, 364–377.

Hyman, C., Hofer, M., Barde, Y. A., Juhasz, M., Yancopoulos, G. D., Squinto, S. P., & Lindsay, R. M. (1991). BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature, 350*, 230–232.

Ikeda, Y., Yahata, N., Ito, I., Nagano, M., Toyota, T., Yoshikawa, T., Okubo, Y., & Suzuki, H. (2008). Low serum levels of brain derived neurotrophic factor and epidermal growth factor in patients with chronic schizophrenia. *Schizophrenia Research, 101*, 58–66.

Impagnatiello, F., Guidotti, A. R., Pesold, C., Dwivedi, Y., Caruncho, H., Pisu, M. G., Uzunov, D. P., Smalheiser, N. R., Davis, J. M., Pandey, G. N., Pappas, G. D., Tueting, P., Sharma, R. P., & Costa, E. (1998). A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 15718–15723.

Jablonka, E., & Raz, G. (2009). Transgenerational epigenetic inheritance: Prevalence, mechanisms, and implications for the study of heredity and evolution. *The Quarterly Review of Biology, 84*, 131–176.

Jacob, C. P., Nguyen, T. T., Dempfle, A., Heine, M., Windemuth-Kieselbach, C., Baumann, K., Jacob, F., Prechtl, J., Wittlich, M., Herrmann, M. J., Gross-Lesch, S., Lesch, K. P., & Reif, A. (2010). A gene-environment investigation on personality traits in two independent clinical sets of adult patients with personality disorder and attention deficit/hyperactivity disorder. *European Archives Psychiatry and Clinical Neuroscience, 260*, 317–326.

Jafari, S., Fernandez-Enright, F., & Huang, X. F. (2012). Structural contributions of antipsychotic drugs to their therapeutic profiles and metabolic side effects. *Journal of Neurochemistry, 120*, 371–384.

Janković, N., Kecmanović, M., Dimitrijević, R., Keckarević Marković, M., Dobricić, V., Keckarević, D., Savić Pavicević, D., & Romac, S. (2008). HD phenocopies – Possible role of Saitohin gene. *The Journal of Neuroscience, 118*, 391–397.

Jaworski, T., Dewachter, I., Lechat, B., Gees, M., Kremer, A., Demedts, D., Borghgraef, P., Devijver, H., Kügler, S., Patel, S., Woodgett, J. R., & Van Leuven, F. (2011). GSK-3α/β kinases and amyloid production in vivo. *Nature, 480*(7376), E4–E5.

Jonsson, S. A., Luts, A., Guldberg-Kjaer, N., & Öhman, R. (1999). Pyramidal neuron size in the hippocampus of schizophrenics correlates with total cell count and degree of cell disarray. *European Archives of Psychiatry and Clinical Neuroscience, 249*, 169–173.

Kaidanovich-Beilin, O., & Woodgett, J. R. (2011). GSK-3: Functional insights from cell biology and animal models. *Frontiers in Molecular Neuroscience, 4*, 40.

Karilampi, U., Helldin, L., Hjärthag, F., Norlander, T., & Archer, T. (2007). Verbal learning in schizopsychotic outpatients and healthy volunteers as a function of cognitive performance levels. *Archives of Clinical Neuropsychology, 22*, 161–174.

Karilampi, U., Helldin, L., & Archer, T. (2011). Cognition and global assessment of functioning in male and female outpatients with schizophrenia spectrum. *The Journal of Nervous and Mental Disease, 199*, 445–448.

Kneeland, R. E., & Fatemi, S. H. (2012). Viral infection inflammation and schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 42*, 35–48. PMID: 22349576.

Konradi, C., Yang, C. K., Zimmerman, E. I., Lohmann, K. M., Gresch, P., Pantazopoulos, H., Berretta, S., & Heckers, S. (2011). Hippocampal interneurons are abnormal in schizophrenia. *Schizophrenia Research, 131*, 165–173.

Lafarga, M., Lerga, A., Andres, M. A., Polanco, J. L., Calle, E., & Berciano, M. T. (1997). Apoptosis induced by methylazoxymethanol in developing rat cerebellum: Organization of the cell nucleus and its relationship to DNA and tRNA degradation. *Cell and Tissue Research, 289*, 25–38.

Lahti, A. C., Weiler, M. A., Holcomb, H. H., Tamminga, C. A., Carpenter, W. T., & McMahon, R. (2006). Correlations between rCBF and symptoms in two independent cohorts of drug-free patients with schizophrenia. *Neuropsychopharmacology, 31*, 221–230.

Large, C. H., Bison, S., Sartori, I., Read, K. D., Gozzi, A., Quarta, D., Antolini, M., Hollands, E., Gill, C. H., Gunthorpe, M. J., Idris, N., Neill, J. C., & Alvaro, G. S. (2011). The efficacy of sodium channel blockers to prevent phencyclidine-induced cognitive dysfunction in the rat: Potential for novel treatments for schizophrenia. *The Journal of Pharmacology and Experimental Therapeutics, 338*, 100–113.

Lavoie, S., Allaman, I., Petit, J. M., Do, K. Q., & Magistretti, P. J. (2011). Altered glycogen metabolism in cultured astrocytes from mice with chronic glutathione deficit: Relevance for neuroenergetics in schizophrenia. *PloS One, 6*, e22875.

Le Pen, G., Jay, T. M., & Krebs, M. O. (2011). Effect of antipsychotics on spontaneous hyperactivity and hypersensitivity to MK-801-induced hyperactivity in rats prenatally exposed to methylazoxymethanol. *Journal of Psychopharmacology, 25*, 822–835.

Lee, M. H., & Rabe, A. (1992). Premature decline in Morris water maze performance of aging microencephalic rats. *Neurotoxicology and Teratology, 14*, 383–392.

Lee, A. H., Lange, C., Ricken, R., Hellweg, R., & Lang, U. E. (2011). Reduced brain-derived neurotrophic factor serum concentrations in acute schizophrenic patients increase during antipsychotic treatment. *Journal of Clinical Psychopharmacology, 31*, 334–336.

Leng, A., Jongen-Rêlo, A. L., Pothuizen, H. H., & Feldon, J. (2005). Effects of prenatal methylazoxymethanol acetate (MAM) treatment in rats on water maze performance. *Behavioural Brain Research, 161*, 291–298.

Li, H. C., Chen, Q. Z., Ma, Y., & Zhou, J. F. (2006). Imbalanced free radicals and antioxidant defense systems in schizophrenia: A comparative study. *Journal of Zhejiang University Science B, 12*, 981–986.

Lillrank, S. M., Lipska, B. K., Kolachana, B. S., & Weinberger, D. R. (1999). Attenuated extracellular dopamine levels after stress and amphetamine in the nucleus accumbens of rats with neonatal ventral hippocampal damage. *Journal of Neural Transmission, 106*, 183–196.

Lipska, B. K., & Weinberger, D. R. (1994). Subchronic treatment with haloperidol and clozapine in rats with neonatal excitotoxic hippocampal damage. *Neuropsychopharmacology, 10*, 199–205.

Lipska, B. K., Jaskiw, G. E., Chrapusta, S., Karoum, F., & Weinberger, D. R. (1992). Ibotenic acid lesions of the ventral hippocampus differentially affects dopamine and its metabolites in the nucleus accumbens and prefrontal cortex in the rat. *Brain Research, 585*, 1–6.

Lipska, B. K., Swerdlow, N. R., Geyer, M. A., Jaskiw, G. E., Braff, D. L., & Weinberger, D. R. (1995). Neonatal excitotoxic lesions hippocampal damage in rats causes post pubertal changes in prepulse inhibition of startle and its disruption by apomorphine. *Psychopharmacology, 122*, 35–43.

Lipska, B. K., Kolb, B., Halim, N., & Weinberger, D. R. (2001). Synaptic abnormalities in prefrontal cortex and nucleus accumbens of adult rats with neonatal hippocampal damage. *Schizophrenia Research, 49*, 47–51.

Lodge, D. J., & Grace, A. A. (2008). Hippocampal dysfunction and disruption of dopamine system regulation in an animal model of schizophrenia. *Neurotoxicity Research, 14*, 97–104.

Lodge, D. J., Behrens, M. M., & Grace, A. A. (2009). A loss of parvalbumin-containing interneurons is associated with loss of oscillatory activity in an animal model of schizophrenia. *The Journal of Neuroscience, 29*, 2344–2354.

Lodge, D. J., & Grace, A. A. (2012). Gestational methylazoxymethanol acetate administration alters proteomic and metabolomics markers of hippocampal glutamatergic transmission. *Neuropsychopharmacology, 37*, 319–320.

Lorenzi, C., Marcone, A., Pirovano, A., Marino, E., Cordici, F., Cerami, C., Delmonte, D., Cappa, S. F., Bramanti, P., & Smeraldi, E. (2010). Serotonin transporter and saitohin genes in risk of Alzheimer's disease and frontotemporal lobar dementia: Preliminary findings. *Neurological Science, 31*, 741–749.

Macêdo, D. S., Araújo, D. P., Sampaio, L. R., Vasconcelos, S. M., Sales, P. M., Sousa, F. C., Hallak, J. E., Crippa, J. A., & Carvalho, A. F. (2012). Animal models of prenatal immune challenge and their contribution to the study of schizophrenia: A systematic review. *Brazilian Journal of Medical and Biological Research, 45*, 179–186.

Maloku, E., Covelo, I. R., Hanbauer, I., Guidotti, A., Kadriu, B., Hu, Q., Davis, J. M., & Costa, E. (2010). Lower number of cerebellar Purkinje neurons in psychosis is associated with reduced reelin expression. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 4407–4411.

Marona-Lewicka, D., Nichols, C. D., & Nichols, D. E. (2011). An animal model of schizophrenia based on chronic LSD administration: Old idea, new results. *Neuropharmacology, 61*, 503–512.

Matricon, J., Bellon, A., Frieling, H., Kebir, O., Le Pen, G., Beuvon, F., Daumas-Dupont, C., Jay, T. M., & Krebs, M.-O. (2010). Neuropathological and reelin deficiencies in the hippocampal formation of rats exposed to MAM: Differences and similarities with schizophrenia. *PloS One, 5*, e1029.

Meyer, U. (2011). Developmental neuroinflammation and schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 42*, 20–34. PMID: 22122877.

Meyer-Lindenberg, A. (2011). Neuroimaging and the question of neurodegeneration in schizophrenia. *Progress in Neurobiology, 95*, 514–516.

Meyer-Lindenberg, A., Olsen, R. K., Kohn, P. D., Brown, T., Egan, M. F., Weinberger, D. R., & Berman, K. F. (2005). Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. *Archives of General Psychiatry, 62*, 379–386.

Miller, J., Williamson, P., Jensen, J. E., Manchanda, R., Menon, R., Neufeld, R., Rajakumar, N., Pavlosky, W., Densmore, M., Schaefer, B., & Drost, D. J. (2009). Longitudinal 4.0 Testa (31)P magnetic resonance spectroscopy changes in the anterior cingulate and left thalamus in first episode schizophrenia. *Psychiatry Research Neuroimaging, 173*, 155–157.

Miller, R., & Chouinard, G. (1993). Loss of striatal cholinergic neurons as a basis for tardive and L-dopa-induced dyskinesias, neuroleptic-induced supersensitivity psychosis and refractory schizophrenia. *Biological Psychiatry, 34*, 713–738.

Miyata, T., Ono, Y., Okamoto, M., Masaoka, M., Sakakibara, A., Kawaguchi, A., Hashimoto, M., & Ogawa, M. (2010). Migration, early axonogenesis, and reelin-dependent layer-forming behavior of early/posterior-born Purkinje cells in the developing mouse lateral cerebellum. *Neural Development, 5*, 23.

Mohammed, A. K., Jonsson, G., Söderberg, U., & Archer, T. (1986a). Impaired selective attention in methylazoxymethanol-induced microencephalic rats. *Pharmacology Biochemistry and Behavior, 24*, 975–981.

Mohammed, A. K., Jonsson, G., Sundström, E., Minor, B. G., Söderberg, U., & Archer, T. (1986b). Selective attention and place navigation in rats treated prenatally with methylazoxymethanol. *Brain Research. Developmental Brain Research, 30*, 145–155.

Moore, H., Jentsch, J. D., Ghajarnia, M., Geyer, M. A., & Grace, A. A. (2006). A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: Implications for the neuropathology of schizophrenia. *Biological Psychiatry, 60*, 253–264.

Morgan, C., Kirkbride, J., Leff, J., Craig, T., Hutchinson, G., McKenzie, K., Morgan, K., Dazzan, P., Doody, G. A., Jones, P., Murray, R., & Fearon, P. (2007). Parental separation, loss and psychosis in different ethnic groups: A case-control study. *Psychological Medicine, 37*, 495–503.

Nielsen, P. R., Laursen, T. M., & Mortensen, P. B. (2011). Association between parental hospital-treated infection and the risk of schizophrenia in adolescence and early adulthood. *Schizophrenia Bulletin, 39*(1), 230–237. PMID: 22021661.

Niitsu, T., Shirayama, Y., Matsuzawa, D., Hasegawa, T., Kanahara, N., Hashimoto, T., Shiraishi, T., Shiina, A., Fukami, G., Fujisaki, M., Watanabe, H., Nakazato, M., Asano, M., Kimura, S., Hashimoto, K., & Iyo, M. (2011). Associations of serum brain-derived neurotrophic factor with cognitive impairments and negative symptoms in schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 35*, 1836–1840.

Niu, C., & Yip, H. K. (2011). Neuroprotective signaling mechanisms of telomerase are regulated by brain-derived neurotrophic factor in rat spinal cord motor neurons. *Journal of Neuropathology and Experimental Neurology, 70*, 634–652.

Niu, S., Yabut, O., & D'Arcangelo, G. (2008). The reelin signaling pathway promotes dendritic spine development in hippocampal neurons. *The Journal of Neuroscience, 28*, 10339–10348.

Nouel, D., Burt, M., Zhang, Y., Harvey, L., & Boksa, P. (2012). Prenatal exposure to bacterial endotoxin reduces the number of GAD67- and reelin-immunoreactive neurons in the hippocampus of rat offspring. *European Neuropsychopharmacology, 22*, 300–307.

O'Donnell, P., Lewis, B. L., Weinberger, D. R., & Lipska, B. K. (2002). Neonatal hippocampal damage alters electrophysiological properties of prefrontal cortical neurons in adult rats. *Cerebral Cortex, 12*, 975–982.

Olas, B., Kedzierska, M., & Wachowicz, B. (2008). Comparative studies on homocysteine and its metabolite – Homocysteine thiolactone action in blood platelets in vitro. *Platelets, 19*, 520–527.

Onishi, T., Iwashita, H., Uno, Y., Kunitomo, J., Saitoh, M., Kimura, E., Fujita, H., Uchiyama, N., Kori, M., & Takizawa, M. (2011). A novel glycogen synthase kinase-3 inhibitor 2-methyl-5-(3-{4-[(S)-methylsulfinyl]phenyl}-1-benzofuran-5-yl)-1,3,4-oxadiazole decreases tau phosphorylation and ameliorates cognitive deficits in a transgenic model of Alzheimer's disease. *Journal of Neurochemistry, 119*, 1330–1340.

Pae, C. U., Chiesa, A., Porcelli, S., Han, C., Patkar, A. A., Lee, S. J., Park, M. H., Serretti, A., & De Ronchi, D. (2012). Influence of BDNF variants on diagnosis and response to treatment in patients with major depression, bipolar disorder and schizophrenia. *Neuropsychobiology, 65*(1), 1–11.

Palomino, A., Vallejo-Illarramendi, A., Gonzalez-Pinto, A., Aldama, A., Gonzalez-Gomez, C., Mosquera, F., González-García, G., & Matute, C. (2006). Decreased levels of plasma BDNF in first-episode schizophrenia and bipolar disorder patients. *Schizophrenia Research, 6*, 321–322.

Park, S. W., Lee, J. G., Kong, B. G., Lee, S. J., Lee, C. H., Kim, J. I., & Kim, Y. H. (2009). Genetic association of BDNF val66met and GSK-3beta-50 T/C polymorphisms with tardive dyskinesia. *Psychiatry and Clinical Neuroscience, 63*, 433–439.

Pascual, M., Pérez-Sust, P., & Soriano, E. (2004). The GABAergic septohippocampal pathway in control and reeler mice: Target specificity and termination onto reelin-expressing interneurons. *Molecular and Cellular Neuroscience, 25*, 679–691.

Pedrini, M., Chendo, I., Grande, I., Lobato, M. I., Belmonte-de-Abreu, P. S., Lersch, C., Walz, J., Kauer-Sant'anna, M., Kapczinski, F., & Gama, C. S. (2011). Serum brain-derived neurotrophic factor and clozapine daily dose in patients with schizophrenia, a positive correlation. *Neuroscience Letters, 491*, 207–210.

Pedrini, M., Massuda, R., Fries, G. R., de Bittencourt Pasquali, M. A., Schnorr, C. E., Moreira, J. C. F., Teixeira, A. L., Lobato, M. I. R., Walz, J. C., Belmonte-de-Abreu, P. S., Kauer-Sant'anna, M., Kapczinski, F., & Gama, C. S. (2012). Similarities in serum oxidative stress markers and inflammatory cytokines in patients with overt schizophrenia at early and late stages of chronicity. *Journal of Psychiatric Research, 46*, 819–824.

Perez-Costas, E., Gandy, J. C., Melendez-Ferro, M., Roberts, R. C., & Bijur, G. N. (2010). Light and electron microscopy study of glycogen synthase kinase-3beta in the mouse brain. *PloS One, 5*, e8911.

Pesold, C., Impagnatiello, F., Pisu, M. G., Uzunov, D. P., Costa, E., Guidotti, A., & Caruncho, H. J. (1998). Reelin is preferentially expressed in neurons synthesizing gamma-aminobutyric acid in cortex and hippocampus of adult rats. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 3221–3226.

Pert, L., Ferriter, M., & Saul, C. (2004). Parental loss before the age of 16 years: A comparative study of patients with personality disorder and patients with schizophrenia in a high secure hospital's population. *Psychology and Psychotherapy, 77*(Pt 3), 403–407.

Pisanté, A., Bronstein, M., Yakir, B., & Darvasi, A. (2009). A variant in the reelin gene increases the risk of schizophrenia and schizoaffective disorder but not bipolar disorder. *Psychiatric Genetics, 19*, 212.

Pollard, M., Varin, C., Hrupka, B., Pemberton, D. J., Steckler, T., & Shaban, H. (2012). Synaptic transmission changes in fear memory circuits underlie key features of an animal model of schizophrenia. *Behavioural Brain Research, 227*, 184–193.

Rice, D., & Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: Evidence from human and animal models. *Environmental Health Perspectives, 108*(Suppl. 3), 511–533.

Richard, M. D., & Brahm, N. C. (2012). Schizophrenia and the immune system: Pathophysiology, prevention, and treatment. *American Journal of Health-System Pharmacy, 69*, 757–766.

Rizos, E. N., Rontos, I., Laskos, E., Arsenis, G., Michalopoulos, P. G., et al. (2008). Investigation of serum BDNF levels in drug-naive patients with schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 32*, 1308–1311.

Rizos, E. N., Papadopoulou, A., Laskos, E., Michalopoulos, P. G., Kastania, D., Vasilopoulos, D., Katsafouros, K., & Lykouras, L. (2010). Reduced serum BDNF levels in patients with chronic schizophrenic disorder in relapse, who were treated with typical or atypical antipsychotics. *The World Journal of Biological Psychiatry, 1*, 251–255.

Rosenberg, S. D., Lu, W., Mueser, K. T., Jankowski, M. K., & Cournos, F. (2007). Correlates of adverse childhood events among adults with schizophrenia spectrum disorders. *Psychiatric Services, 58*, 245–253.

Sachdev, P. S., Valenzuela, M., Wang, X. L., Looi, J. C., & Brodaty, H. (2002). Relationship between plasma homocysteine levels and brain atrophy in healthy elderly individuals. *Neurology, 58*, 1539–1541.

Sanderson, T. M., Cotel, M. C., O'Neill, M. J., Tricklebank, M. D., Collingridge, G. L., & Sher, E. (2012). Alterations in hippocampal excitability, synaptic transmission and synaptic plasticity in a neurodevelopmental model of schizophrenia. *Neuropharmacology, 62*, 1349–1358.

Sawa, A., & Snyder, S. H. (2002). Schizophrenia: Diverse approaches to a complex disease. *Science, 296*, 692–695.

Schutte, D. L., Reed, D., Decrane, S., & Ersig, A. L. (2011). Saitohin and APOE polymorphisms influence cognition and function in persons with advanced alzheimer disease. *Dementia and Geriatric Cognitive Disorders, 32*, 94–102.

Shinohara, M., Ybanez, M. D., Win, S., Than, T. A., Jain, S., Gaarde, W. A., Han, D., & Kaplowitz, N. (2010). Silencing glycogen synthase kinase-3beta inhibits acetaminophen hepatotoxicity and attenuates JNK activation and loss of glutamate cysteine ligase and myeloid cell leukemia sequence 1. *The Journal of Biological Chemistry, 285*, 8244–8255.

Singh, K. K., De Rienzo, G., Drane, L., Mao, Y., Flood, Z., Madison, J., Ferreira, M., Bergen, S., King, C., Sklar, P., Sive, H., & Tsai, L. H. (2011). Common DISC1 polymorphisms disrupt Wnt/GSK3β signaling and brain development. *Neuron, 72*, 545–558.

Somenarain, L., & Jones, L. B. (2010). A comparative study of MAP2 immunostaining in areas 9 and 17 in schizophrenia and huntington chorea. *Journal of Psychiatric Research, 44*, 694–699.

Song, H., Ueno, S., Numata, S., Iga, J., Shibuya-Tayoshi, S., Nakataki, M., Tayoshi, S., Yamauchi, K., Sumitani, S., Tomotake, T., Tada, T., Tanahashi, T., Itakura, M., & Ohmori, T. (2007). Association between PNPO and schizophrenia in the Japanese population. *Schizophrenia Research, 97*, 264–270.

Steiner, J., Bogerts, B., Schroeter, M. L., & Bernstein, H. G. (2011). S100B Protein in neurodegenerative disorders. *Clinical Chemistry and Laboratory Medicine, 49*, 409–424.

Stone, J. M., Day, F., Tsagaraki, H., Valli, M. L. M. A., Lythgoe, D. J., O'Gorman, R. L., Barker, G. J., McGuire, P. K., & OASIS. (2009). Glutamate dysfunction in people with prodromal symptoms of psychosis: Relationship to grey matter volume. *Biological Psychiatry, 66*, 533–539.

Sullivan, E. M., & O'Donnell, P. (2012). Inhibitory interneurons, oxidative stress, and schizophrenia. *Schizophrenia Bulletin, 38*, 373–376.

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003). Schizophrenia as a complex trait: Evidence from a meta-analysis of twin studies. *Archives of General Psychiatry, 60*, 1187–1192.

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., & Alberini, C. M. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell, 144*, 810–823.

Suzuki, K., Nakamura, K., Iwata, Y., Sekine, Y., Kawai, M., Sugihara, G., Tsuchiya, K. J., Suda, S., Matsuzaki, H., Takei, N., Hashimoto, K., & Mori, N. (2008). Decreased expression of reelin receptor VLDLR in peripheral lymphocytes of drug-naive schizophrenic patients. *Schizophrenia Research, 98*, 148–156.

Swanson, R. A. (1992). Physiologic coupling of glial glycogen metabolism to neuronal activity in brain. *Canadian Journal of Physiology and Pharmacology, 70*(Suppl), S138–S144.

Tan, Y. L., Zhou, D. F., & Zhang, X. Y. (2005). Decreased plasma brain-derived neurotrophic factor levels in schizophrenic patients with tardive dyskinesia: Association with dyskinetic movements. *Schizophrenia Research, 74*, 263–270.

Tayoshi, S., Sumitani, S., Taniguchi, K., Shibuya-Tayoshi, S., Numata, S., Iga, J., Nakataki, M., Ueno, S., Harada, M., & Ohmori, T. (2009). Metabolite changes and gender differences in schizophrenia using 3-Testa proton magnetic resonance spectroscopy (^{1}H-MRS). *Schizophrenia Research, 108*, 69–77.

Tedla, Y., Shibre, T., Ali, O., Tadele, G., Woldeamanuel, Y., Asrat, D., Aseffa, A., Mihret, W., Abebe, M., Alem, A., Medhin, G., & Habte, A. (2011). Serum antibodies to toxoplasma gondii and herpesvidae family viruses in individuals with schizophrenia and bipolar disorder: A case-control study. *Ethiopian Medical Journal, 49*, 211–220.

Théberge, J., Williamson, K. E., Aoyama, N., Drost, D. J., Manchanda, R., Malla, A. K., Northcott, S., Menon, R. S., Neufeld, R. W., Rajakumar, N., Pavlosky, W., Densmore, M., Schaefer, B., & Williamson, P. C. (2007). Longitudinal grey-matter and glutamatergic losses in first-episode schizophrenia. *The British Journal of Psychiatry, 191*, 325–334.

Thoma, R. J., Monnig, M., Hanlon, F. M., Miller, G. A., Petropoulos, H., Mayer, A. R., Yeo, R., Euler, M., Lysne, P., Moses, S. N., & Cañive, J. M. (2009). Hippocampus volume and episodic memory in schizophrenia. *Journal of the International Neuropsychological Society, 15*, 182–195.

Tibbo, P., Hanstock, C., Valiakalayil, A., & Allen, P. (2004). 3-T proton MRS investigation of glutamate and glutamine in adolescents at high genetic risk for schizophrenia. *The American Journal of Psychiatry, 161*, 1116–1118.

Tosic, M., Ott, J., Barral, S., Bovet, P., Deppen, P., Gheorghita, F., Matthey, M. L., Parnas, J., Preisig, M., Saraga, M., Solida, A., Timm, S., Wang, A. G., Werge, T., Cuénod, M., & Do, K. Q. (2006). Schizophrenia and oxidative stress: Glutamate cysteine ligase modifier as a susceptibility gene. *The American Journal of Human Genetics, 79*, 586–592.

Traver, S., Marien, M., Martin, E., Hirsch, E. C., & Michel, P. P. (2006). The phenotypic differentiation of locus coeruleus noradrenergic neurons mediated by brain-derived neurotrophic factor is enhanced by corticotrophin releasing factor through the activation of a cAMP-dependent signaling pathway. *Molecular Pharmacology, 70*, 30–40.

Tseng, K. Y., Lewis, B. L., Lipska, B. K., & O'Donnell, P. (2007). Post-pubertal disruption of medial prefrontal cortical dopamine-glutamate interactions in a developmental animal model of schizophrenia. *Biological Psychiatry, 62*, 730–738.

Tseng, K. Y., Chambers, R. A., & Lipska, B. K. (2009). The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. *Behavioural Brain Research, 204*, 295–305.

Tueting, P., Doueiri, M. S., Guidotti, A., Davis, J. M., & Costa, E. (2006). Reelin down-regulation in mice and psychosis endophenotypes. *Neuroscience and Biobehavioral Reviews, 30*, 1065–1077.

Turetsky, B. I., Moberg, P. J., Roalf, D. R., Arnold, S. E., & Gur, R. E. (2003). Decrements in volume of anterior ventromedial temporal lobe and olfactory dysfunction in schizophrenia. *Archives of General Psychiatry, 60*, 1193–1200.

Valenti, O., Cifalli, P., Gill, K. M., & Grace, A. A. (2011). Antipsychotic drugs rapidly induce dopamine neuron depolarization block in a developmental rat model of schizophrenia. *The Journal of Neuroscience, 31*, 12330–12338.

Ventruti, A., Kazdoba, T. M., Niu, S., & D'Arcangelo, G. (2011). Reelin deficiency cause specific defects in the molecular composition of the synapses in the adult brain. *Neuroscience, 189*, 32–42.

Virgili, M., Vandi, M., & Contestabile, A. (1997). Ischemic and excitotoxic damage to brain slices from normal and microencephalic rats. *Neuroscience Letters, 233*, 53–57.

Walton, N. M., Shin, R., Tajinda, K., Heusner, C. L., Kogan, J. H., Miyake, S., Chen, Q., Tamura, K., & Matsumoto, M. (2012). Adult neurogenesis transiently generates oxidative stress. *PloS One, 7*(4), e35264.

Wang, Y., Gao, L., Conrad, C. G., & Andreadis, A. (2011). Saitohin, which is nested within the tau gene, interacts with tau and Abl and its human-specific allele influences Abl phosphorylation. *Journal of Cellular Biochemistry, 112*, 3482–3488.

Welch, K. A., McIntosh, A. M., Job, D. E., Whalley, H. C., Moorhead, T. W., Hall, J., Owens, D. G., Lawrie, S. M., & Johnstone, E. C. (2011). The impact of substance use on brain structure in people at high risk of developing schizophrenia. *Schizophrenia Bulletin, 37*, 1066–1076.

Wender, R., Brown, A. M., Fern, R., Swanson, R. A., Farrell, K., & Ransom, B. R. (2000). Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. *The Journal of Neuroscience, 20*, 6804–6810.

Wexler, B. E., Zhu, H., Bell, M. D., Nicholls, S. S., Fulbright, R. K., Gore, J. C., Colibazzi, T., Amat, J., Bansal, R., & Peterson, B. S. (2009). Neuropsychological near normality and brain structure abnormality in schizophrenia. *The American Journal of Psychiatry, 166*, 189–195.

Wexler, E. M., & Geschwind, D. H. (2011). DISC1: A schizophrenia gene with multiple personalities. *Neuron, 72*, 501–503.

Yang, Y. Q., Sun, S., Yu, Y. Q., Li, W. J., Zhang, X., & Xiu, M. H. (2011). Decreased serum brain-derived neurotrophic factor levels in schizophrenic patients with tardive dyskinesia. *Neuroscience Letters, 502*, 37–40.

Yao, J. K., Reddy, R., Meelhinny, L. G., & Van Kammen, D. P. (1998). Reduced status of plasma total antioxidant capacity in schizophrenia. *Schizophrenia Research, 55*, 33–34.

Yao, J. K., Reddy, R., Meelhinny, L. G., & Van Kammen, D. P. (2001). Oxidative damage and schizophrenia: An overview of the evidence and its therapeutic implications. *CNS Drugs, 15*, 287–310.

Yoshimura, R., Hori, H., Sugita, N., Ueda, N., & Kakihara, S. (2007). Treatment with risperidone for 4 weeks increased plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels, but did not alter plasma brain-derived neurotrophic factor (BDNF) levels in schizophrenic patients. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 31*, 1072–1077.

Yoshimura, R., Ueda, N., Hori, H., Ikenouchi-Sugita, A., Umene-Nakano, W., et al. (2010). Different patterns of longitudinal changes in plasma levels of catecholamine metabolites and brain-derived neurotrophic factor after administration of atypical antipsychotics in first episode untreated schizophrenic patients. *The World Journal of Biological Psychiatry, 11*, 256–261.

Yu, Q., Plis, S. M., Erhardt, E. B., Allen, E. A., Sui, J., Kiehl, K. A., Pearlson, G., & Calhoun, V. D. (2011). Modular organization of functional network connectivity in healthy controls and patients with schizophrenia during the resting state. *Frontiers in Systems Neuroscience, 5*, 103.

Zai, C. C., Tiwari, A. K., De Luca, V., Muller, D. J., Bulgin, N., Hwang, R., Zai, G. C., King, N., Voineskos, A. N., Meltzer, H. Y., Lieberman, J. A., Potkin, S. G., Remington, G., & Kennedy, J. L. (2009). Genetic study of BDNF, DRD3, and their interaction in tardive dyskinesia. *European Neuropsychopharmacology, 19*, 317–328.

Zaidel, D. W., Esiri, M. M., & Harrison, P. J. (1997). Size, shape and orientation of neurons in the left and right hippocampus: Investigation of normal asymmetries and alterations in schizophrenia. *The American Journal of Psychiatry, 154*, 812–818.

Zhang, X. Y., Xiu, M. H., da Chen, C., Zhu, F. Y., Wu, G. Y., Haile, C. N., Lu, L., Kosten, T. A., & Kosten, T. R. (2010). Increased S100B serum levels in schizophrenic patients with tardive dyskinesia: Association with dyskinetic movements. *Journal of Psychiatric Research, 44*, 429–433.

Zhang, X. Y., Chen, D. C., Xiu, M. H., Hui, L., Liu, H., Luo, X., Zuo, L., Zhang, H., Kosten, T. A., & Kosten, T. R. (2012a). Association of functional dopamine-beta-hydroxylase (DBH) 19 bp insertion/deletion polymorphism with smoking severity in male schizophrenic smokers. *Schizophrenia Research, 141*(1), 48–53. PMID: 22871345.

Zhang, X. Y., Liang, J., Chen, D. C., Xiu, M. H., De Yang, F., Kosten, T. A., & Kosten, T. R. (2012b). Low BDNF is associated with cognitive impairment in chronic patients with schizophrenia. *Psychopharmacology (Berlin), 222*(2), 277–284. PMID: 22274000.

Neurotoxicity of Methamphetamine

Rosario Moratalla, Sara Ares-Santos, and Noelia Granado

Contents

Abstract

Recreational consumption of the highly addictive psychostimulant methamphetamine is becoming a serious public health problem worldwide. Recent estimates indicate that methamphetamine abuse has increased in the last decade and that only cannabis is used by a greater number of consumers. Despite its popularity, methamphetamine is a known neurotoxin that damages dopaminergic terminals

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in the striatum, as indicated by reductions in striatal levels of dopamine and its metabolites and a sustained decrease in the expression of markers for dopaminergic terminals such as TH and DAT. In addition, methamphetamine affects the cell bodies of these same dopaminergic neurons in the substantia nigra, resulting in cell loss. The mechanisms underlying dopaminergic neurotoxicity are the focus of intense research, and knowledge in this area has expanded in recent decades. Evidence from previous studies points to dysregulation of dopamine, oxidative stress, DNA damage, and mitochondrial dysfunction as the main causes of methamphetamine neurotoxicity. The dopamine receptors D1 and D2 also play an important role in methamphetamine-induced neurotoxicity since inactivation of either receptor is neuroprotective against methamphetamine. Recent results from clinical research indicate that methamphetamine abusers have a higher risk of developing Parkinson's disease; this is in keeping with results in laboratory animals and confirms the persistence of methamphetamine-induced dopaminergic injury. These findings suggest that neuroprotective strategies that are effective against methamphetamine-induced toxicity are also promising candidates for preventive therapy for Parkinson's disease and other persistent dopaminergic injuries.

Keywords

Amphetamine derivatives • Designer drugs • Dopamine • Drug addiction • Methamphetamine • Neurotoxicity • Parkinson's disease • Psychomotor stimulants

List of Abbreviations

ATS	Amphetamine-type stimulants
$D1R^{-/-}$	D1R knockout mice
D1R	Dopamine D1 receptor
$D2R^{-/-}$	D2R knockout mice
D2R	Dopamine D2 receptor
DAT	Dopamine transporter
DOPAC	3,4-Dihydroxyphenylacetic acid
Gclm	γ-Cysteine ligase modulatory subunit
Gclc	γ-Cysteine ligase catalytic subunit
GPx	Glutathione peroxidase
HVA	Homovanillic acid
MDMA	3,4-Methylendioxymethamphetamine also called "ecstasy"
Nrf2	Nuclear factor-erythroid 2-related factor 2
PD	Parkinson's disease
ROS	Reactive oxygen species
SNpc	Substantia nigra pars compacta
TH	Tyrosine hydroxylase
VMAT2	Vesicular monoamine transporter

1 Introduction

1.1 Background, Medical Use, and Epidemiology

Methamphetamine (*N*-methyl-1-phenylpropan-2-amine) is a synthetic drug first used clinically at the beginning of the twentieth century. Therapeutic use of methamphetamine was based on its sympathicomimetic properties, but its current illicit use as a recreational drug in several countries around the world is due to its psychostimulant effects. It is also used for weight loss and for enhancing alertness, focus, motivation, and mental clarity for extended periods of time. Methamphetamine is structurally related to the neurotransmitters dopamine and phenylethylamine, and to other psychostimulant drugs like amphetamines (Fig. 1).

Methamphetamine has proven to be highly addictive and its abuse can result in severe psychological and physical dependence. It is therefore classified as a Schedule II drug under the Convention on Psychotropic Substances. Methamphetamine abuse is increasingly recognized as a major global health problem (Degenhardt et al. 2008). The 2012 World Drug Report (UNODC 2012) suggests that the global use of amphetamine-type stimulants (ATS; methamphetamine,

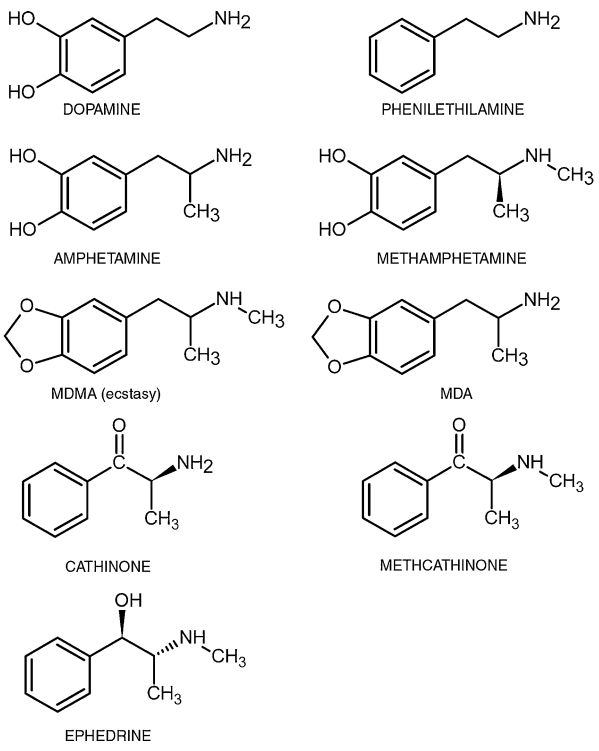

Fig. 1 Chemical structures of methamphetamine and related amphetamine compounds. All compounds share chemical structure with dopamine and therefore have strong effects on the dopamine system

amphetamine, and methcathionine) (excluding "ecstasy") is second only to cannabis. They were used by 14–53 million people in 2010 equivalent to 0.3–1.2 % of the global population aged 15–64 years.

1.2 Administration Routes and Patterns of Methamphetamine Use

Methamphetamine comes in several forms. The hydrochloride salt of methamphetamine is a white, crystalline, bitter, odorless powder. It is water-soluble and strongly hygroscopic (absorbs water quickly). The common street names "speed," "Meth," or "chalk" refer to the salt, while "crystal," "crystal Meth," and "ice" refer to crystalline methamphetamine, a form purer than the powder. Methamphetamine is also known by a variety of other names, including shabu, batu, D-meth, tina, and glass. Methamphetamine freebase is oily and is uncommon on the street.

Methamphetamine can be taken orally (in pill form), by intravenous injection, smoking, snorting (in powder form), or by anal or vaginal insertion of a suppository. The effects experienced by the user last 6–8 h, depending on the rate at which methamphetamine reaches the blood, which depends on the route of administration. The faster the drug reaches the blood, the greater the "high" and other effects experienced by the user.

Following administration by any route, methamphetamine is distributed through most organs, including the lungs, liver, and stomach. Moderate levels reach the brain by crossing the blood–brain barrier. It also crosses the placenta and is secreted into breast milk. Methamphetamine is metabolized in the liver, with the main metabolites including the active compounds amphetamine, 4-hydrooxyamphetamine, and norephedrine. The concentration of amphetamine metabolite peaks at 10–24 h post-administration.

Methamphetamine abuse has two distinct use patterns. The first, characterized by low intensity use, does not confer psychological dependence. The second, known as "binge" use, consists of repeated redosing, usually by inhalation or injection, for several days in a row; generally, withdrawal symptoms occur when drug delivery is stopped abruptly.

1.3 Methamphetamine: Mechanism of Action and Effects

Methamphetamine's effects on the body are due to its structural resemblance to the neurotransmitter dopamine (Fig. 1). It easily crosses the blood–brain barrier, reaches the brain, and enters the axons of dopaminergic neurons through the dopamine transporter DAT and by passive diffusion (Granado et al. 2011a; Krasnova and Cadet 2009). Once inside the axon, methamphetamine triggers the release of dopamine from synaptic storage vesicles, resulting in an unusually high concentration of dopamine in the cytoplasm (Krasnova and Cadet 2009). Methamphetamine cannot directly activate dopamine receptors; rather, it acts as an indirect

dopamine agonist that increases dopamine concentration in the synapse by increasing dopamine release and blocking dopamine uptake. Methamphetamine also releases norepinephrine and serotonin by a similar mechanism; however, in the brain, methamphetamine is selectively concentrated in norepinephrine and dopamine nerve terminals because it is a substrate for the molecular transporters present in these terminals.

As a consequence of this increased release of dopamine in several areas of the brain, methamphetamine produces a number of acute psychological effects including euphoria (also known as "flash" or "rush", and lasting only several minutes). After this first short period, other feelings and behaviors may appear, including a false sense of self-confidence and power (delusions of grandeur), loquacity, moodiness, irritability, anxiousness, nervousness, aggressiveness, and violent behavior. Methamphetamine consumption has many acute adverse physical effects, including hyperthermia, increase in blood pressure and heart rate, mydriasis (pupil dilatation), logorrhoea, grinding teeth (trismus and bruxia), gastrointestinal irritation, appetite loss, itching, welts on skin, hyperactivity, involuntary body movements, irreversible damage to blood vessels in the brain resulting in cerebrovascular accidents, arrhythmia, tachycardia, cardiovascular collapse, and death. The most common symptoms of chronic methamphetamine abuse include temporomandibular joint syndrome, tooth erosion, and myofacial pain, all manifestations of acute trismus and bruxia. Other long-term symptoms are loss of appetite, weight loss, accelerated aging, nose bleeding, and "Meth mouth," an oral disease characterized by tooth erosion, extensive caries, decayed surfaces, missing teeth, tooth wear, plaque, and calculus (Fig. 2). Methamphetamine is highly addictive, and its use can result in tolerance: The effects decrease gradually with chronic use; thus, increased dosages are required to achieve the desired effects.

2 Methamphetamine Induces Neurotoxicity

Repeated methamphetamine administration results in neurotoxicity, primarily affecting dopaminergic neurons in the nigrostriatal system as reflected by long-lasting reductions in levels of dopamine and its metabolites (DOPAC and HVA), dopaminergic markers such as tyrosine hydroxylase (TH) (the rate-limiting enzyme for dopamine synthesis), and DAT (Krasnova and Cadet 2009) (Fig. 3).

Several studies have demonstrated dopaminergic axon loss in the striatum after repeated methamphetamine use, indicated by loss of TH and DAT immunoreactivity. Although there is partial recovery of axonal TH and DAT immunoreactivity, some loss persists for long periods. Other amphetamine compounds such as ecstasy (MDMA) also produce this persistent axonal loss, which correlates with dopaminergic cell body loss in the substantia nigra pars compacta (SNpc), as demonstrated by rigorous stereology/cell counts with TH and Nissl staining and by use of cell death markers such as Fluorojade (Granado et al. 2008a). Apoptotic cell bodies, an irrefutable marker of cell death, have also been observed in the SNpc of methamphetamine-treated mice (Ares-Santos et al. 2012, 2013; Granado et al. 2011a).

Fig. 2 Methamphetamine abuse can produce accelerated aging, "METH mouth," and "METH mites." (**a**). Physical aspect of a woman at several time points during 4 years of methamphetamine abuse. (**b**). Case of "Meth Mouth". (**c**). Sores on the skin known as "meth mites," which result from scratching to relieve the feeling of having small bugs under the skin that methamphetamine abusers may experience (Taken from drug enforcement administration www.dea.gov)

Interestingly, the compartments of the mouse striatum – the striosomes and matrix – differ in their vulnerabilities to methamphetamine, (Granado et al. 2010) (see Fig. 4). Striosomes, which are connected to the limbic system and are functionally associated with reward-related behaviors and emotional events (White and Hiroi 1998), are more vulnerable to methamphetamine-induced dopaminergic terminal loss than the matrix, which is connected to sensorimotor regions of the brain and is more closely associated with normal motor functions.

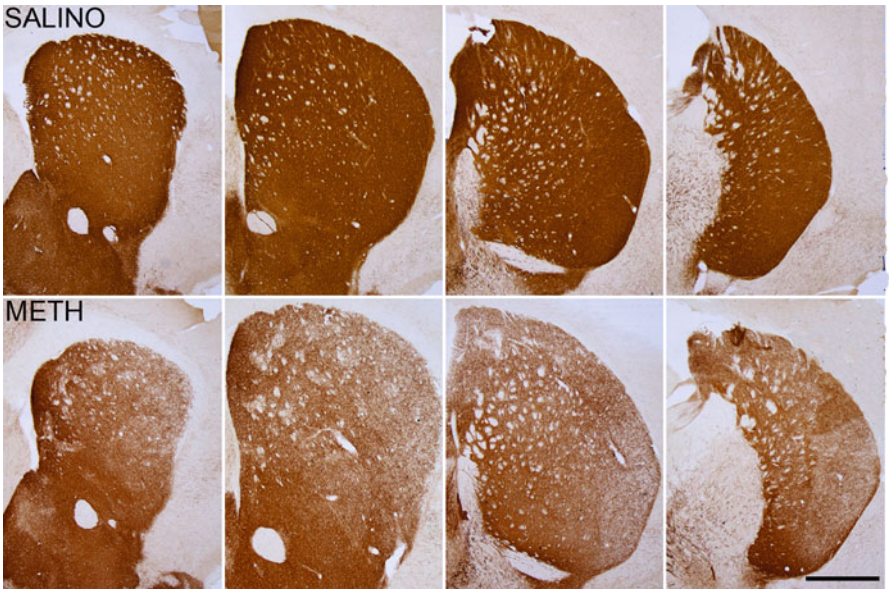

Fig. 3 Methamphetamine induces a loss of striatal TH-ir, indicative of dopamine fiber loss. Photomicrographs of striatal sections from mice treated with saline or methamphetamine stained for TH. METH produced a marked loss in striatal TH-ir that persists 7 days after treatment. Scale bar indicates 500 μm

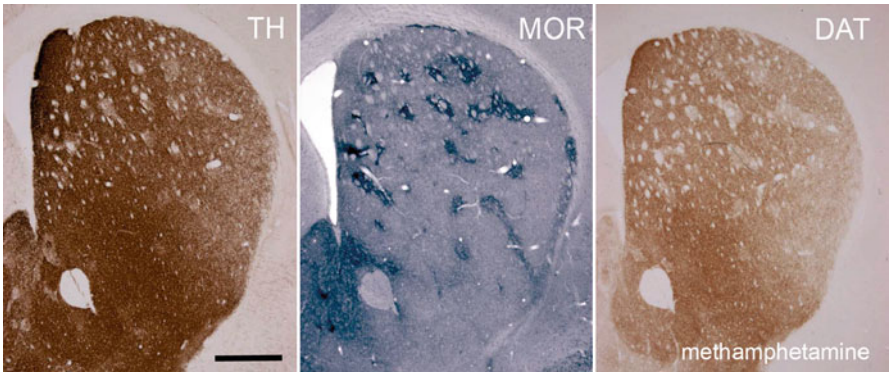

Fig. 4 Striatal vulnerability to methamphetamine. Methamphetamine toxicity occurs primarily in the striatum. Methamphetamine produces a preferential loss of TH and DAT in the striosomal compartment compared to the matrix. Photomicrographs of mouse brain sections 7 days after saline or methamphetamine treatment were stained for TH, MOR-1, and DAT. MOR-1 is a marker for striosomes. Scale bar indicates 500 μm (Modified from Granado et al. (2010))

The pattern of dopamine degeneration in the striatum is similar to that observed in the early stages of other neurodegenerative diseases such as Huntington's disease, hypoxic ischemic injury, and treatment with MPTP, a selective neurotoxin for dopaminergic neurons that is frequently used as a model of Parkinson's disease.

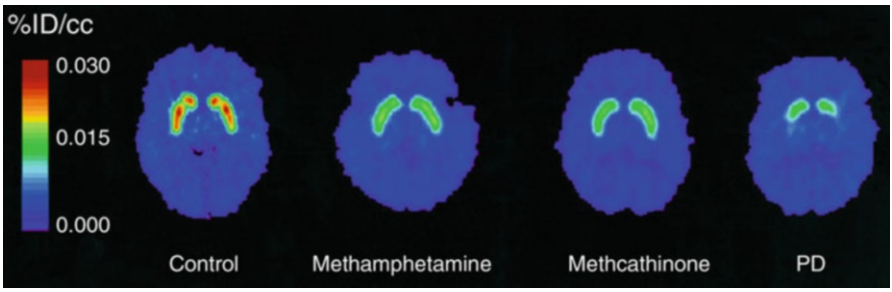

Fig. 5 Reduced DAT function in methamphetamine users. PET images showing accumulation of [11C] WIN-35,428 in the striatum of a control subject, an abstinent methamphetamine subject, an abstinent methcathinone subject, and a PD patient 70–90 min after injection of [11C] WIN-35,428 (Taken from McCann et al. (1998))

The nucleus accumbens is resistant to methamphetamine-induced dopaminergic axon loss (Granado et al. 2010), paralleling the effects of Parkinson's disease (Hurtig et al. 2000).

Methamphetamine neurotoxicity has also been demonstrated in humans (Fig. 5). PET studies in methamphetamine abusers found a reduction in DAT density in the caudate nucleus (26 % loss) and the putamen (21 % loss) after a short period of abstinence (Volkow et al. 2001a). Other authors reported similar DAT loss even 3 years after methamphetamine withdrawal (McCann et al. 1998). Studies in postmortem striatal tissue from chronic methamphetamine abusers showed a significant dopamine reduction concomitant with loss of DAT and TH immunoreactivity, indicative of dopamine nerve fiber loss. These effects have been related to loss of neurological function, including memory loss and motor and verbal learning impairments (Volkow et al. 2001b). Methamphetamine also causes neurotoxicity in other brain areas including somatosensory parietal, frontal, and piriform cortex, olfactory bulb, and hippocampus, where apoptotic neurons have been found following exposure to the drug.

These studies in animals and in human abusers suggest that methamphetamine consumers may be more susceptible to neurodegenerative diseases like Parkinson's disease (PD), raising important concerns about the use and abuse of amphetamines clinically and recreationally. A very recent clinical study shows that methamphetamine users have a 76 % greater risk of developing PD than normal subjects. Although these studies have not yet confirmed neuronal damage in the substantia nigra of human methamphetamine abusers, results in animals support this loss and are consistent with the idea that repeated methamphetamine abuse predisposes the abuser to PD. Methamphetamine also causes neuronal loss in other brain areas such as the olfactory bulb, cortex, hippocampus, and striatum, as indicated by increased apoptosis, increased numbers of TUNEL-positive cells, and decreased numbers of neurons in the brains of methamphetamine-treated laboratory animals (Krasnova and Cadet 2009).

3 Mechanisms of Methamphetamine-Induced Neurotoxicity

3.1 Role of Dopamine

Methamphetamine consumption greatly increases the dopamine concentration in brain synapses. Excessive dopamine in the synaptic cleft is responsible for most of the physical and psychological effects of the drug, including addiction and psychomotor stimulant effects. An imbalance in the distribution of dopamine in the brain also seems to give rise to neurotoxicity, a fact that explains the localization of drug-induced degeneration to dopaminergic terminals (Fig. 6).

Following synthesis in dopaminergic neurons, dopamine is first released to the cytosol before being stored in vesicles where it is protected from metabolism and auto-oxidation. Methamphetamine induces a redistribution of dopamine inside the terminal, releasing dopamine from the vesicles into the cytosol where it is a substrate for metabolic and oxidative reactions, resulting in the production of dopamine quinones, superoxide anions, and hydrogen peroxide and hydroxyl radical species. This can further promote the oxidation of cytosolic dopamine, generating oxidative stress and leading to mitochondrial dysfunction and damage in the dopaminergic terminal (Cadet and Krasnova 2009; Thomas et al. 2008). The detrimental role of excessive cytosolic dopamine and its implication in the neurotoxic effects of methamphetamine is supported by the fact that when dopamine synthesis is inhibited by αMPT, protection against methamphetamine toxicity is observed (Albers and Sonsalla 1995; Ares-Santos et al. 2012). Moreover, pretreatment with L-DOPA, a precursor of dopamine, and treatment with reserpine, which releases dopamine from vesicles to the cytoplasm, both potentiate methamphetamine toxicity (Albers and Sonsalla 1995; Granado et al. 2011a).

3.2 Implications of Oxidative Stress

Reactive nitrogen species and reactive oxygen species (ROS) are by-products of normal physiological metabolism in the brain, but excessive production of these reactive species can damage cell components, including lipids by lipid peroxidation, proteins by formation of protein carbonyls, and mitochondrial and nuclear DNA by peroxidation of these macromolecules. These reactive species impair mitochondrial respiratory chain enzymes and inhibit sodium-potassium ATPase, generating oxidative and nitrosative stress that leads to metabolic collapse and necrotic or apoptotic cell death. These oxidative stress cascades occur in several neurodegenerative disorders including Parkinson's disease.

Methamphetamine administration increases levels of extra-vesicular dopamine in the cytosol, where it can be metabolized by MAO or auto-oxidized in a process that generates toxic dopamine quinones. These dopamine quinones can damage cell proteins by binding to cysteine residues or by generating hydrogen peroxide (H_2O_2) and superoxide anions ($O_2\bullet-$), considered a major culprit in methamphetamine toxicity. The methamphetamine-induced increase in

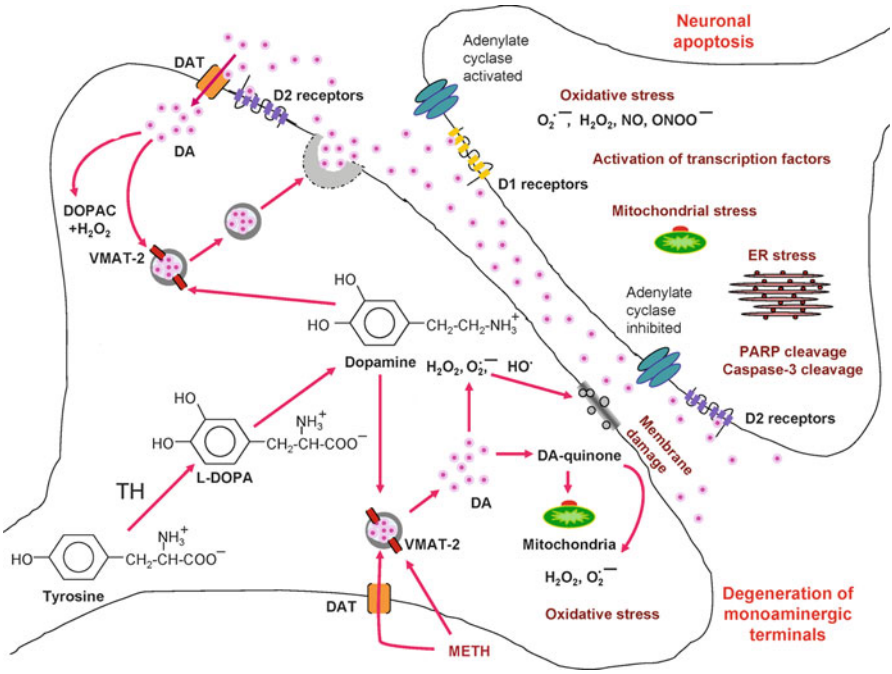

Fig. 6 Schematic representation of cellular and molecular events involved in methamphetamine-induced dopamine terminal degeneration and neuronal apoptosis within the striatum. The figure summarizes findings of various studies that have addressed the role of dopamine, oxidative stress, and other mechanisms in methamphetamine toxicity. Methamphetamine enters dopaminergic neurons via DAT and passive diffusion. Within these neurons, methamphetamine enters synaptic vesicles through VMAT2 and causes dopamine release into the cytoplasm via changes in pH balance. In the cytoplasm, dopamine auto-oxidizes to form toxic dopamine quinones, generating superoxide radicals and hydrogen peroxides via quinone cycling. Subsequent formation of hydroxyl radicals through interactions of superoxides and hydrogen peroxide with transition metals leads to oxidative stress, mitochondrial dysfunctions, and peroxidative damage to presynaptic membranes. The toxic effects of released dopamine might occur through activation of dopamine receptors, as dopamine receptor antagonists block degeneration of dopamine terminals (Modified from Krasnova and Cadet (2009))

interaction of superoxides and hydrogen peroxide with transition metals like iron can lead to the formation of hydroxyl radicals (•OH) that cause oxidative stress, mitochondrial dysfunction, and peroxidative damage to dopaminergic terminal membranes (Krasnova and Cadet 2009).

The methamphetamine-induced increase in oxidative stress likely results from an imbalance between ROS production and the capacity of antioxidant enzyme systems to scavenge ROS: Methamphetamine both increases ROS production and reduces levels of the ROS scavengers CuZnSOD, catalase, glutathionine, and peroxiredoxins in the brain (Jayanthi et al. 1998; Li et al. 2008). Oxidative stress increases the susceptibility of the striosomal compartment to methamphetamine-induced dopaminergic toxicity (Granado et al. 2010). SOD is less abundant in

striosomes than in the matrix (Medina et al. 1996), which may explain the greater vulnerability of the striosomes to methamphetamine neurotoxicity. This is supported by the finding that transgenic mice overexpressing CuZnSOD are resistant to methamphetamine-induced striatal neuronal damage (Hirata et al. 1996). Furthermore, antioxidants like ascorbic acid (vitamin C), vitamin E, bromocriptine (a hydroxyl radical scavenger), and coenzyme Q10 (antioxidant and mitochondrial energy enhancer) attenuate methamphetamine toxicity (Wagner et al. 1986). Free radical scavengers like PBN (α-phenyl-*N*-terbutil nitrone) also reduce neurotoxic damage (Yamamoto and Zhu 1998), without altering the hyperthermic response that follows methamphetamine administration and that contributes to methamphetamine neurotoxicity (see Sect. 3.3 below).

3.3 Role of Hyperthermia

Methamphetamine usually produces a hyperthermic response in experimental animals and human meth abusers directly proportional to the dosage of the drug and to the ambient temperature in the place of treatment. This hyperthermia can be lethal (it is the first cause of methamphetamine-induced deaths) and can promote long-term neurotoxicity, as a relationship has been observed between the hyperthermic response and the neurotoxicity induced by the drug (Ares-Santos et al. 2012; Bowyer et al. 1994; Granado et al. 2010, 2011a). Strategies that reduce or avoid this hyperthermic response after methamphetamine treatment, like administering the drug at low ambient temperatures (4 °C) or pretreatment with pharmacologic agents like diclocipine or haloperidol, prevent or attenuate drug-induced neurotoxicity (Albers and Sonsalla 1995; O'Callaghan and Miller 1994). Furthermore, methamphetamine administration at high ambient temperatures promotes the hyperthermic response and increases neurotoxicity (Ares-Santos et al. 2012; Bowyer et al. 1994; Granado et al. 2011a; Miller and O'Callaghan 2003).

The correlation between hyperthermia and neurotoxicity is believed to be due to the fact that hyperthermia can potentiate DAT function (Xie et al. 2000), increasing free radicals and dopamine oxidation in the brain (Krasnova and Cadet 2009; LaVoie and Hastings 1999). Conversely, hypothermia has inhibitory effects on oxidative stress, as it reduces dopamine oxidation (LaVoie and Hastings 1999) and the formation of hydroxyl radicals (Fleckenstein et al. 1997; Krasnova and Cadet 2009). Moreover, neurons in animals maintained at low body temperature have reduced energetic demand, which may be protective since administration of methamphetamine produces striatal loss of ATP, possibly as a consequence of metabolic stress in dopaminergic neurons (Chan et al. 1994).

In contrast to these results, other pharmacological and genetic studies indicate that while hyperthermia contributes to methamphetamine-induced dopaminergic neurotoxicity, it is not required. For example, reserpine, a pharmacologic agent that dramatically lowers body temperature, strongly potentiates methamphetamine-induced neurotoxicity while blocking the hyperthermic response (Albers and Sonsalla 1995; Ares-Santos et al. 2012; Granado et al. 2011a). These results

indicate that blocking the hyperthermic response is not sufficient to protect against neuronal damage. Moreover, total or partial inactivation of DAT, nNOS, IL-6, or c-jun protects against methamphetamine-induced toxicity without altering the hyperthermic response. Thus, methamphetamine-induced hyperthermia contributes to, but is not required for, the neurotoxic effects of the drug.

3.4 Role of Dopamine Receptors and Dopaminergic System

As evidence points to dysregulation of dopamine as the primary cause of methamphetamine-induced neurotoxicity, the role of the dopaminergic system in this process has also been evaluated. In particular, as methamphetamine acts as an indirect dopamine agonist, several studies have focused on elucidating the role of dopamine receptors. There are five different dopamine receptors (D1–D5), which fall into two families based on pharmacologic classification: the D1-like receptors (D1 and D5) and the D2-like receptors (D2, D3, and D4). Both families are involved in behavior and cognition, voluntary movement, motivation, punishment and reward, attention, working memory and learning, and in several neurodegenerative diseases like PD (Darmopil et al. 2009; Granado et al. 2008b; Ortiz et al. 2010).

Pharmacologic studies with the D2 receptor antagonists sulpiride, eticlopride, and raclopride have shown a dose-dependent prevention of methamphetamine toxicity in mice (Albers and Sonsalla 1995; Eisch and Marshall 1998). However, these compounds do not differentiate between members of the D2 receptor family, so it was not clear which receptor(s) mediate the protective effect. Recent studies using genetically modified mice lacking dopamine receptor D2 demonstrated that it is specifically the D2 receptor that is involved in methamphetamine toxicity as its genetic inactivation prevented the loss of dopaminergic striatal markers and inhibited the loss of dopaminergic neurons in the substantia nigra (Granado et al. 2011a).

D2 receptors are localized pre- and postsynaptically. At presynaptic locations, D2 receptors control dopamine release and thereby regulate extra-synaptic dopamine levels, which are involved in non-dopaminergic toxicity, such as in striatal medium spiny neurons, cortical and hippocampal neurons and neuropil. In addition, D2 receptors form heteromeric protein-protein complexes with DAT localized in the dopaminergic terminals that potentiate DAT activity. Blockade or inactivation of the D2R decreases striatal DAT activity. Since DAT knockout mice exhibit full protection against methamphetamine-induced dopaminergic toxicity (Fumagalli et al. 1998), indicating that active DAT is required for this neurotoxicity, the decrease in DAT is likely a major factor in the reduction of methamphetamine-induced dopaminergic toxicity induced by blockade or inactivation of the D2R. Moreover, fast scan cyclic voltammetry indicates that dopamine D2R$^{-/-}$ mice have lower vesicular dopamine content, resulting in lower cytosolic dopamine levels. This also contributes to the reduction in methamphetamine-induced toxicity because cytosolic dopamine levels determine the severity of the toxicity (Granado et al. 2011a).

Receptors from the D1 family are also involved in methamphetamine-induced neurotoxicity as their pharmacologic inactivation with antagonists like SCH23390 also confers protection (Sonsalla et al. 1986). Genetic inactivation of the dopamine D1 receptor (D1R) also protected against reductions in striatal TH and DAT expression and against loss of dopaminergic neurons in the substantia nigra following methamphetamine administration, indicating that the D1R in particular is involved in methamphetamine neurotoxicity (Ares-Santos et al. 2012). Neuroprotection afforded by D1R inactivation is due in part to inhibition of hyperthermia, but also to the redistribution of dopamine inside the terminal. Animals lacking the D1R store more dopamine in vesicles and therefore have a reduced cytosolic dopamine pool compared to WT mice (Ares-Santos et al. 2012). Blockade of D1/D5R also suppresses activation of caspases 3 and 8, mediators of the calcineurin/NFAT/FasL-dependent apoptotic cell death pathway (Jayanthi et al. 2005; Krasnova and Cadet 2009); this may also contribute to the neuroprotective effects of D1/D5 blockade or inactivation. Finally, SCH23390 decreases dopamine-induced oxidation and cytotoxicity mediated by ERK and JNK activation (Chen et al. 2004).

Other components of the dopaminergic system are also involved in methamphetamine-induced toxicity. Vesicular monoamine transporter (VMAT2) takes up dopamine from the cytosol to store it in synaptic vesicles, decreasing dopamine oxidation. Methamphetamine interacts with VMAT2 to cause a possible association of vesicles inside the dopaminergic terminal, increasing the release of dopamine to the cytosol and thereby increasing oxidative stress (Sulzer et al. 2005). VMAT2 knockout mice, with higher levels of cytosolic dopamine, are more sensitive to methamphetamine dopaminergic toxicity and show greater expression of oxidative stress markers than WT animals (Larsen et al. 2002). Other results are in line with these findings, showing that VMAT2 becomes nitrated 1 h after methamphetamine administration, which may reduce its activity (Eyerman and Yamamoto 2005), and that methamphetamine also reduces VMAT2 expression in the striatum (Krasnova and Cadet 2009).

3.5 Role of Glutamate and Nitric Oxide

Methamphetamine produces excitotoxicity by increasing glutamate release in the striatum (Nash and Yamamoto 1992), activating *N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. Stimulation of these receptors increases intracellular levels of Ca^{2+}, causing activation of kinases, lipases, and proteases that damage the cytoskeleton, generating free radicals and DNA damage (Sattler and Tymianski 2000). Pharmacological studies using MK801, a noncompetitive NMDA receptor antagonist, prevented the long-term dopamine loss induced by methamphetamine (Sonsalla et al. 1989).

Moreover, NMDA receptor overactivation results in the production of superoxide radicals (O_2•−) and nitric oxide (NO). When these two species react with each other, peroxynitrite ($ONOO^-$), a more potent oxidative species, is formed, further

potentiating neurotoxicity. To examine the contribution of this strong oxidizing agent to methamphetamine toxicity, a previous study measured the formation of neural 3-nitrotyrosine (3-NT), a product of tyrosine nitration that indicates irreversible structural modification of proteins (Butterfield et al. 2011) that can lead to loss of physiological cell functions, appearance of abnormal functions, and eventually cell death. A single injection of methamphetamine produced a significant rise in 3-NT concentrations in the striatum, signifying the involvement of $ONOO^-$ in the destructive effects methamphetamine abuse. Genetic or pharmacological inactivation of nNOS, the enzyme that produces nitric oxide in the brain, considerably reduced methamphetamine neurotoxicity without affecting the hyperthermic response (Itzhak et al. 1998, 2002), likely due to loss of peroxynitrite production.

3.6 Role of Astroglial and Microglial Activation

The central nervous system (CNS), consisting of the brain and the spinal cord, is an "immune privileged" area with an immune system distinct from that in the rest of the body. Microglia, a type of glial cell, are the resident macrophages of the central nervous system, and so act as its first and main form of active immune defense. These cells are normally in a resting state, but become activated after certain types of CNS damage, as a part of the innate immune response. Activated microglia migrate rapidly to the damage sites and secrete reactive species including proinflammatory cytokines (IL-1β, IL-6, TNF-α) chemokines, prostaglandins, reactive oxygen species (ROS), nitric oxide, and superoxide to protect the brain. However, overactivation can be detrimental, producing cell death and astroglial dysfunction.

Amphetamines increase microglia activation in the striatum, hippocampus, cortex, and SN, with populations peaking 1 day after administration. Following administration of methamphetamine or other amphetamine derivatives, activated microglia are found in the areas in which dopaminergic neurotoxicity occurs, and the intensity of activation seems to be correlated with the level of dopaminergic damage. The highest levels of microglial activation occur in the dorsal striatum, an area highly affected by methamphetamine treatment, while the nucleus accumbens, more resistant than the striatum to methamphetamine-induced dopaminergic toxicity, has relatively few reactive microglia. Methamphetamine administration also results in increased levels of the three principal proinflammatory cytokines: IL-1β, IL-6, TNF-α, that in large part result from microglial activation (Clark et al. 2013). Thus, microglial activation represents a direct response to damage by amphetamines and is part of the cascade leading to neuronal damage (Thomas et al. 2008). Although many of the molecules secreted by activated microglia have been implicated in methamphetamine-induced neurotoxicity, and some anti-inflammatory drugs like ketoprofen, indometacin, tetracycline, and minocycline can protect against methamphetamine-induced microglial activation and neurotoxicity, attenuating microglial activation is not sufficient to protect against methamphetamine neurotoxicity (Sriram et al. 2006).

CNS damage is also accompanied by reactive gliosis, the injury-induced activation of astrocytes. The most abundant cells in the human brain, astrocytes, provide nutrients to nervous tissue, maintain extracellular ion balance, support other brain cells, and play an important role in repair and scar formation in the CNS after injury.

Recently, the immunomodulatory role of astrocytes has begun to emerge. Astrocytes can be protective, increasing levels of glutathionine (an antioxidant), facilitating sprouting, and providing growth factors, guidance molecules, and scaffolding for axonal regeneration, but they can also initiate several neuroinflammatory pathways and release inflammatory cytokines, some of which are neurotoxic. However, it seems likely that astrocytes play a positive role in limiting neuroinflammation and the balance between the activation of microglia and astrocytes leads to a detrimental or beneficial outcome (Clark et al. 2013).

Reactive gliosis is considered a universal reaction to CNS damage and is used as a sensitive marker of neuronal damage. Methamphetamine increases astrocyte activation in the striatum, as seen by increased expression of the marker glial fibrillary acidic protein (GFAP), which reaches maximal levels between 3 and 7 days after drug administration (Fig. 7). However, there are much earlier indications of reactive gliosis: Within a few minutes of methamphetamine delivery, there is already a 20 % increase in the magnitude of the Ca^{2+} fluorescence signal in striatal astrocytes, and a 50–60 % increase in the number of responding astrocytes, indicating a primary astrocytic response (Granado et al. 2011b). As with microglia, activation of astrocytes takes place in the areas the most affected by methamphetamine, while the astrocyte population does not increase in the nucleus accumbens, where dopaminergic damage is normally not significant. Briefly, neuroinflammatory mechanism could in part contribute to the gradually escalating deleterious effects of methamphetamine.

3.7 Nrf2 and Inflammation Play a Role in Methamphetamine-Induced Neurotoxicity

The transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2) has been recently shown to have a protective role against methamphetamine-induced dopaminergic neurotoxicity (Granado et al. 2011b). Nrf2 is considered a master regulator of redox homeostasis, as it regulates the expression of a group of genes that encode the phase 2 detoxification enzymes, including heme oxygenase-1 (HO-1), NADPH quinone oxidoreductase (NQO1), and the catalytic and modulatory subunits of γ-glutamyl synthase (GCLM, GCLC) (Clark and Simon 2009; Johnson et al. 2008). In normal conditions, Nrf2 has a very short half-life because of its interaction with the BTB-Kelch protein Keap1, which promotes Nrf2 degradation by the proteasome (Lo et al. 2006). However, oxidant molecules are able to disrupt the Keap1/Nrf2 complex, rescuing Nrf2 from proteasomal degradation and allowing its entry into the nucleus and transcriptional activity.

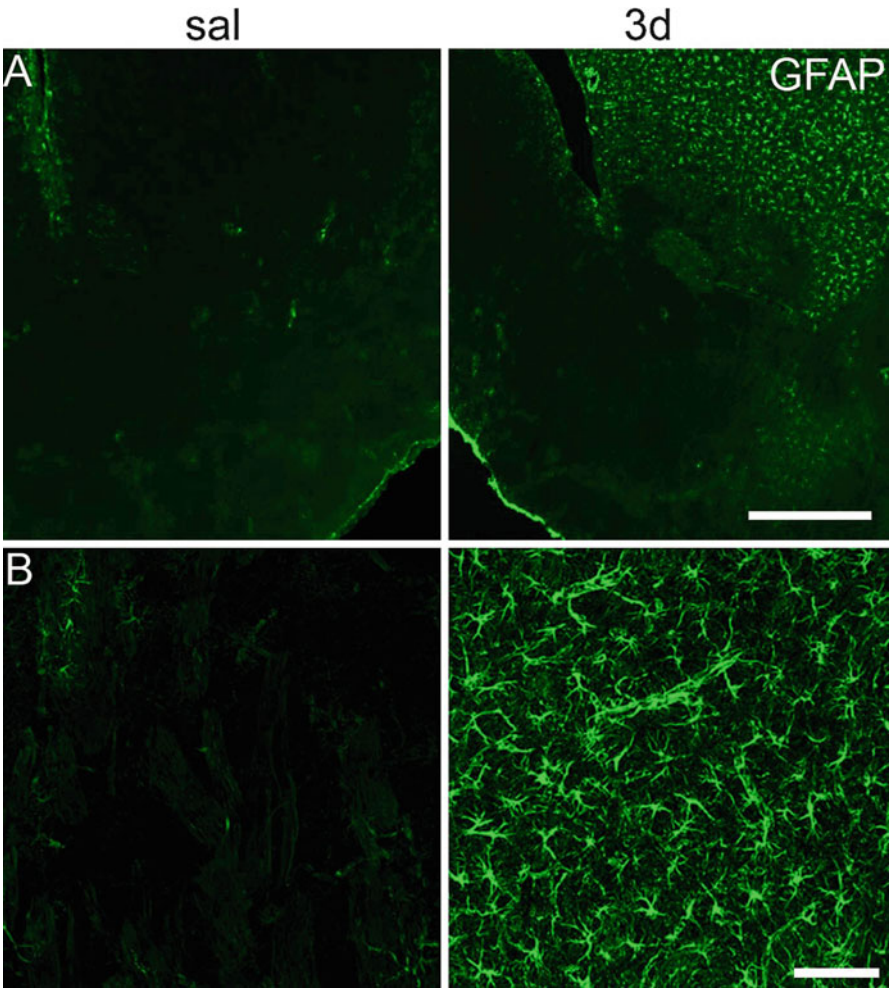

Fig. 7 Methamphetamine induces astrogliosis in mouse striatum but not in nucleus accumbens. (**a**) Photomicrographs of striatal sections from methamphetamine-treated mice stained for GFAP. (**b**) Enlargements of striatum sections shown in (**a**). Animals were killed 3 days after methamphetamine treatment. Methamphetamine increased GFAP staining in the striatum but not in nucleus accumbens. Bar indicates 500 μm for (**a**) and 100 μm for (**b**) (Modified from Granado et al. (2010))

Recent studies have shown that Nrf2 is activated by methamphetamine administration (Jayanthi et al. 2009) and that it plays a crucial role in the protection of dopaminergic neurons against oxidative stress by detoxifying mitochondrial complex I inhibitors and downregulating genes involved in the brain innate immune response. Mice lacking Nrf2 are more susceptible than WT mice to methamphetamine toxicity (Granado et al. 2011b), showing exacerbated hyperthermia, enhanced striatal TH and DAT-fiber loss, and greater decrease in dopamine levels,

and increased dopamine and nigrostriatal dopaminergic alterations and gliosis following administration of methamphetamine (Granado et al. 2011b). This is due to the fact that in the absence of Nrf2, ARE-regulated genes in the striatum, including HO-1 and other antioxidant genes, are not induced by methamphetamine as they are in WT mice, leading to increased oxidative stress, accumulation of ROS (Chen et al. 2009), and ultimately to dopamine fiber loss. In addition, cytokine mRNA levels (TNF-α, IL-1β), gliosis, and astrocytosis in the striatum were elevated to a greater extent in methamphetamine-treated $Nrf2^{-/-}$ mice than WT mice, indicating that the proinflammatory effects of methamphetamine treatment are potentiated in the absence of Nrf2.

Moreover, $Nrf2^{-/-}$ mice treated with methamphetamine presented significantly lower levels of modulatory subunits of γ-cysteine ligase (Gclm) and glutathione peroxidase (GPx) than WT animals, meaning that the detoxificating response is reduced in absence of Nrf2, a fact that could contribute to the increased detrimental effects induced by the drug.

Our finding that Nrf2-deficient mice were more sensitive than WT mice to methamphetamine-induced striatal damage further demonstrates that Nrf2 activation is part of a defensive response to methamphetamine neurotoxicity that involves modulation of methamphetamine-induced inflammation and oxidative stress (Granado et al. 2011b). This defensive modulation of inflammation and oxidative stress by Nrf2 is also seen following administration of other ROS-generating toxins like MPTP (Chen et al. 2009) and lipopolysaccharide (Rangasamy et al. 2004). Intriguingly, Nrf2 deficiency potentiates methamphetamine-induced neurotoxicity in the striatum but not in the SN. It is possible that methamphetamine differentially activates Nrf2-ARE transcription pathways in the striatum and the SN. Thus, differential regulation of Nrf2 by methamphetamine in SN and striatum might explain the lack of effect of $Nrf2^{-/-}$ on neurotoxicity in the SN in our study. These results strongly support the hypothesis that methamphetamine produces dopaminergic neurotoxicity through a process involving inflammation and oxidative stress (Granado et al. 2011b).

3.8 Role of Mitochondrial Dysfunction and DNA Damage

Methamphetamine-induced neurotoxicity also causes mitochondrial dysfunction and DNA damage. Mitochondria are the main source of cellular energy through activation of the ATP-producing mitochondrial respiratory chain, or electron transport chain, composed of a series of four complexes (I–IV). Methamphetamine is a cationic and lipophilic molecule that can diffuse into the mitochondria, where it is able to inhibit ATP synthesis (Asanuma et al. 2000; Krasnova and Cadet 2009). Administration of high doses of methamphetamine in rats decreases the activity of complexes II and IV of the respiratory chain in the striatum and prefrontal cortex, even in the absence of hyperthermia (Brown et al. 2005). Moreover, methamphetamine reduces ATP accumulation, resulting

in mitochondrial dysfunction. The increase in reactive oxygen and nitrogen species may also contribute to the observed mitochondrial dysfunction.

Elevated production of oxygen- and nitrogen-based radicals and related non-radical products leads to the oxidation of essential macromolecules, including DNA. DNA damage plays a role in the pathogenic mechanism of methamphetamine, as the drug increases DNA oxidation in the striatum, hippocampus, substantia nigra, and olfactory bulb (Jeng et al. 2006), causing apoptotic cell death in experimental animals (Deng and Cadet 2000). In particular, the 8-oxoguanine (8-oxoG) produced in DNA lesion may mispair with adenine, causing transversions or mutations, altering the DNA binding of nuclear transcription factors or blocking RNA polymerase, resulting in altered or delayed transcription of proteins. DNA repair genes, including members of the BER pathway, are upregulated in adult mice after methamphetamine administration, suggesting that increased repair activity is induced to counteract the oxidative DNA damage induced by the drug.

4 Neuroprotective Strategies Against Methamphetamine-Induced Neurotoxicity

As basic and clinical research findings begin to elucidate mechanisms of methamphetamine-induced neurotoxicity, potential neuroprotective strategies are being proposed. Since oxidative stress and mitochondrial dysfunction are important factors in methamphetamine-induced neurodegeneration, pretreatment with antioxidants like *N*-acetyl-L-cysteine, ascorbic acid, vitamin E, or coenzyme Q_{10} was evaluated and shown to have protective effects against depletion of monoaminergic axons (Krasnova and Cadet 2009). Melatonin, another antioxidant compound that also has antiapoptotic effects, also reduces methamphetamine-induced depletion of dopaminergic markers in the striatum (Hirata et al. 1998).

Other strategies for avoiding an increase in oxidative stress, such as preventing methamphetamine-induced hyperthermia by pharmacologic treatment or by maintaining animals at cool ambient temperatures during drug administration, also reduce mortality and neurotoxicity.

Controlling the amount of dopamine in the cytosol, where it is susceptible to oxidation, causing oxidative stress, is another neuroprotective strategy. Increasing VMAT2, the vesicular monoamine transporter that sequesters dopamine into vesicles, and administering dopamine uptake inhibitors or dopamine receptor antagonist, all provide protection against methamphetamine -induced degeneration of striatal dopamine terminals. In addition, some trophic factors provide protection against the toxic effects of methamphetamine. Administration of glial cell line-derived neurotrophic factor (GDNF) or brain-derived neurotrophic factor (BDNF) can prevent methamphetamine-mediated reduction in dopamine in the striatum and caspase activation (Dluzen 2004; Matsuzaki et al. 2004; Melega et al. 2000).

Since neuroinflammation may contribute to methamphetamine neurotoxicity, some cytokines, such as interferon gamma, TNF-alpha among others, have been

evaluated and were shown to protect against methamphetamine toxicity. Estrogen can also protect against methamphetamine-induced damage to the nigrostriatal dopamine system (D'Astous et al. 2005). Pretreatment with tamoxifen, a selective estrogen receptor modulator, showed neuroprotective effect against methamphetamine-induced toxicity and attenuated inflammatory response in the striatum when used alone but abolishes estrogen's positive effects when combined with this hormone. While both treatments prevented dopamine decrease, estrogen protected more efficiently other dopaminergic parameters, suggesting that overall estrogen is more effective than tamoxifen as a neuroprotectant of the nigrostriatal dopaminergic system (Bourque et al. 2007; D'Astous et al. 2005).

5 Conclusion

Methamphetamine is a synthetic drug used worldwide, mostly for recreational purposes, due to its powerful psychostimulant effects. It has addictive effects due to its structural analogy with dopamine, but also has neurotoxic effects on the dopaminergic system. The drug causes a reduction in dopamine markers in the striatum similar to that seen in the early stages of neurodegenerative diseases like PD, Huntington's disease, and hypoxic/ischemic injury. In experimental animals, methamphetamine also causes neuron loss in the substantia nigra. This may explain why patients who abuse methamphetamine have a predisposition to future development of PD. Among the mechanisms responsible for methamphetamine's neurotoxic effects are oxidative stress, hyperthermia, microglia and astroglia activation, mitochondrial dysfunction, DNA damage, and elevated levels of glutamate and nitric oxide. The recent implication of the D1 and D2 receptors in methamphetamine-induced neurotoxicity suggests that targeting these receptors may be a promising strategy for development of new approaches to prevention and treatment of methamphetamine addiction and its neurotoxic effects. In addition, the neuropathological similarities between methamphetamine neurotoxicity and PD and the demonstrated predisposition of methamphetamine abusers to developing PD indicate that similar therapeutic approaches may be useful in the early stages of PD and related neurodegenerative diseases.

References

Albers, D. S., & Sonsalla, P. K. (1995). Methamphetamine-induced hyperthermia and dopaminergic neurotoxicity in mice: Pharmacological profile of protective and nonprotective agents. *The Journal of Pharmacology and Experimental Therapeutics*, *275*, 1104–1114. http://www.ncbi.nlm.nih.gov/pubmed/8531070.

Ares-Santos, S., Granado, N., Oliva, I., O'Shea, E., Martin, E. D., Colado, M. I., & Moratalla, R. (2012). Dopamine D(1) receptor deletion strongly reduces neurotoxic effects of methamphetamine. *Neurobiology of Disease*, *45*(2), 810–820. http://www.ncbi.nlm.nih.gov/pubmed/22115942.

Ares-Santos S, Granado N, Moratalla R. (2013). The role of dopamine receptors in the neurotoxicity of methamphetamine. *Journal of Internal Medicine, 273*, 437–453.

Asanuma, M., Hayashi, T., Ordonèz, S. V., Ogawa, N., & Cadet, J. L. (2000). Direct interactions of methamphetamine with the nucleus. *Brain Research. Molecular Brain Research*, *80*(2), 237–243. http://www.ncbi.nlm.nih.gov/pubmed/11038257

Bourque, M., Bin Liu, Dluzen, D. E., & Di Paolo, T. (2007). Tamoxifen protects male mice nigrostriatal dopamine against methamphetamine-induced toxicity. *Biochemical Pharmacology*, *74*(9), 1413–1423. http://www.ncbi.nlm.nih.gov/pubmed/17825264.

Bowyer, J. F., Davies, D. L., Schmued, L., Broening, H. W., Newport, G. D., Slikker, W., & Holson, R. R. (1994). Further studies of the role of hyperthermia in methamphetamine neurotoxicity. *The Journal of Pharmacology and Experimental Therapeutics*, *268*(3), 1571–1580. http://www.ncbi.nlm.nih.gov/pubmed/8138969.

Brown, J. M., Quinton, M. S., & Yamamoto, B. K. (2005). Methamphetamine-induced inhibition of mitochondrial complex II: Roles of glutamate and peroxynitrite. *Journal of Neurochemistry*, *95*(2), 429–436. http://www.ncbi.nlm.nih.gov/pubmed/16086684.

Butterfield, D. A, Reed, T., & Sultana, R. (2011). Roles of 3-nitrotyrosine- and 4-hydroxynonenal-modified brain proteins in the progression and pathogenesis of Alzheimer's disease. *Free Radical Research, 45*(1), 59–72. http://www.ncbi.nlm.nih.gov/pubmed/20942567.

Cadet, J. L., & Krasnova, I. N. (2009). Molecular bases of methamphetamine-induced neurodegeneration. *International Review of Neurobiology*, *88*, 101–119. (1st ed.). Elsevier. http://www.ncbi.nlm.nih.gov/pubmed/19897076.

Chan, P., Di Monte, D. A., Luo, J. J., DeLanney, L. E., Irwin, I., & Langston, J. W. (1994). Rapid ATP loss caused by methamphetamine in the mouse striatum: Relationship between energy impairment and dopaminergic neurotoxicity. *Journal of Neurochemistry*, *62*(6), 2484–2487. http://www.ncbi.nlm.nih.gov/pubmed/8189253.

Chen, J., Rusnak, M., Luedtke, R. J., & Sidhu, A. (2004). D1 dopamine receptor mediates dopamine-induced cytotoxicity via the ERK signal cascade. *The Journal of Biological Chemistry*, *279*(38), 39317–3930. http://www.ncbi.nlm.nih.gov/pubmed/15247297.

Chen, P.-C., Vargas, M. R., Pani, A. k., Smeyne, R. J., Johnson, D. A., Yuet Wai Kan, & Johnson, J. A. (2009). Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(8), 2933–2938. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2650368&tool=pmcentrez&rendertype=abstract.

Clark, J., & Simon, D. K. (2009). Transcribe to survive: Transcriptional control of antioxidant defense programs for neuroprotection in Parkinson's disease. *Antioxidants & Redox Signaling*, *11*(3), 509–528. http://www.ncbi.nlm.nih.gov/pubmed/18717631.

Clark, K. H., Wiley, C. A., & Bradberry, C. W. (2013). Psychostimulant abuse and neuroinflammation: Emerging evidence of their interconnection. *Neurotoxicity Research, 23*(2), 174–188. http://www.ncbi.nlm.nih.gov/pubmed/22714667.

D'Astous, M., Mickley, K. R., Dluzen, D. E., & Di Paolo, T. (2005). Differential protective properties of estradiol and tamoxifen against methamphetamine-induced nigrostriatal dopaminergic toxicity in mice. *Neuroendocrinology*, *82*(2), 111–120. http://www.ncbi.nlm.nih.gov/pubmed/16446547.

Darmopil, S., Martín, A. B., De Diego, I. R., Ares, S., & Moratalla, R. (2009). Genetic inactivation of dopamine D1 but not D2 receptors inhibits L-DOPA-induced dyskinesia and histone activation. *Biological Psychiatry*, *66*(6), 603–613. http://www.ncbi.nlm.nih.gov/pubmed/19520364.

Degenhardt, L., Baker, A., & Maher, L. (2008). Methamphetamine: Geographic areas and populations at risk, and emerging evidence for effective interventions. *Drug and Alcohol Review*, *27*(3), 217–219. http://www.ncbi.nlm.nih.gov/pubmed/18368601.

Deng, X., & Cadet, J. L. (2000). Methamphetamine-induced apoptosis is attenuated in the striata of copper-zinc superoxide dismutase transgenic mice. *Brain Research. Molecular Brain Research*, *83*(1–2), 121–124. http://www.ncbi.nlm.nih.gov/pubmed/11072101.

Dluzen, D. E. (2004). The effect of gender and the neurotrophin, BDNF, upon methamphetamine-induced neurotoxicity of the nigrostriatal dopaminergic system in mice. *Neuroscience letters*, *359*(3), 135–138. http://www.ncbi.nlm.nih.gov/pubmed/15050682.

Eisch, A. J., & Marshall, J. F. (1998). Methamphetamine neurotoxicity: Dissociation of striatal dopamine terminal damage from parietal cortical cell body injury. *Synapse (New York, N.Y.)*, *30*(4), 433–445. http://www.ncbi.nlm.nih.gov/pubmed/9826235.

Eyerman, D. J., & Yamamoto, B. K. (2005). Lobeline attenuates methamphetamine-induced changes in vesicular monoamine transporter 2 immunoreactivity and monoamine depletions in the striatum. *The Journal of Pharmacology and Experimental Therapeutics*, *312*(1), 160–169. http://www.ncbi.nlm.nih.gov/pubmed/15331654.

Fleckenstein, A. E., Metzger, R. R., Beyeler, M. L., Gibb, J. W., & Hanson, G. R. (1997). Oxygen radicals diminish dopamine transporter function in rat striatum. *European Journal of Pharmacology*, *334*(1), 111–114. http://www.ncbi.nlm.nih.gov/pubmed/9346337.

Fumagalli, F., Gainetdinov, R. R., Valenzano, K. J., & Caron, M. G. (1998). Role of dopamine transporter in methamphetamine-induced neurotoxicity: Evidence from mice lacking the transporter. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *18*(13), 4861–4869. http://www.ncbi.nlm.nih.gov/pubmed/9634552.

Granado, N., O'Shea, E., Bove, J., Vila, M., Colado, M. I., & Moratalla, R. (2008a). Persistent MDMA-induced dopaminergic neurotoxicity in the striatum and substantia nigra of mice. *Journal of Neurochemistry*, *107*(4), 1102–1112. http://www.ncbi.nlm.nih.gov/pubmed/18823368.

Granado, N., Ortiz, O., Suárez, L. M., Martín, E. D., Ceña, V., Solís, J. M., & Moratalla, R. (2008b). D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-Induced arc and zif268 expression in the hippocampus. *Cerebral Cortex (New York, N.Y.: 1991)*, *18*(1), 1–12. http://www.ncbi.nlm.nih.gov/pubmed/17395606.

Granado, N., Ares-Santos, S., O'Shea, E., Vicario-Abejón, C., Colado, M. I., & Moratalla, R. (2010). Selective vulnerability in striosomes and in the nigrostriatal dopaminergic pathway after methamphetamine administration: Early loss of TH in striosomes after methamphetamine. *Neurotoxicity Research*, *18*(1), 48–58. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2875475&tool=pmcentrez&rendertype=abstract.

Granado, N., Ares-Santos, S., Oliva, I., O'Shea, E., Martin, E. D., Colado, M. I., & Moratalla, R. (2011a). Dopamine D2-receptor knockout mice are protected against dopaminergic neurotoxicity induced by methamphetamine or MDMA. *Neurobiology of Disease*, *42*(3), 391–403. http://www.ncbi.nlm.nih.gov/pubmed/21303698.

Granado, N., Lastres-Becker, I., Ares-Santos, S., Oliva, I., Martin, E., Cuadrado, A., & Moratalla, R. (2011b). Nrf2 deficiency potentiates methamphetamine-induced dopaminergic axonal damage and gliosis in the striatum. *Glia*, *59*(12), 1850–1863. http://www.ncbi.nlm.nih.gov/pubmed/21882243.

Hirata, H., Ladenheim, B., Carlson, E., Epstein, C., & Lud, J. (1996). Dopaminergic loss in mouse brain: Attenuation in CuZn-superoxide dismutase transgenic mice. *Brain Res, 714*, 95–103.

Hirata, H., Asanuma, M., & Cadet, J. L. (1998). Superoxide radicals are mediators of the effects of methamphetamine on Zif268 (Egr-1, NGFI-A) in the brain: Evidence from using CuZn superoxide dismutase transgenic mice. *Brain Research. Molecular Brain Research*, *58*(1–2), 209–216. http://www.ncbi.nlm.nih.gov/pubmed/9685645

Hurtig, H. I., Trojanowski, J. Q., Galvin, J., Ewbank, D., Schmidt, M. L., Lee, V. M., Clark, C. M., Glosser, G., Stern, M. B., Gollomp, S. M., & Arnold, S. E. (2000). Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease. *Neurology*, *54*(10), 1916–1921. http://www.ncbi.nlm.nih.gov/pubmed/10822429.

Itzhak, Y., Gandia, C., Huang, P. L., & Ali, S. F. (1998). Resistance of neuronal nitric oxide synthase-deficient mice to methamphetamine-induced dopaminergic neurotoxicity. *The Journal of Pharmacology and Experimental Therapeutics*, *284*(3), 1040–1047. http://www.ncbi.nlm.nih.gov/pubmed/9495865

Itzhak, Y., Martin, J. L., & Ali, S. F. (2002). Methamphetamine-induced dopaminergic neurotoxicity in mice: Long-lasting sensitization to the locomotor stimulation and desensitization to the rewarding effects of methamphetamine. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *26*(6), 1177–1183. http://www.ncbi.nlm.nih.gov/pubmed/12452543.

Jayanthi, S., Ladenheim, B., & Cadet, J. L. (1998). Methamphetamine-induced changes in antioxidant enzymes and lipid peroxidation in copper/zinc-superoxide dismutase transgenic mice. *Annals of the New York Academy of Sciences, 844*, 92–102. http://www.ncbi.nlm.nih.gov/pubmed/9668667.

Jayanthi, S., McCoy, M. T., Beauvais, G., Ladenheim, B., Gilmore, K., Wood, W., Becker, K., & Cadet, J. L. (2009). Methamphetamine induces dopamine D1 receptor-dependent endoplasmicreticulum stress-related molecular events in the rat striatum. *PloS One, 4*(6), e6092. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2699544&tool=pmcentrez&rendertype=abstract.

Jayanthi, S., Xiaolin Deng, Ladenheim, B., McCoy, M. T., Cluster, A., Ning-Sheng Cai, & Cadet, J. L. (2005). Calcineurin/NFAT-induced up-regulation of the Fas ligand/Fas death pathway is involved in methamphetamine-induced neuronal apoptosis. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(3), 868–873. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=545515&tool=pmcentrez&rendertype=abstract.

Jeng, W., Ramkissoon, A., Parman, T., & Wells, P. G. (2006). Prostaglandin H synthase-catalyzed bioactivation of amphetamines to free radical intermediates that cause CNS regional DNA oxidation and nerve terminal degeneration. *FASEB Journal: official publication of the Federation of American Societies for Experimental Biology, 20*(6), 638–650. http://www.ncbi.nlm.nih.gov/pubmed/16581972.

Johnson, J. A., Johnson, D. A., Kraft, A. D., Calkins, M. J., Jakel, R. J., Vargas, M. R., & Pei-Chun Chen. (2008). The Nrf2-ARE pathway: An indicator and modulator of oxidative stress in neurodegeneration. *Annals of the New York Academy of Sciences, 1147*, 61–69. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2605641&tool=pmcentrez&rendertype=abstract.

Krasnova, I.N., & Cadet, J. L. (2009). Methamphetamine toxicity and messengers of death. *Brain Research Reviews, 60*(2), 379–407. http://dx.doi.org/10.1016/j.brainresrev.2009.03.002.

Larsen, K. E., Fon, E. A., Hastings, T. G., Edwards, R. H., & Sulzer, D. (2002). Methamphetamine-induced degeneration of dopaminergic neurons involves autophagy and upregulation of dopamine synthesis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 22*(20), 8951–8960. http://www.ncbi.nlm.nih.gov/pubmed/12388602

LaVoie, M. J., & Hastings, T. G. (1999). Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: Evidence against a role for extracellular dopamine. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 19*(4), 1484–1491. http://www.ncbi.nlm.nih.gov/pubmed/9952424.

Li, Yan-hong, Hui-jun Wang, & Dong-fang Qiao. (2008). Effect of methamphetamine on the microglial cells and activity of nitric oxide synthases in rat striatum. *Nan fang yi ke da xue xue bao = Journal of Southern Medical University, 28*(10), 1789–1791. http://www.ncbi.nlm.nih.gov/pubmed/18971173.

Lo, Shih-Ching, Xuchu Li, Henzl, M. T., Beamer, L. J., & Hannink, M. (2006). Structure of the Keap1:Nrf2 interface provides mechanistic insight into Nrf2 signaling. *The EMBO Journal, 25*(15), 3605–3617. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1538563&tool=pmcentrez&rendertype=abstract.

Matsuzaki, H., Namikawa, K., Kiyama, H., Mori, N., & Sato, K. (2004). Brain-derived neurotrophic factor rescues neuronal death induced by methamphetamine. *Biological Psychiatry, 55*(1), 52–60. http://www.ncbi.nlm.nih.gov/pubmed/14706425.

McCann, U. D., Wong, D. F., Yokoi, F., Villemagne, V., Dannals, R. F., & Ricaurte, G. A. (1998). Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: Evidence from positron emission tomography studies with [11C]

WIN-35, 428. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 18*(20), 8417–8422. http://www.ncbi.nlm.nih.gov/pubmed/9763484

Medina, L., Figueredo-Cardenas, G., & Reiner, A. (1996). Differential abundance of superoxide dismutase in interneurons versus projection neurons and in matrix versus striosome neurons in monkey striatum. *Brain Research, 708*(1–2), 59–70. http://www.ncbi.nlm.nih.gov/pubmed/8720860

Melega, W. P., Lacan, G., Desalles, A. A., & Phelps, M. E. (2000). Long-term methamphetamine-induced decreases of [(11)C]WIN 35,428 binding in striatum are reduced by GDNF: PET studies in the vervet monkey. *Synapse (New York, N.Y.), 35*(4), 243–249. http://www.ncbi.nlm.nih.gov/pubmed/10657033.

Miller, D. B., & O'Callaghan, J. P. (2003). Elevated environmental temperature and methamphetamine neurotoxicity. *Environmental Research, 92*(1), 48–53. http://www.ncbi.nlm.nih.gov/pubmed/12706754.

Nash, J. F., & Yamamoto, B. K. (1992). Methamphetamine neurotoxicity and striatal glutamate release: Comparison to 3,4-methylenedioxymethamphetamine. *Brain Research, 581*(2), 237–243. http://www.ncbi.nlm.nih.gov/pubmed/1356579.

O'Callaghan, J. P., & Miller, D. B. (1994). Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse. *The Journal of Pharmacology and Experimental Therapeutics, 270*(2), 741–751. http://www.ncbi.nlm.nih.gov/pubmed/8071867.

Ortiz, O., Delgado-García, J. M., Espadas, I., Bahí, A., Trullas, R., Dreyer, J. L., Gruart, A., & Moratalla, R. (2010). Associative learning and CA3-CA1 synaptic plasticity are impaired in D1R null, Drd1a−/− mice and in hippocampal siRNA silenced Drd1a mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 30*(37), 12288–12300. http://www.ncbi.nlm.nih.gov/pubmed/20844125.

Rangasamy, T., Chung Y. Cho, Thimmulappa, R. K., Lijie Zhen, Sorachai S. Srisuma, Kensler, T. W., Masayuki Yamamoto, Petrache, I., Tuder, R. M., & Biswal, S. (2004). Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *The Journal of Clinical Investigation, 114*(9), 1248–1259. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=524225&tool=pmcentrez&rendertype=abstract.

Sattler, R., & Tymianski, M. (2000). Molecular mechanisms of calcium-dependent excitotoxicity. *Journal of Molecular Medicine (Berlin, Germany), 78*(1), 3–13. http://www.ncbi.nlm.nih.gov/pubmed/10759025

Sonsalla, P. K., Gibb, J. W., & Hanson, G. R. (1986). Roles of D1 and D2 dopamine receptor subtypes in mediating the methamphetamine-induced changes in monoamine systems. *The Journal of Pharmacology and Experimental Therapeutics, 238*(3), 932–937. http://www.ncbi.nlm.nih.gov/pubmed/2943891.

Sonsalla, P. K., Nicklas, W. J., & Heikkila, R. E. (1989). Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity. *Science (New York, N.Y.), 243*(4889), 398–400. http://www.ncbi.nlm.nih.gov/pubmed/2563176.

Sriram, K., Miller, D. B., & O'Callaghan, J. P. (2006). Minocycline attenuates microglial activation but fails to mitigate striatal dopaminergic neurotoxicity: Role of tumor necrosis factor-alpha. *Journal of Neurochemistry, 96*(3), 706–718. http://www.ncbi.nlm.nih.gov/pubmed/16405514.

Sulzer, D., Sonders, M. S., Poulsen, N. W., & Galli, A. (2005). Mechanisms of neurotransmitter release by amphetamines: A review. *Progress in Neurobiology, 75*(6), 406–33. http://www.ncbi.nlm.nih.gov/pubmed/15955613.

Thomas, D. M., Francescutti-Verbeem, D. M., & Kuhn, D. M. (2008). The newly synthesized pool of dopamine determines the severity of methamphetamine-induced neurotoxicity. *Journal of Neurochemistry, 105*(3), 605–616. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2668123&tool=pmcentrez&rendertype=abstract.

UNODC. (2012). *World drug report 2012*. United Nations publication, Sales No. E.12.XI.1.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Franceschi, D., Sedler, M., Gatley, S. J., Miller, E., Hitzemann, R., Ding, Y. S., & Logan, J. (2001a). Loss of dopamine transporters in

methamphetamine abusers recovers with protracted abstinence. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 21*(23), 9414–9418. http://www.ncbi.nlm.nih.gov/pubmed/11717374

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Leonido-Yee, M., Franceschi, D., Sedler, M. J., Gatley, S. J., Hitzemann, R., Ding, Y. S., Logan, J., Wong, C., & Miller, E. N. (2001b). Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *The American Journal of Psychiatry, 158*(3), 377–382. http://www.ncbi.nlm.nih.gov/pubmed/11229977.

Wagner, G. C., Carelli, R. M., & Jarvis, M. F. (1986). Ascorbic acid reduces the dopamine depletion induced by methamphetamine and the 1-methyl-4-phenyl pyridinium ion. *Neuropharmacology, 25*(5), 559–561. http://www.ncbi.nlm.nih.gov/pubmed/3488515.

White, N. M., & Hiroi, N. (1998). Preferential localization of self-stimulation sites in striosomes/patches in the rat striatum. *Proceedings of the National Academy of Sciences of the United States of America, 95*(11), 6486–6491. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=27819&tool=pmcentrez&rendertype=abstract

Xie, T., McCann, U. D., Kim, S., Yuan, J., & Ricaurte, G. A. (2000). Effect of temperature on dopamine transporter function and intracellular accumulation of methamphetamine: Implications for methamphetamine-induced dopaminergic neurotoxicity. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 20*(20), 7838–7845. http://www.ncbi.nlm.nih.gov/pubmed/11027249

Yamamoto, B. K., & Zhu, W. (1998). The effects of methamphetamine on the production of free radicals and oxidative stress. *The Journal of Pharmacology and Experimental Therapeutics, 287*(1), 107–114. http://www.ncbi.nlm.nih.gov/pubmed/9765328.

Pathophysiology of Obsessive-Compulsive Disorder: Insights from Normal Function and Neurotoxic Effects of Drugs, Infection, and Brain Injury

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Contents

Abstract

The clinical presentation of obsessive–compulsive disorder (OCD) is summarized and a theoretical model of the disorder and its neurobiology are described. The model is used as a framework to account for the observed pattern of clinical symptoms, for the pathophysiology of OCD, and for medical reports of OCD-like symptoms from neurotoxic effects of drugs, brain injury, and infection. The essential clinical feature of OCD is preoccupation with ideas and behaviors called "obsessions" and "compulsions," respectively. The characteristic content of obsessions and compulsions is concern about dangers that might happen in the

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future – as opposed to actual threats that are direct and immediate. Hence, OCD is a clinical preoccupation with *potential* threats. A model is considered where normal concerns regarding potential danger and security are handled by a biologically ancient hard-wired motivational system, called security motivation system (SMS), which has as its output the same repertoire of precautionary behaviors that characterize OCD compulsions. In the model, OCD symptoms emerge because security motivation is normally deactivated by a negative feedback signal generated by performance of precautionary behaviors, but in patients with OCD, this signal is inoperative, resulting in continued motivation to perform security-related behaviors. The neurobiology of SMS consists of functional loops involving a cascade of cortico-striato-pallido-thalamo-cortical connections, with inhibitory connections from the brainstem. Accordingly, OCD pathophysiology is overly persistent and uncontrolled neural activity in SMS, possibly due to a dopamine–serotonin imbalance. Evidence of OCD symptoms from drugs, brain injury, and infection is consistent with disturbed basal ganglia regulation as the pathophysiology of OCD.

1 Introduction

A comprehensive understanding of the pathophysiology of obsessive–compulsive disorder (OCD) is lacking because the cause of this disease is unknown. In general, if the cause of a disease is known then the course of pathogenesis can be traced forward from a well-defined starting point. In this way, even when the pathophysiology and presentation of symptoms are varied, it is nevertheless certain that they all belong to the same disease (stem from the same cause). However, if the disease cause is unknown, and one observes variations in pathophysiology and symptoms, it is not certain whether those variations belong to one disease with several presentations or in fact reflect several different diseases with different causes.

Criteria for disease causality were laid down by Robert Koch in 1882 when he proved the causation of tuberculosis, a devastating disease then believed to result from societal woes (Barnes 2000). Koch established that a bacterium (which he called "tubercle bacillus") is the causal agent of tuberculosis and he proved this through experiments demonstrating:

> (1) The parasite occurs in every case of the disease. (2) The parasite does not occur in other diseases or nonpathogenically. (3) After being fully isolated and repeatedly grown in pure culture the parasite can induce the disease by being introduced into a healthy animal. (Barnes 2000, p. 433)

As noted, when it comes to OCD, its cause – its agent of origin – is unknown. In fact, whether there are multiple sufficient causes that result in OCD is also unknown. However, information exists regarding the nature of the behavioral systems affected by OCD, and this information can be integrated into a theoretical model that provides a useful framework for considering the pathophysiology of OCD and how the function of these systems can be affected by neurotoxicity.

Below, the clinical presentation of OCD is summarized and a theoretical model is described that provides a framework to explain the symptoms of OCD and their neurobiological underpinnings. This framework is followed by a review of case reports to illustrate the neurotoxic effects of drugs, brain injury, and infection as causal agents producing OCD-like behavior.

2 Description of OCD

Given that the cause and pathophysiology of OCD are unknown, there are no biological tests for OCD, and, hence, a diagnosis of OCD is made solely from evidence of a particular behavioral profile (American Psychiatric Association. Task Force on DSM-IV 2000). The essential features of this behavioral profile are preoccupation by the individual with certain types of ideas and behaviors. The preoccupation is clinically significant because it is (a) *excessive* (it consumes more than 1 h in a day and interferes with normal daily function), (b) *ego-dystonic* (it gives no pleasure to the individual but the subject feels driven to be so engaged), and (c) *unreasonable* (the person acknowledges the preoccupation may appear to others as disconnected from objective reality). The ideas and behaviors that preoccupy the individual are called "obsessions" and "compulsions," respectively. According to Reed (Reed 1985), the content of obsessions may include thoughts involving (a) self-depreciation, self-denigration, and unworthiness either with reference to perceived social norms or the individual's own standards; (b) undue concern with one's bodily functions; (c) fears, doubts, and preoccupations about dirt and the spread of disease to self; and (d) fears of harm to a friend or relative and being responsible for the imagined event. The range of compulsive behaviors includes (Reed 1985) (a) excessive *checking* activities, characterized by repeated re-doings of actions supposedly related to security, orderliness, or accuracy; (b) *avoidance behaviors*, which are "activities engaged in to avoid feared objects, places, or situations" (p. 37); and (c) *washing and cleaning*, generally of hands but sometimes also compulsive washing of clothes, teeth cleaning, or the cleaning of possessions or parts of the home. Individuals may be preoccupied with more than one obsession and/or compulsion. The two most common characteristics of obsessions are doubts and indecision, and the two compulsive behaviors performed most often are checking and washing (Rasmussen and Eisen 1992).

It is remarkable that despite being aware that their preoccupation is exaggerated, afflicted individuals feel unable for any extended period of time to control their obsessions and compulsions; indeed, powerlessness over time spent engaged in obsessions and compulsions is very often the immediate reason for seeking help. But OCD does not reflect weaknesses of the will (Huoranszki 2011) or intellect. Most revealingly, there are individuals with OCD who despite the distresses of their illness manage very prolific and intellectually exceptional careers, one famous example being Samuel Johnson, the eighteenth century English writer and literary critic who counts among his major works the sole authorship of "A Dictionary of the English Language." There are many such

examples of highly successful individuals with OCD where clearly neither weak will nor poor intellect are the reasons for OCD symptoms (the interested reader may view video testimonials and descriptions of OCD on YouTube, e.g., http://www.youtube.com/watch?v=Rn1OYlYzgm8 or http://www.youtube.com/watch?v=dSZNnz9SM4g). Rather, the striking disconnection in OCD between knowing rationally that some actions are unreasonable, but nevertheless feeling that they must be performed, demonstrates the presence of a powerful "feeling" system as a determinant of behavior (Kahneman 2011), a notion which is elaborated upon in the next section.

It is should be noted that the DSM-IV manual indicates criteria for the differential diagnosis of OCD. Specifically, it states that OCD "must be distinguished from Anxiety Disorder Due to a General Condition" or from a "Substance-Induced Anxiety Disorder." Those diagnoses apply, respectively, "when the obsessions or compulsions are judged to be a direct physiological consequence of a specific general medical condition" or "when a substance (i.e., a drug of abuse, a medication, or exposure to a toxin) is judged to be etiologically related to the obsessions or compulsions" (American Psychiatric Association. Task Force on DSM-IV 2000). At first glance, the differential diagnosis suggests the presence of another disease, with a pathophysiology distinctly different from the pathophysiology of OCD diagnosed solely from behavioral evidence. However, the utility of the differential diagnosis probably applies to the clinical management of the symptoms rather than indicating that the differently labeled disorders have their own distinct pathophysiology. Indeed, as argued later, the medical conditions producing compulsions and obsessions reveal a plausible pathophysiology of OCD.

3 Security Motivation System as the Framework to Explain OCD

Disease in an organism was seen for a long time as due to invasion by unseen spirits, and it is relatively recently that the unseen forces have been given a physical reality, as demonstrated by Robert Koch above. The history of psychiatric disorders is no different, and even today mental illness is often viewed as a creation of outside forces, such as possession by various spirits or demons, environmental evils, abnormal learning, or mutation of genes. Nevertheless, it is crucial to appreciate that whatever the actual agent may turn out to be, and regardless of how bizarre the psychiatric symptoms may seem, the psychiatric condition is not a de novo creation but a phenomenon already in the repertoire of the organism. After all, the agent must act on the body and consequently the observed behavioral output, regardless of how bizarre, is a reflection of the functions the brain is built to perform. Accordingly, psychiatric disorders, including OCD, reflect the exaggerated operation of normal brain system(s) present in the population. This perspective raises the following two defining and related questions regarding OCD:

(i) Is there a common theme to the symptoms of OCD, or are the symptoms merely a collection of unrelated acts?

(ii) What are the normal constitutive processes that can lead an individual to feel driven to engage in irrational acts (obsessions and compulsion) but without showing a disconnection from objective reality?

With regard to a possible relationship among OCD symptoms, a perusal of the various obsessions and compulsions (Reed 1985) suggests that they can be conceptualized as belonging to a specific domain. In particular, the content of most OCD thoughts and behaviors seems to revolve around concerns related to security or safety, either of the self or of others. Hence, following an expression used by Paul MacLean (MacLean 1973), the core theme of OCD obsessions and compulsions may be described as concerns regarding "self-preservation or preservation of the species" (Szechtman and Woody 2004). Importantly, because the concerns in obsessions and compulsions are about dangers that *might* happen in the future – as opposed to actual threats that are direct and immediate – it is ***potential* threats** that are proposed to constitute the domain of OCD concerns (Szechtman and Woody 2004; Woody and Szechtman 2011).

The domain of potential threats belongs to the normal concerns of everyone and is not unique to OCD (Muris et al. 1997; Rachman and De Silva 1978; Rassin and Muris 2007; Reed 1985; Salkovskis and Harrison 1984). Within this normal domain several classes of potential threats are present, but of most salience to OCD are the security concerns engendered by predation (attack by others) and contamination–contagion. Interestingly, these security concerns are among the key normal fears of children, with a clear developmental time-course (Boyer and Bergstrom 2011). The two classes of potential dangers normally evoke appropriate and specific precautionary responses, namely, checking and washing, respectively. As noted, the exaggerated performance of checking and washing are the two most common OCD compulsions (Rasmussen and Eisen 1992). Conceivably, OCD subtypes of checkers and washers map onto the predation and the contamination–contagion classes of security concerns, respectively.

There are crucial distinctions between threats that are potential and those that are direct and immediate, with profound implications for the design of systems that process them (Boyer and Lienard 2006; Szechtman and Woody 2004; Woody and Boyer 2011; Woody and Szechtman 2011). One important distinction is that the two types of threat necessitate different types of responses. Already-present threats require fast responses to confront the danger, the faster the better – whereas potential ones require behavioral adjustments that can take more time to develop and perform. Furthermore, behaviors evoked by immediate threats are directed and constrained by moment-to-moment interaction with the source of danger. In contrast, potential threats are countered by indirect precautionary measures, such as avoidance or probing, and are free of constraint by an actual confrontation.

Another important distinction between potential and immediate threats relates to feedback from external environment regarding elimination of the threat. With actual threats, cues of danger confront the senses and thus there is no ambiguity about the presence of the threat or its successful elimination. In contrast, for potential threats there is an information asymmetry regarding presence versus elimination of threat. Potential dangers such as contagion or predator presence

are detected on the basis of particular partial cues, such as rotting smells or predator spoor. However, once the organism has engaged in precautionary behaviors, there is no positive evidence that these did actually eliminate or sufficiently reduce the danger. For example, the absence of a predator smell is no positive evidence for the predator's absence. Thus, taking precautions does not create clear signals that the danger is removed. Hence, with potential threats, the external environment provides no actual feedback either that the danger is eliminated or that the precautionary behavior was appropriate and effective.

This peculiar property inherent to potential threat – no feedback from external information that performance of precautionary behaviors achieved security – creates an obvious design challenge for a system handling potential threats: (1) what ensures that precautionary behaviors are initiated and performed and (2) what decides that a satisfactory level of security has been achieved? As explained below, it is hypothesized that this challenge is met through a biologically "hard-wired" special motivation – the security motivation system (SMS) (Szechtman and Woody 2004, 2006; Woody and Szechtman 2005, 2011).

The challenge of ensuring that precautionary behaviors are initiated and performed is answered by employing a dedicated motivational system. A defining design property of motivation is the urge to engage in species-typical preprogrammed responses when the motivation is activated. Accordingly, a mechanism to process potential threats through a dedicated security motivation system has a built-in urge to perform security-related precautionary behaviors in response to potential threats.

The next challenge – how to satisfy security without external feedback – is answered by utilizing signals generated internally. Most motivations are deactivated through contact with the appropriate goal object; for example, hunger motivation is deactivated by eating food, which generates a negative feedback satiety signal that terminates the motivation to attain food (Toates 1986). Because no external goal objects exist to satisfy an activated security motivation, then it must be that motivation gets deactivated through the very act of performance of security-related precautionary behaviors. In other words, self-generated signals from engagement in precautionary behaviors serve as the negative feedback satiety signal that terminates security motivation. Such an arrangement is functionally plausible because species-typical motor activity per se is rewarding (Glickman and Schiff 1967). Clearly, the built-in reward for security-related engagement contributes also to maintenance of precautionary responses to potential threats.

The model of SMS shown in Fig. 1 makes comprehensible how OCD can represent the exaggerated activity of a normal system and how normal constitutive processes can indeed compel performance of seemingly irrational acts (obsessions and compulsion) despite awareness of a seemingly inconsistent objective reality. The design in Fig. 1 of SMS follows the generic blueprint for a special motivation and consists of four modules shown in the middle row: a specialized module for detection of potential threats (*green* circle labeled "appraisal of potential danger") that turns on a "security motivation" module, which generates a coordinated set of processes to energize and focus brain resources on attaining a specified goal.

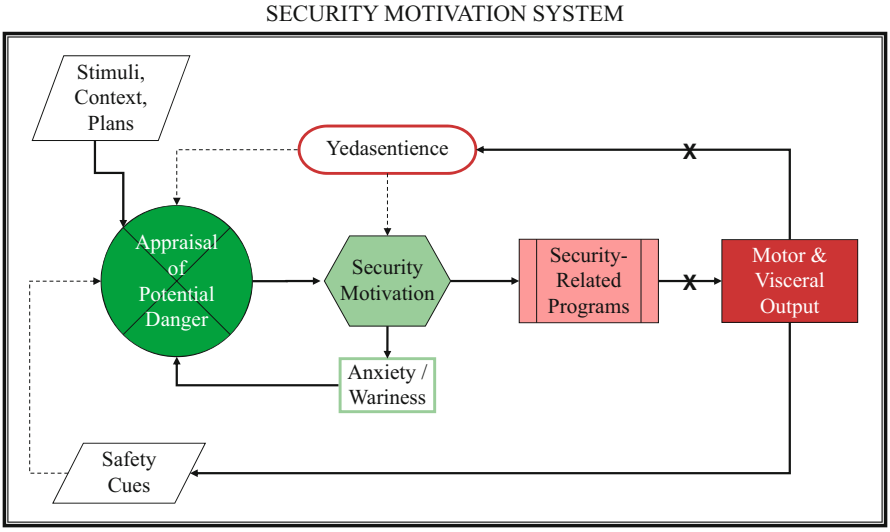

Fig. 1 A conceptual model of the security motivation system and sites of dysfunction producing OCD. Solid arrows indicate excitatory and dashed arrows inhibitory stimulation, respectively. Yedasentience output does not act on environmental input but rather on the appraisal of potential danger and the security motivation processors to inhibit their activity. Exposure through motor output to "safety" stimuli provides inhibitory stimulation to appraisal of potential danger [Modified from (Szechtman and Woody 2004)]

The signal from "security motivation" selects from the "security-related programs" module the plan of programs appropriate for the particular class of potential threat (e.g., washing and cleaning behaviors for the contamination–contagion class, probing and checking for the predation class). The "security-related programs" module in turn activates the "motor and visceral output" module, which executes and implements the actual performance of the species-typical motor actions. Execution of precautionary responses generates also internal feedback signals, including the signal labeled "yedasentience" shown in red in the top row [*yedasentience* = "feeling of knowing" (Szechtman et al. 2002; Szechtman and Woody 2004; Woody and Szechtman 2000)]. This yedasentience signal inhibits the "security motivation" and "appraisal of potential danger" modules, thereby stopping SMS activity.

Termination of security motivation activity by the self-generated yedasentience negative feedback signal provides an explanatory mechanism for OCD compulsions and obsessions. Specifically, failure to generate yedasentience (or respond to this signal) leaves SMS activity on for a prolonged period of time, driving persistent performance of precautionary behaviors – this continued performance constitutes OCD obsessions and compulsions. Two potential sites where damage may lead to a failure to generate yedasentience and yield obsessions and compulsions are shown by X in Fig. 1.

Lack of yedasentience feedback also explains how seemingly unreasonable obsessions and compulsions can exist alongside a normal sense of reality. Two particular characteristics of SMS contribute to the explanation. The first relevant property is the hierarchical dominance of an activated SMS. Specifically, when SMS is activated, it functions at the top of the hierarchy among competing systems controlling brain resources and actions. This is a design feature of special motivations which are engaged to perform functions vital for survival of the organism (Hebb 1966). Hence, it follows that when security motivation is stimulated, the individual becomes preoccupied with security, at the expense of other cues in the environment, and in this respect such preoccupation is normal and does not reflect any loss of a sense of reality.

The second relevant property is peculiar to SMS: an inherent disconnection of SMS deactivation from objective reality. As noted above, the external environment provides no reliable feedback either that potential threat was eliminated or that precautionary behaviors were appropriate and effective in achieving security. Consequently, as shown in the model in Fig. 1, information from the external environment has no access to security motivation to shut it down – only the self-generated yedasentience feedback can turn off security motivation. Hence, the drive to engage in obsessions and compulsions can coexist with a logically conflicting sense of reality.

4 Neurobiology of SMS and Substrate of OCD Pathophysiology

Framing OCD as a disturbance of security motivation simplifies the task of identifying OCD pathophysiology because it reduces the pathophysiology of OCD to a dysfunction in the neurobiology of SMS, and a fairly good model of a functional SMS neurobiology exists (Szechtman and Woody 2004, 2006; Woody and Szechtman 2011). The model follows the generic plan of motivational systems derived from behavioral neuroscience studies on motivation. Accordingly, the proposed schematics of SMS neurocircuitry and physiology are shown in Figs. 2 and 3.

Like other neuroanatomical models of motivation [e.g., (Brown and Pluck 2000; Everitt and Wolf 2002)], the SMS diagram in Fig. 2 consists of functional loops involving cortico-striato-pallido-thalamo-cortical connections, as advanced by Alexander et al. (1986) and others (Groenewegen et al. 1999; Haber et al. 2000; Joel and Weiner 2000; Penney and Young 1983). Importance of the yedasentience signal is highlighted by inhibitory connections from the brainstem. There are four cascading neural loops in Fig. 2, each mediating one of the four functional components in Fig. 1 – the color codes of the loops correspond to the functional modules in Fig. 1. Accordingly, the circuits in Fig. 2 are labeled: appraisal of potential danger loop (*green*), security motivation and affect loop (*light green*), security-related programs loop (*light red*), and brainstem output network (*red*), where each loop contains specific brain structures that are interconnected to form a specialized neurocircuit mediating a distinct motivational task.

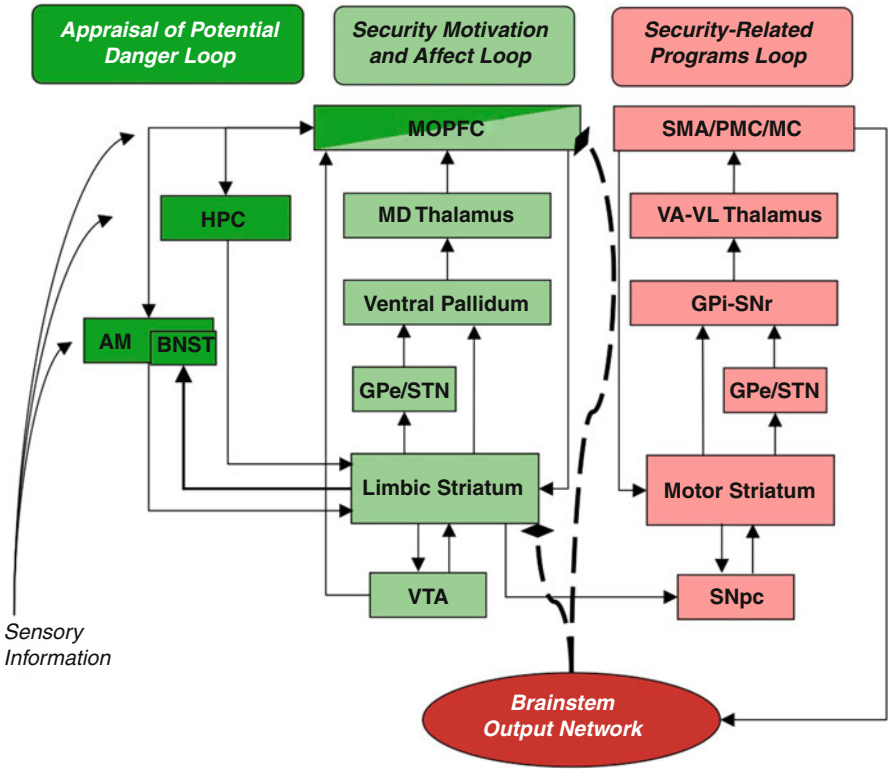

Fig. 2 A neural circuit model of the security motivation system. Each of the four distinct subcircuits (*loops*) subserves one of the functional components in Fig. 1 and is identified by corresponding colors. The dashed line indicates possible sites of yedasentience feedback inhibition. Abbreviations: *AM* amygdala, *BNST* bed nucleus of the stria terminalis, *GPe* external segment of the globus pallidus, *GPi* internal segment of the globus pallidus, *HPC* hippocampus, *MC* motor cortex, *MD* Thalamus, mediodorsal thalamic nucleus, *MOPFC* medial prefrontal cortex and orbital prefrontal cortex, *PMC* premotor cortex, SMA, supplementary motor area, *SNpc* substantia nigra pars compacta, *SNr* substantia nigra pars reticulata, *STN* subthalamic nucleus, *VA* ventroanterior thalamic nucleus, *VL* ventrolateral thalamic nucleus, *VTA* ventral tegmental area [Reprinted by permission from (Szechtman and Woody 2004)]

The first motivational task – detection of potential danger – is handled by the appraisal of potential danger loop composed of the interconnected green-coded brain regions in Fig. 2. The indicated interconnected limbic regions – the hippocampus, the amygdala, and the bed nucleus of the stria terminalis (BNST), as well as the medial and orbital prefrontal cortex (MOPFC) – process motivation-related input, including security-related information (Bechara et al. 2000; Fiddick 2011; LeDoux 2000; Woody and Szechtman 2011). Thus, this detection circuit has the computational functions to quickly process signalling cues (e.g., rotting smells, predator spoor, novelty) and more complex information ("context," "plans") and determine whether a change of present circumstance could result in a future harm.

If computational outcome is indeed potential danger, the appraisal of potential danger loop has neuroanatomical links needed for activation of the next motivational task, handled by the security motivation and affect loop (*light green*-colored boxes). Specifically, the hippocampus and the amygdala have projections to the ventral striatum, a key area in the security motivation and affect loop; moreover, the MOPFC (denoted by the box with *green* and *light green* colors) is a component of both the appraisal of potential danger loop and the security motivation and affect loop, providing an interface between the two circuits.

The next motivational task is the engagement of motivational drive and affect and is handled by the security motivation and affect loop (light green-coded brain regions in Fig. 2). The neuroanatomical sites of this loop are linked as a prototypical basal ganglia–thalamocortical circuit (Alexander et al. 1986) and form, specifically, the "limbic striatum loop" (Brown and Pluck 2000; Joel and Weiner 2000). Three attributes of this neurocircuit seem especially relevant for the designated job of the security motivation and affect loop. First, it contains dopamine innervation from the VTA onto the limbic striatum and the prefrontal cortex. Dopamine input can supply the tonic ignition of the loop and this dopamine stimulation is likely the implementation of a salient characteristic of motivations, namely, the urge to engage in goal-directed behaviors. Indeed, stimulation of the mesolimbic dopamine system yields approach and investigatory behaviors (Ikemoto and Panksepp 1999), and such motor activity is part of the repertoire of many motivations, including security motivation, where it manifests itself as probing the environment for potential danger. Second, the loop involves connections with limbic regions implicated with processing of affect accompanying motivation. In the case of security motivation, it has been suggested that the connection from the limbic striatum to BNST (Davis and Shi 1999; Davis et al. 1997; Lang et al. 2000; Woody and Szechtman 2011) is especially important in mediating the feelings of anxiety and wariness, the phenomenological cues of potential danger (Masterson and Crawford 1982; Woody and Szechtman 2011). Finally, the neural arrangement of the regions within the loop is such as to prolong and sustain reverberatory activity (Alexander et al. 1986; Penney and Young 1983) needed to maintain motivational drive and also activate the next motivational task.

The next motivational task is the selection and engagement of an appropriate security-related response. This job is executed by the security-related programs loop (light-red-coded brain regions in Fig. 2); this loop is also a prototypical basal ganglia neurocircuit but interconnected with the dorsal (or "motor" striatum) and innervated by dopamine from the substantia nigra. The circuit has design features especially useful for "selection" of appropriate responses (Mink 1996; Penney and Young 1983; Wolgin 2012). Every motivation has its own set of species-typical behavioral actions; typically, the neural instructions of how to implement the organization of a species-typical pattern of behavior are held in the striatum in the form of a precoded "program" (Aldridge et al. 2004; MacLean 1978; Penney and Young 1983; Wise and Rapoport 1989). Accordingly, depending on the domain of potential danger, the security-related programs loop engages the appropriate program (e.g., for probing or checking the environment in case of potential threat of

attack and for washing or grooming in case of contamination–contagion), and at the same time it inhibits and prevents competing behavioral programs from access to motor output. Dopamine activity from the nigrostriatal system probably helps to maintain the neural operation of the selected program and intensity of its execution, reflected in the vigor of motor performance (Dvorkin et al. 2010).

Finally, the program selected by the security-related programs loop implements those instructions by activating the brainstem output network (*red*-coded area in Fig. 2), and stimulating brainstem output neurons that convey neural signals for the actual contraction of muscles that move the body and viscera in performance of the species-typical actions. In addition to patterned movements, the brainstem output network generates the yedasentience negative feedback signal, which shuts down security motivation via inhibitory input possibly to the ventral striatum and MOPFC (heavy dashed line in Fig. 2). This negative feedback pathway is probably serotonergic, given that the serotonin neurotransmitter serves widely as a "stop" or "satiety" signal in many functions (e.g., Blundell 1991; Lorrain et al. 1999; Soubrie 1986) and serotonin reuptake inhibitors have therapeutic effects in OCD (DeVeaugh-Geiss 1994).

Every motivation which has a characteristic neural network possesses also a characteristic physiology that facilitates network operation at its optimum. The model in Fig. 3 shows that physiological mechanisms that support an activated security motivation operate through stimulation of the hypothalamic–pituitary–adrenocortical (HPA) axis and regulation of the parasympathetic nervous system (PNS). These systems contribute peripherally and centrally to enhance security motivation, which is turned on by potential threat and thus geared for action – for doing *something* that will relax the psychological tension of uncertain danger. To support those SMS prerequisites, HPA and PNS engage peripheral mechanisms to mobilize energy for physical work and also exert central effects on the *appraisal of potential danger* and *security motivation and affect* loops to potentiate threat detection and motivation [Fig. 3; for details, see (Woody and Szechtman 2011)].

5 Pathophysiology of OCD

Using the above framework of a fully functional SMS (Figs. 2 and 3), the pathophysiology of OCD is relatively clear cut – OCD pathophysiology is normal neural activity in SMS loops that becomes overly persistent and uncontrolled (Saxena et al. 1998; Wise and Rapoport 1989). While there are many possible neuroanatomical spots where a malfunction (regardless of cause) can produce this pathophysiology, two salient malfunctions likely to yield prolonged and uncontrolled SMS neural activity are excessive dopaminergic stimulation, insufficient serotonergic negative feedback, or possibly both. Indeed, there is evidence for each possibility in OCD (Denys et al. 2004; Westenberg et al. 2007), including dysfunction in both dopamine and serotonin (Nikolaus et al. 2010) attested by current clinical practice of combining dopamine blockers with serotonin reuptake inhibitors for treatment of OCD (Bloch et al. 2006; Dougherty et al. 2004).

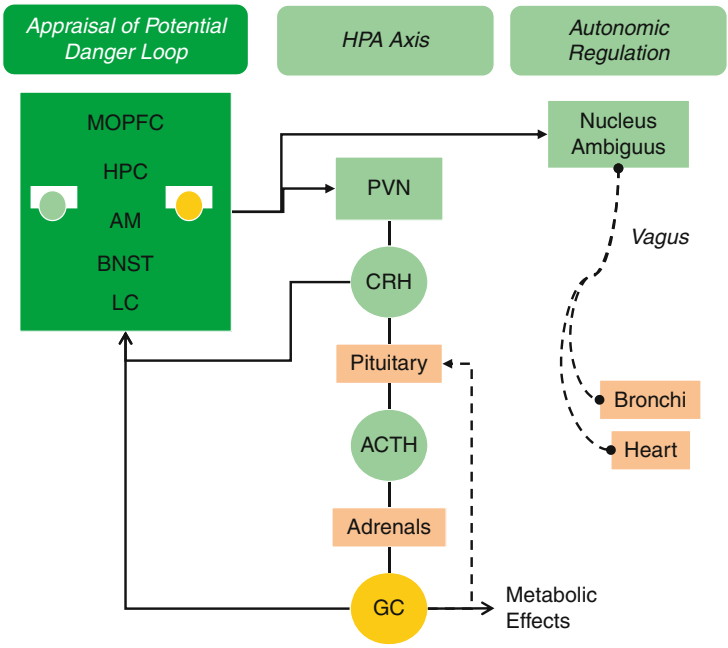

Fig. 3 The physiological network associated with security motivation. The appraisal of potential danger loop of Fig. 2 connects with the HPA Axis, which has both input from and output to the Appraisal Loop. Within the Appraisal Loop there are receptors for CRH and GC, denoted by the respective circles in white rectangles, by which HPA can modulate it; CHR also acts as a neurotransmitter within the Appraisal Loop. Finally, the Appraisal Loop affects Autonomic Regulation, by which the myelinated vagus modulates the bronchi and heart. Abbreviations: *AM* amygdala, *BNST*, bed nucleus of the stria terminalis, *CRH* corticotropin-releasing hormone, *GC* glucocorticoids, *HPC* hippocampus, *LC* locus coeruleus, *MOPFC* medial prefrontal cortex and orbital prefrontal cortex, *PVN* paraventricular nucleus [Reprinted by permission from (Woody and Szechtman 2011)]

One manner by which dopamine–serotonin imbalance can yield excessive SMS activity and thus OCD pathophysiology is as follows. OCD concerns are the normal security-related concerns of everyone and indeed their incidence does not differ between OCD and non-patient populations (Muris et al. 1997; Rachman and De Silva 1978; Rassin and Muris 2007; Reed 1985; Salkovskis and Harrison 1984). Consequently, individuals are often presented with some potential threat that activates the dopaminergic motivational drive of the security motivation circuit. However, in individuals with a constitutively hyperfunctional dopamine system, activation of security-related dopaminergic motivational drive produces excessive stimulation of dopamine receptors thereby driving the SMS circuit continually and overpowering any negative feedback to shutdown SMS activity. Similarly, in individuals with a constitutively ineffective serotonergic negative feedback,

activation of security motivation also yields OCD pathophysiology but here SMS activity stays prolonged not because of an intrinsic or pathologic dopamine hyperfunction. Instead, SMS activity is prolonged because serotonergic negative feedback is the normal mechanism to deactivate the dopaminergic motivational drive, and consequently, without a serotonergic "stop" signal, SMS activity continues unabated.

6 Evidence for Overactive SMS Circuit in OCD

The foundational line of evidence for overactive functional loops involving cortico-striato-pallido-thalamo-cortical connections came from early positron emission tomography (PET) studies measuring the brain distribution of glucose metabolism in patients with OCD. Such PET studies showed metabolic hyperactivity in orbitofrontal cortex and caudate nucleus in patients with OCD, a hyperactivity that resolved upon disappearance of OCD symptoms with therapy (Baxter 1992; Baxter et al. 1992; Benkelfat et al. 1990; Swedo et al. 1992). Subsequent brain imaging studies have also implicated hyperfunction of the orbitofrontal cortex, basal ganglia, and the limbic system in OCD (Adler et al. 2000; Friedlander and Desrocher 2006; Kim et al. 2001; Mataix-Cols et al. 2004; McGuire et al. 1994; Menzies et al. 2008; Phillips and Mataix-Cols 2004; Rauch et al. 1994; Rotge et al. 2008; Saxena et al. 1998; van den Heuvel et al. 2004).

Deep brain stimulation (DBS) has been found effective for some patients with symptoms of OCD (Alegret et al. 2001; Anderson and Ahmed 2003; Fontaine et al. 2004; Mallet et al. 2002; Nuttin et al. 1999, 2003; Piasecki and Jefferson 2004), with the three sites targeted being the subthalamic nucleus (Fontaine et al. 2004; Mallet et al. 2002), the anterior limbs of the internal capsules (Anderson and Ahmed 2003; Nuttin et al. 2003), and the nucleus accumbens (Denys et al. 2010; Huff et al. 2010; Sturm et al. 2003). Although it is not well understood how DBS yields therapeutic effects, it may involve disruption of reverberatory activity in basal ganglia loops (Aouizerate et al. 2004; Bourne et al. 2012; Haynes and Mallet 2010; Tass et al. 2003), consistent with OCD pathophysiology. A similar disruption of overactivity may be expected from strategically placed lesions, and indeed severe cases of OCD may be improved with psychosurgery, in particular with anterior capsulotomy or cingulotomy (Baer et al. 1995; Chiocca and Martuza 1990; Dougherty et al. 2002; Jenike et al. 1991; Kettl and Marks 1986).

7 Neurotoxic Effects Producing OCD Pathophysiology

The pathophysiology of OCD – persistence of activity in SMS functional loops – could result from many types of insults and probably at several neuroanatomical locations in the neurocircuit. This section consults the clinical literature for medical conditions with a presentation of OCD phenomenology and asks whether those

medical perturbations could have yielded unregulated activity in basal ganglia functional loops. Three types of perturbations are considered: by drugs, brain injury, and infection.

7.1 Drugs

Given the prominent role of dopamine systems in SMS circuit (Fig. 2), it is expected that dopamine-activating drugs should provoke OCD phenomenology, at least with prolonged use. The clinical literature does indeed support this expectation with case reports of OCD symptoms from chronic exposure to amphetamines (Marchesi et al. 2009; Semple et al. 2011; Woolley and Heyman 2003), cocaine (Crum and Anthony 1993), and other direct dopamine receptor agonists (Ahlskog 2011). In animals, too, dopamine agonists produce compulsive behaviors considered to model OCD (De Carolis et al. 2011; Szechtman et al. 1999, 1998).

At first blush, a contradictory literature seemingly exists as well, because drugs used in treatment of OCD, dopamine receptors blockers (Bloch et al. 2006; Dougherty et al. 2004), may themselves induce OCD symptoms. There are clinical reports that atypical antipsychotics, such as aripiprazole (Desarkar et al. 2007), clozapine, risperidone, and olanzapine (Lykouras 2003), can produce or exacerbate OCD symptoms, as evidenced by the appearance and disappearance of symptoms with the introduction and removal of the drug. Clearly, those drug effects may involve an action on other than dopamine receptors. However, a possible mechanism via dopamine receptors should not be ignored, given that dopamine receptor supersensitivity may develop with exposure to dopamine blockers (Kostrzewa 1995) and given that dopamine D2 receptors play a role not only in the initiation of behavior but also in arresting ongoing activity (Ghahremani et al. 2012; Hoffman and Rueda Morales 2012). Indeed, the intricate circuitry of basal ganglia loops contains many inhibitory and excitatory connection points, not just dopaminergic or serotonergic (Bourdy and Barrot 2012; Pittenger et al. 2011; Rotge et al. 2010), and a disruption at any such point can yield overly sustained reverberatory activity.

7.2 Brain Injury

Symptoms of OCD often appear associated with neurologic disorders. These include postencephalitis Parkinsonian syndrome (Schilder 1938), Parkinson's disease (Kurlan 2004), striatal (Ercan et al. 2008) or globus pallidus necrosis (Laplane et al. 1989), Huntington's chorea (Cummings and Cunningham 1992), and Gilles de la Tourette's syndrome (TS) (Pauls et al. 1986). All of these conditions involve a disturbance of the basal ganglia, suggesting that upon activation of security motivation those neurologic disturbances may also yield the characteristic pathophysiology of OCD, namely, prolonged overactivity in the SMS neurocircuit. A similar inference of SMS circuit perturbation may relate to cases of OCD following acquired brain injury (Coetzer 2004).

7.3 Infection

Some instances of infection may result in OCD symptoms that resolve upon disappearance of the infection. For instance, there are such reported cases for cerebral malaria (Idro et al. 2005), *Mycoplasma pneumoniae* infection (Ercan et al. 2008), *Borna disease virus* (BDV) infection (Rotge et al. 2010), as well as streptococcal infection, the latter infection having a designation on its own for the ensuing OCD – PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) (Swedo et al. 1998, 1989). Some autoimmune disorders, including Sydenham's chorea (Swedo et al. 1989) and systemic lupus erythematosus (Yu et al. 2008), may also present with OCD symptoms. More generally, various immune markers may be altered in patients with OCD (da Rocha et al. 2008). Such findings suggest three types of mechanisms whereby infection and immune activation could yield OCD pathophysiology.

One type of mechanism follows the disease model of tuberculosis, namely, that a specific pathogen causes OCD, just like *tubercle bacillus* is the causal agent of tuberculosis. In tuberculosis, the symptoms and the pathophysiology of the disease are the outcomes of the bacterium lodging preferentially in certain body organs (e.g., lungs) and its interaction with immune processes to contain it. This battle yields "collateral damage" in the afflicted organs and physiology, and such particular perturbations are manifested as disease-specific symptoms. Similarly, for OCD, Rotge and colleagues (Rotge et al. 2010) elaborated a plausible scenario for "the process by which infection becomes disease" (Barnes 2000, p. 439). Following up on reports (Bode et al. 1995) that BDV infection may play a role in psychiatric disorders, Rotge and colleagues (Rotge et al. 2010) noted that the virus' preferential neurotropism for brain regions of relevance to OCD would mount an immune response there, leading possibly to a region-specific malfunction as a by-product of the immune response. While there are many plausible mechanisms by which immune reactions can perturb neuronal function (for a recent review, see Kapadia and Sakic (2011)), the authors highlight a mechanism which involves glutamate neuroexcitotoxicity, in light of genetic findings of a glutamate transporter gene SLC1A1 association with OCD (Arnold et al. 2006; Wu et al. 2012) and recognition that glutamate is both a neurotransmitter and an immunomodulator. According to the authors, genetic alterations of the glutamatergic system in some individuals would facilitate BDV-induced immunopathological reactions in the OCD-relevant brain regions (Rotge et al. 2010), with consequent hyperactivity of basal ganglia loops (Wu et al. 2012) that constitute the SMS neurocircuit.

Another type of mechanism is closely related to the above in that it also invokes immune activation as the agent producing neural dysfunction. However, this mechanism is related to autoimmune processes rather than stemming from local reactions to neurotropic pathogens. An example of such an autoimmune-induced OCD-related neural dysfunction is PANDAS. The mechanism underlying the OCD of PANDAS is considered to involve an autoimmune response triggered by Group A beta-hemolytic streptococcal infection. In individuals

positive for the D8/17 antigen on the surface of peripheral blood mononuclear cells, the antistreptococcal antibodies are thought to cross-react with neuronal basal ganglia antigens (Eisen et al. 2001; Swedo et al. 1997). This autoantibodies basal ganglia cross-reactivity can produce various forms of altered cellular function, including perturbation of neuronal signal transduction (Kapadia and Sakic 2011). Accordingly, OCD will ensue when humoral immunity targets neuronal elements of the SMS neurocircuit, and the resultant malfunction consists of overly persistent and uncontrolled circuit activity characteristic of OCD pathophysiology.

The last mechanism that can produce OCD pathophysiology is different: while still related to immune function, it does not involve an immunopathogenic effect on neuronal function. Rather, the OCD pathophysiology is produced by a sensitization-related mechanism that renders the SMS neurocircuit hyperfunctional. This may occur in the course of normal physiological function and not necessarily due to some immunopathogenic process. An example of such a phenomenon is "compensatory behavioral prophylaxis" (Fessler 2001). At times of relatively high progesterone levels, women are more susceptible to illness from infection because the immune system is suppressed, and a behavioral compensation at such times is enhanced precaution about contamination. Through a process of sensitization (Woolf 2011), this behavioral compensation mechanism can lead to SMS hyperactivity, producing OCD symptoms.

8 Conclusion

Because the cause or causes of OCD are unknown, it is uncertain whether variations in pathophysiology and symptoms constitute different manifestations of one disease or different diseases with distinct causes. However, whatever the causal agent may turn out to be, OCD is not a de novo creation but instead must reflect an exaggeration of the operation of a normal brain system. As argued here, information about the nature of the behavioral system affected by OCD can be integrated into a theoretical model of this normal system, called the security motivation system.

Recognizing both the similarities and differences between this system and other hard-wired motivational systems, a neurobiological model of the SMS may be derived from behavioral neuroscience studies of motivation. It consists of functional loops involving a cascade of cortico-striato-pallido-thalamo-cortical connections, with inhibitory connections from the brainstem. The pathophysiology of OCD can then be understood as normal neural activity in this system that becomes overly persistent and uncontrolled, e.g., due to a dopamine–serotonin imbalance. Three types of medical perturbations have been reported to produce OCD symptoms: drugs, brain injuries, and infections. The available evidence on these effects is broadly consistent with disturbances of regulation in the activity of the hypothesized basal ganglia functional loops. Thus, the SMS model provides a unifying framework for understanding the likely mechanisms of these neurotoxic effects.

References

Adler, C. M., McDonough-Ryan, P., Sax, K. W., Holland, S. K., Arndt, S., & Strakowski, S. M. (2000). FMRI of neuronal activation with symptom provocation in unmedicated patients with obsessive compulsive disorder. *Journal of Psychiatric Research, 34*(4–5), 317–324.

Ahlskog, J. E. (2011). Pathological behaviors provoked by dopamine agonist therapy of Parkinson's disease. *Physiology & Behavior, 104*(1), 168–172.

Aldridge, J. W., Berridge, K. C., & Rosen, A. R. (2004). Basal ganglia neural mechanisms of natural movement sequences. *Canadian Journal of Physiology and Pharmacology, 82*(8–9), 732–739.

Alegret, M., Junque, C., Valldeoriola, F., Vendrell, P., Marti, M. J., & Tolosa, E. (2001). Obsessive-compulsive symptoms in Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry, 70*(3), 394–396.

Alexander, G. E., Delong, M. R., & Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience, 9*, 357–381.

American Psychiatric Association. Task Force on DSM-IV. (2000). *Diagnostic and statistical manual of mental disorders: DSM-IV-TR* (4th ed.). Washington, DC: American Psychiatric Association.

Anderson, D., & Ahmed, A. (2003). Treatment of patients with intractable obsessive-compulsive disorder with anterior capsular stimulation. Case report. *Journal of Neurosurgery, 98*(5), 1104–1108.

Aouizerate, B., Cuny, E., Martin-Guehl, C., Guehl, D., Amieva, H., Benazzouz, A., Fabrigoule, C., Allard, M., Rougier, A., Bioulac, B., Tignol, J., & Burbaud, P. (2004). Deep brain stimulation of the ventral caudate nucleus in the treatment of obsessive-compulsive disorder and major depression. Case report. *Journal of Neurosurgery, 101*(4), 682–686.

Arnold, P. D., Sicard, T., Burroughs, E., Richter, M. A., & Kennedy, J. L. (2006). Glutamate transporter gene SLC1A1 associated with obsessive-compulsive disorder. *Archives of General Psychiatry, 63*(7), 769–776.

Baer, L., Rauch, S. L., Ballantine, H. T., Martuza, R., Cosgrove, R., Cassem, E., Giriunas, I., Manzo, P. A., Dimino, C., & Jenike, M. A. (1995). Cingulotomy for intractable obsessive-compulsive disorder – Prospective long-term follow-up of 18 patients. *Archives of General Psychiatry, 52*, 384–392.

Barnes, D. S. (2000). Historical perspectives on the etiology of tuberculosis. *Microbes and Infection, 2*(4), 431–440.

Baxter, L. R., Jr. (1992). Neuroimaging studies of obsessive compulsive disorder. *The Psychiatric Clinics of North America, 15*(4), 871–884.

Baxter, L. R., Jr., Schwartz, J. M., Bergman, K. S., Szuba, M. P., Guze, B. H., Mazziotta, J. C., Alazraki, A., Selin, C. E., Ferng, H. K., & Munford, P. (1992). Caudate glucose metabolic rate changes with both drug and behavior therapy for obsessive-compulsive disorder. *Archives of General Psychiatry, 49*(9), 681–689.

Bechara, A., Damasio, H., & Damasio, A. R. (2000). Emotion, decision making and the orbitofrontal cortex. *Cerebral Cortex, 10*(3), 295–307.

Benkelfat, C., Nordahl, T. E., Semple, W. E., King, A. C., Murphy, D. L., & Cohen, R. M. (1990). Local cerebral glucose metabolic rates in obsessive-compulsive disorder. Patients treated with clomipramine. *Archives of General Psychiatry, 47*(9), 840–848.

Bloch, M. H., Landeros-Weisenberger, A., Kelmendi, B., Coric, V., Bracken, M. B., & Leckman, J. F. (2006). A systematic review: Antipsychotic augmentation with treatment refractory obsessive-compulsive disorder. *Molecular Psychiatry, 11*(7), 622–632.

Blundell, J. (1991). Pharmacological approaches to appetite suppression. *Trends in Pharmacological Sciences, 12*, 147–157.

Bode, L., Zimmermann, W., Ferszt, R., Steinbach, F., & Ludwig, H. (1995). Borna-disease virus genome transcribed and expressed in psychiatric-patients. *Nature Medicine, 1*(3), 232–236.

Bourdy, R., & Barrot, M. A. (2012). A new control center for dopaminergic systems: Pulling the VTA by the tail. *Trends in Neurosciences, 35*(11), 681–690.

Bourne, S. K., Eckhardt, C. A., Sheth, S. A., & Eskandar, E. N. (2012). Mechanisms of deep brain stimulation for obsessive compulsive disorder: Effects upon cells and circuits. *Frontiers in Integrative Neuroscience, 6*, 29.

Boyer, P., & Bergstrom, B. (2011). Threat-detection in child development: An evolutionary perspective. *Neuroscience and Biobehavioral Reviews, 35*(4), 1034–1041.

Boyer, P., & Lienard, P. (2006). Why ritualized behavior? Precaution systems and action parsing in developmental, pathological and cultural rituals. *The Behavioral and Brain Sciences, 29*(6), 595–613.

Brown, R. G., & Pluck, G. (2000). Negative symptoms: The "pathology" of motivation and goal-directed behaviour. *Trends in Neurosciences, 23*(9), 412–417.

Chiocca, E. A., & Martuza, R. L. (1990). Neurosurgical therapy of obsessive-compulsive disorder. In M. A. Jenike, L. Baer, & W. E. Minichiello (Eds.), *Obsessive-compulsive disorders: Theory and management* (Vol. 2nd, pp. 283–294). Chicago: Year Book Medical Publishers.

Coetzer, B. R. (2004). Obsessive-compulsive disorder following brain injury: A review. *International Journal of Psychiatry in Medicine, 34*(4), 363–377.

Crum, R. M., & Anthony, J. C. (1993). Cocaine use and other suspected risk factors for obsessive-compulsive disorder: A prospective study with data from the Epidemiologic Catchment Area surveys. *Drug and Alcohol Dependence, 31*(3), 281–295.

Cummings, J. L., & Cunningham, K. (1992). Obsessive-compulsive disorder in Huntington's disease. *Biological Psychiatry, 31*(3), 263–270.

da Rocha, F. F., Correa, H., & Teixeira, A. L. (2008). Obsessive–compulsive disorder and immunology: A review. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 32*(5), 1139–1146.

Davis, M., & Shi, C. (1999). The extended amygdala: are the central nucleus of the amygdala and the bed nucleus of the stria terminalis differentially involved in fear versus anxiety? *Annals of the New York Academy of Sciences, 877*, 281–291.

Davis, M., Walker, D. L., & Lee, Y. (1997). Amygdala and bed nucleus of the stria terminalis: Differential roles in fear and anxiety measured with the acoustic startle reflex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 352*(1362), 1675–1687.

De Carolis, L., Schepisi, C., Milella, M., Nencini, P. (2011). Clomipramine, but not haloperidol or aripiprazole, inhibits quinpirole-induced water contrafreeloading, a putative animal model of compulsive behavior. *Psychopharmacology, 218*(4), 749–759

Denys, D., Mantione, M., Figee, M., van den Munckhof, P., Koerselman, F., Westenberg, H., Bosch, A., & Schuurman, R. (2010). Deep brain stimulation of the nucleus acccumbens for treatment-refractory obsessive-compulsive disorder. *Archives of General Psychiatry, 67*(10), 1061–1068.

Denys, D., Zohar, J., & Westenberg, H. G. (2004). The role of dopamine in obsessive-compulsive disorder: Preclinical and clinical evidence. *The Journal of Clinical Psychiatry, 65*(Suppl 14), 11–17.

Desarkar, P., Das, A., & Nizamie, S. H. (2007). Aripiprazole-induced obsessive-compulsive disorder: A report of 2 cases. *Journal of Clinical Psychopharmacology, 27*(3), 305–306.

DeVeaugh-Geiss, J. (1994). Pharmacologic therapy of obsessive compulsive disorder. *Advances in Pharmacology, 30*, 35–52.

Dougherty, D. D., Baer, L., Cosgrove, G. R., Cassem, E. H., Price, B. H., Nierenberg, A. A., Jenike, M. A., & Rauch, S. L. (2002). Prospective long-term follow-up of 44 patients who received cingulotomy for treatment-refractory obsessive-compulsive disorder. *The American Journal of Psychiatry, 159*(2), 269–275.

Dougherty, D. D., Rauch, S. L., & Jenike, M. A. (2004). Pharmacotherapy for obsessive-compulsive disorder. *Journal of Clinical Psychology, 60*(11), 1195–1202.

Dvorkin, A., Silva, C., McMurran, T., Bisnaire, L., Foster, J., & Szechtman, H. (2010). Features of compulsive checking behavior mediated by nucleus accumbens and orbital frontal cortex. *The European Journal of Neuroscience, 32*(9), 1552–1563.

Eisen, J. L., Leonard, H. L., Swedo, S. E., Price, L. H., Zabriskie, J. B., Chiang, S. Y., Karitani, M., & Rasmussen, S. A. (2001). The use of antibody D8/17 to identify B cells in adults with obsessive-compulsive disorder. *Psychiatry Research, 104*(3), 221–225.

Ercan, T. E., Ercan, G., Severge, B., Arpaozu, M., & Karasu, G. (2008). Mycoplasma pneumoniae infection and obsessive-compulsive disease: A case report. *Journal of Child Neurology, 23*(3), 338–340.

Everitt, B. J., & Wolf, M. E. (2002). Psychomotor stimulant addiction: A neural systems perspective. *The Journal of Neuroscience, 22*(9), 3312–3320.

Fessler, D. M. (2001). Luteal phase immunosuppression and meat eating. *Rivista di Biologia, 94*(3), 403–426.

Fiddick, L. (2011). There is more than the amygdala: Potential threat assessment in the cingulate cortex. *Neuroscience and Biobehavioral Reviews, 35*(4), 1007–1018.

Fontaine, D., Mattei, V., Borg, M., von Langsdorff, D., Magnie, M. N., Chanalet, S., Robert, P., & Paquis, P. (2004). Effect of subthalamic nucleus stimulation on obsessive-compulsive disorder in a patient with Parkinson disease. Case report. *Journal of Neurosurgery, 100*(6), 1084–1086.

Friedlander, L., & Desrocher, M. (2006). Neuroimaging studies of obsessive-compulsive disorder in adults and children. *Clinical Psychology Review, 26*(1), 32–49.

Ghahremani, D. G., Lee, B., Robertson, C. L., Tabibnia, G., Morgan, A. T., De Shetler, N., Brown, A. K., Monterosso, J. R., Aron, A. R., Mandelkern, M. A., Poldrack, R. A., & London, E. D. (2012). Striatal dopamine D2/D3 receptors mediate response inhibition and related activity in frontostriatal neural circuitry in humans. *The Journal of Neuroscience, 32*(21), 7316–7324.

Glickman, S. E., & Schiff, B. B. (1967). A biological theory of reinforcement. *Psychological Review, 74*(2), 81–109.

Groenewegen, H. J., Wright, C. I., Beijer, A. V. J., & Voorn, P. I. E. (1999). Convergence and segregation of ventral striatal inputs and outputs. *Annals of the New York Academy of Sciences, 877*(1), 49–63.

Haber, S. N., Fudge, J. L., & McFarland, N. R. (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *The Journal of Neuroscience, 20*(6), 2369–2382.

Haynes, W. I. A., & Mallet, L. (2010). High-frequency stimulation of deep brain structures in obsessive-compulsive disorder: The search for a valid circuit. *The European Journal of Neuroscience, 32*(7), 1118–1127.

Hebb, D. O. (1966). *A Textbook of Psychology* (Vol. 2d). Philadelphia: Saunders.

Hoffman, K. L., & Rueda Morales, R. I. (2012). D1 and D2 dopamine receptor antagonists decrease behavioral bout duration, without altering the bout's repeated behavioral components, in a naturalistic model of repetitive and compulsive behavior. *Behavioural Brain Research, 230*(1), 1–10.

Huff, W., Lenartz, D., Schormann, M., Lee, S. H., Kuhn, J., Koulousakis, A., Mai, J., Daumann, J., Maarouf, M., Klosterkotter, J., & Sturm, V. (2010). Unilateral deep brain stimulation of the nucleus accumbens in patients with treatment-resistant obsessive-compulsive disorder: Outcomes after one year. *Clinical Neurology and Neurosurgery, 112*(2), 137–143.

Huoranszki, F. (2011). Weakness and compulsion: The essential difference. *Philosophical Explorations, 14*(1), 81–97.

Idro, R., Maling, S., & Byarugaba, J. (2005). Compulsive behavior and coprolalia after cerebral malaria. *Journal of Pediatric Neurology, 3*, 107–108.

Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: A unifying interpretation with special reference to reward-seeking. *Brain Research Reviews, 31*(1), 6–41.

Jenike, M. A., Baer, L., Ballantine, T., Martuza, R. L., Tynes, S., Giriunas, I., Buttolph, M. L., & Cassem, N. H. (1991). Cingulotomy for refractory obsessive-compulsive disorder. A long-term follow-up of 33 patients. *Archives of General Psychiatry, 48*(6), 548–555.

Joel, D., & Weiner, I. (2000). The connections of the dopaminergic system with the striatum in rats and primates: An analysis with respect to the functional and compartmental organization of the striatum. *Neuroscience, 96*(3), 451–474.

Kahneman, D. (2011). *Thinking, Fast and Slow*. Toronto: Doubleday Canada.

Kapadia, M., & Sakic, B. (2011). Autoimmune and inflammatory mechanisms of CNS damage. *Progress in Neurobiology, 95*(3), 301–333.

Kettl, P. A., & Marks, I. M. (1986). Neurological factors in obsessive compulsive disorder. Two case reports and a review of the literature. *The British Journal of Psychiatry, 149*, 315–319.

Kim, J. J., Lee, M. C., Kim, J., Kim, I. Y., Kim, S. I., Hee, H. M., Chang, K. H., & Kwon, J. S. (2001). Grey matter abnormalities in obsessive-compulsive disorder: Statistical parametric mapping of segmented magnetic resonance images. *The British Journal of Psychiatry, 179*, 330–334.

Kostrzewa, R. M. (1995). Dopamine receptor supersensitivity. *Neuroscience and Biobehavioral Reviews, 19*, 1–17.

Kurlan, R. (2004). Disabling repetitive behaviors in Parkinson's disease. *Movement Disorders, 19*(4), 433–437.

Lang, P. J., Davis, M., & Ohman, A. (2000). Fear and anxiety: Animal models and human cognitive psychophysiology. *Journal of Affective Disorders, 61*(3), 137–159.

Laplane, D., Levasseur, M., Pillon, B., Dubois, B., Baulac, M., Mazoyer, B., Tran, D. S., Sette, G., Danze, F., & Baron, J. C. (1989). Obsessive-compulsive and other behavioural changes with bilateral basal ganglia lesions. A neuropsychological, magnetic resonance imaging and positron tomography study. *Brain, 112*(Pt 3), 699–725.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*(1), 155–184.

Lorrain, D. S., Riolo, J. V., Matuszewich, L., & Hull, E. M. (1999). Lateral hypothalamic serotonin inhibits nucleus accumbens dopamine: Implications for sexual satiety. *The Journal of Neuroscience, 19*(17), 7648–7652.

Lykouras, L. (2003). Obsessive–compulsive symptoms induced by atypical antipsychotics. A review of the reported cases. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27*(3), 333–346.

MacLean, P. D. (1973). Special award lecture: new findings on brain function and sociosexual behavior. In J. Zubin & J. Money (Eds.), *Contemporary sexual behavior: Critical issues in the 1970s* (pp. 53–74). Baltimore: The John Hopkins University Press.

MacLean, P. D. (1978). Effects of lesions of globus pallidus on species-typical display behavior of squirrel monkeys. *Brain Research, 149*(1), 175–196.

Mallet, L., Mesnage, V., Houeto, J. L., Pelissolo, A., Yelnik, J., Behar, C., Gargiulo, M., Welter, M. L., Bonnet, A. M., Pillon, B., Cornu, P., Dormont, D., Pidoux, B., Allilaire, J. F., & Agid, Y. (2002). Compulsions, Parkinson's disease, and stimulation. *Lancet, 360*(9342), 1302–1304.

Marchesi, C., Tonna, M., & Maggini, C. (2009). Obsessive-compulsive disorder followed by psychotic episode in long-term ecstasy misuse. *The World Journal of Biological Psychiatry: The Official Journal of the World Federation of Societies of Biological Psychiatry, 10*(4 Pt 2), 599–602.

Masterson, F. A., & Crawford, M. (1982). The defense motivation system – A theory of avoidance-behavior. *The Behavioral and Brain Sciences, 5*(4), 661–675.

Mataix-Cols, D., Wooderson, S., Lawrence, N., Brammer, M. J., Speckens, A., & Phillips, M. L. (2004). Distinct neural correlates of washing, checking, and hoarding symptom dimensions in obsessive-compulsive disorder. *Archives of General Psychiatry, 61*(6), 564–576.

McGuire, P. K., Bench, C. J., Frith, C. D., Marks, I. M., Frackowiak, R. S., & Dolan, R. J. (1994). Functional anatomy of obsessive-compulsive phenomena. *The British Journal of Psychiatry, 164*(4), 459–468.

Menzies, L., Chamberlain, S. R., Laird, A. R., Thelen, S. M., Sahakian, B. J., & Bullmore, E. T. (2008). Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: The orbitofronto-striatal model revisited. *Neuroscience and Biobehavioral Reviews, 32*(3), 525–549.

Mink, J. W. (1996). The basal ganglia: Focused selection and inhibition of competing motor programs. *Progress in Neurobiology, 50*(4), 381–425.

Muris, P., Merckelbach, H., & Clavan, M. (1997). Abnormal and normal compulsions. *Behaviour Research and Therapy, 35*(3), 249–252.

Nikolaus, S., Antke, C., Beu, M., & Muller, H. W. (2010). Cortical GABA, striatal dopamine and midbrain serotonin as the key players in compulsive and anxiety disorders–results from in vivo imaging studies. *Reviews in the Neurosciences, 21*(2), 119–139.

Nuttin, B., Cosyns, P., Demeulemeester, H., Gybels, J., & Meyerson, B. (1999). Electrical stimulation in anterior limbs of internal capsules in patients with obsessive-compulsive disorder. *Lancet, 354*(9189), 1526.

Nuttin, B. J., Gabriels, L. A., Cosyns, P. R., Meyerson, B. A., Andreewitch, S., Sunaert, S. G., Maes, A. F., Dupont, P. J., Gybels, J. M., Gielen, F., & Demeulemeester, H. G. (2003). Long-term electrical capsular stimulation in patients with obsessive-compulsive disorder. *Neurosurgery, 52*(6), 1263–1272.

Pauls, D. L., Towbin, K. E., Leckman, J. F., Zahner, G. E., & Cohen, D. J. (1986). Gilles de la Tourette's syndrome and obsessive-compulsive disorder. Evidence supporting a genetic relationship. *Archives of General Psychiatry, 43*(12), 1180–1182.

Penney, J. B., Jr., & Young, A. B. (1983). Speculations on the functional anatomy of basal ganglia disorders. *Annual Review of Neuroscience, 6*, 73–94.

Phillips, M. L., & Mataix-Cols, D. (2004). Patterns of neural response to emotive stimuli distinguish the different symptom dimensions of obsessive-compulsive disorder. *CNS Spectrums, 9*(4), 275–283.

Piasecki, S. D., & Jefferson, J. W. (2004). Psychiatric complications of deep brain stimulation for Parkinson's disease. *The Journal of Clinical Psychiatry, 65*(6), 845–849.

Pittenger, C., Bloch, M. H., & Williams, K. (2011). Glutamate abnormalities in obsessive compulsive disorder: Neurobiology, pathophysiology, and treatment. *Pharmacology & Therapeutics, 132*(3), 314–332.

Rachman, S., & De Silva, P. (1978). Abnormal and normal obsessions. *Behaviour Research and Therapy, 16*(4), 233–248.

Rasmussen, S. A., & Eisen, J. L. (1992). The epidemiology and differential-diagnosis of obsessive-compulsive disorder. *The Journal of Clinical Psychiatry, 53*, 4–10.

Rassin, E., & Muris, P. (2007). Abnormal and normal obsessions: A reconsideration. *Behaviour Research and Therapy, 45*(5), 1065–1070.

Rauch, S. L., Jenike, M. A., Alpert, N. M., Baer, L., Breiter, H. C., Savage, C. R., & Fischman, A. J. (1994). Regional cerebral blood flow measured during symptom provocation in obsessive-compulsive disorder using oxygen 15-labeled carbon dioxide and positron emission tomography. *Archives of General Psychiatry, 51*(1), 62–70.

Reed, G. F. (1985). *Obsessional experience and compulsive behaviour: A cognitive-structural approach*. Orlando: Academic Press.

Rotge, J. Y., Aouizerate, B., Tignol, J., Bioulac, B., Burbaud, P., & Guehl, D. (2010). The glutamate-based genetic immune hypothesis in obsessive-compulsive disorder. An integrative approach from genes to symptoms. *Neuroscience, 165*(2), 408–417.

Rotge, J. Y., Guehl, D., Dilharreguy, B., Cuny, E., Tignol, J., Bioulac, B., Allard, M., Burbaud, P., & Aouizerate, B. (2008). Provocation of obsessive-compulsive symptoms: A quantitative voxel-based meta-analysis of functional neuroimaging studies. *Journal of Psychiatry & Neuroscience, 33*(5), 405–412.

Salkovskis, P. M., & Harrison, J. (1984). Abnormal and normal obsessions–a replication. *Behaviour Research and Therapy, 22*(5), 549–552.

Saxena, S., Brody, A. L., Schwartz, J. M., & Baxter, L. R. (1998). Neuroimaging and frontal-subcortical circuitry in obsessive-compulsive disorder. *The British Journal of Psychiatry. Supplement, 35*, 26–37.

Schilder, P. (1938). The organic background of obsessions and compulsions. *The American Journal of Psychiatry, 94*, 1397–1414.

Semple, S. J., Strathdee, S. A., Zians, J., McQuaid, J., & Patterson, T. L. (2011). Correlates of obsessive-compulsive disorder in a sample of HIV-positive, methamphetamine-using men who have sex with men. *AIDS and Behavior, 15*(6), 1153–1160.

Soubrie, P. (1986). Reconciling the role of central serotonin neurons in human and animal behavior. *The Behavioral and Brain Sciences, 9*, 319–364.

Sturm, V., Lenartz, D., Koulousakis, A., Treuer, H., Herholz, K., Klein, J. C., & Klosterkotter, J. (2003). The nucleus accumbens: A target for deep brain stimulation in obsessive-compulsive- and anxiety-disorders. *Journal of Chemical Neuroanatomy, 26*(4), 293–299.

Swedo, S. E., Leonard, H. L., Garvey, M., Mittleman, B., Allen, A. J., Perlmutter, S., Dow, S., Zamkoff, J., Dubbert, B. K., & Lougee, L. (1998). Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: Clinical description of the first 50 cases. *The American Journal of Psychiatry, 155*(2), 264–271.

Swedo, S. E., Leonard, H. L., Mittleman, B. B., Allen, A. J., Rapoport, J. L., Dow, S. P., Kanter, M. E., Chapman, F., & Zabriskie, J. (1997). Identification of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections by a marker associated with rheumatic fever. *The American Journal of Psychiatry, 154*(1), 110–112.

Swedo, S. E., Pietrini, P., Leonard, H. L., Schapiro, M. B., Rettew, D. C., Goldberger, E. L., Rapoport, S. I., Rapoport, J. L., & Grady, C. L. (1992). Cerebral glucose metabolism in childhood-onset obsessive-compulsive disorder. Revisualization during pharmacotherapy. *Archives of General Psychiatry, 49*(9), 690–694.

Swedo, S. E., Rapoport, J. L., Cheslow, D. L., Leonard, H. L., Ayoub, E. M., Hosier, D. M., & Wald, E. R. (1989). High prevalence of obsessive-compulsive symptoms in patients with Sydenham's chorea. *The American Journal of Psychiatry, 146*(2), 246–249.

Szechtman, H., Culver, K., & Eilam, D. (1999). Role of dopamine systems in obsessive-compulsive disorder (OCD): implications from a novel psychostimulant-induced animal model. *Polish Journal of Pharmacology, 51*(1), 55–61.

Szechtman, H., Nahmias, C., & Woody, E. Z. (2002). Alucinaciones, compulsiones y "yedasentience": hacia una biología de la epistemologia (hallucinations, compulsions and "yedasentience": Toward a biology of epistemology). In T. Palomo, R. J. Beninger, M. A. Jiménez Arriero, E. Huertas, & T. Archer (Eds.), *Avances neurocientíficos y realidad clínica V, neuroimagen en psiquiatría* (1st ed., pp. 173–194). Madrid: Fundación Cerebro y Mente.

Szechtman, H., Sulis, W., & Eilam, D. (1998). Quinpirole induces compulsive checking behavior in rats: A potential animal model of obsessive-compulsive disorder (OCD). *Behavioral Neuroscience, 112*(6), 1475–1485.

Szechtman, H., & Woody, E. (2004). Obsessive-compulsive disorder as a disturbance of security motivation. *Psychological Review, 111*(1), 111–127.

Szechtman, H., & Woody, E. Z. (2006). Obsessive-compulsive disorder as a disturbance of security motivation: Constraints on comorbidity. *Neurotoxicity Research, 10*(2), 103–112.

Tass, P. A., Klosterkotter, J., Schneider, F., Lenartz, D., Koulousakis, A., & Sturm, V. (2003). Obsessive-compulsive disorder: Development of demand-controlled deep brain stimulation with methods from stochastic phase resetting. *Neuropsychopharmacology, 28*(S1), S27–S34.

Toates, F. M. (1986). *Motivational systems*. Cambridge Cambridgeshire: Cambridge University Press.

van den Heuvel, O. A., Veltman, D. J., Groenewegen, H. J., Dolan, R. J., Cath, D. C., Boellaard, R., Mesina, C. T., van Balkom, A. J., van Oppen, P., Witter, M. P., Lammertsma, A. A., & van Dyck, R. (2004). Amygdala activity in obsessive-compulsive disorder with contamination fear: A study with oxygen-15 water positron emission tomography. *Psychiatry Research, 132*(3), 225–237.

Westenberg, H. G., Fineberg, N. A., & Denys, D. (2007). Neurobiology of obsessive-compulsive disorder: Serotonin and beyond. *CNS Spectrums, 12*(2 Suppl 3), 14–27.

Wise, S., & Rapoport, J. L. (1989). Obsessive compulsive disorder – Is it a basal ganglia dysfunction? In J. Rapoport (Ed.), *Obsessive compulsive disorder in children and adolescence* (pp. 327–344). Washington, DC: American Psychiatric Press.

Wolgin, D. L. (2012). Amphetamine stereotypy, the basal ganglia, and the "selection problem". *Behavioural Brain Research, 231*(2), 297–308.

Woody, E. Z., & Boyer, P. (2011). Threat-detection and precaution: Introduction to the special issue preface. *Neuroscience and Biobehavioral Reviews, 35*(4), 989–990.

Woody, E. Z., & Szechtman, H. (2000). Hypnotic hallucinations and yedasentience. *Contemporary Hypnosis, 17*, 26–31.

Woody, E. Z., & Szechtman, H. (2005). Motivation, time course, and heterogeneity in obsessive-compulsive disorder: Response to Taylor, McKay, and Abramowitz (2005). *Psychological Review, 112*(3), 658–661.

Woody, E. Z., & Szechtman, H. (2011). Adaptation to potential threat: The evolution, neurobiology, and psychopathology of the security motivation system. *Neuroscience and Biobehavioral Reviews, 35*(4), 1019–1033.

Woolf, C. J. (2011). Central sensitization: Implications for the diagnosis and treatment of pain. *Pain, 152*(3, Supplement), S2–S15.

Woolley, J. B., & Heyman, I. (2003). Dexamphetamine for obsessive-compulsive disorder. *The American Journal of Psychiatry, 160*(1), 183.

Wu, M., Hanna, G. L., Rosenberg, D. R., & Arnold, P. D. (2012). The role of glutamate signaling in the pathogenesis and treatment of obsessive-compulsive disorder. *Pharmacology, Biochemistry, and Behavior, 100*(4), 726–735.

Yu, C.-H., Lee, M.-B., Tseng, M. M.-C., & Liao, S.-C. (2008). Obsessive-compulsive symptoms as a manifestation of neuropsychiatric systemic lupus erythematosus. *Journal of the Formosan Medical Association, 107*(1), 68–72.

Physical Exercise as Intervention in Parkinsonism

Trevor Archer and Anders Fredriksson

Contents

Abstract

Physical exercise, particularly for individuals with sedentary occupations and independent of activity type, offers probably the most effective health-ensuring policy available. The associations between physical exercise and symptoms/biomarkers of idiopathic Parkinsonism and animal models of Parkinson's disease (PD), quality of life and self-reliance, disorder progression, and risk factors all support the contention that activity provides for an improved prognosis. In the present treatise, mice treated with the selective dopamine neurotoxin (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 3 × 30 mg/kg once each week over three weeks), or Vehicle, were given access to running-wheel exercise from the week

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following the first injection of MPTP onwards: the MPTP-Exercise group received four 30-min sessions in the running wheels (Mondays to Thursdays), whereas the MPTP No-exercise and Vehicle groups received a single session each week (Wednesdays). It was observed that the MPTP+Exercise group increased the distance run on each Wednesday 30-min session incrementally, whereas the MPTP No-exercise and Vehicle groups remained at the same distance throughout; similarly, during the 10-min test session on Fridays, prior to the tests of motor activity, the MPTP+Exercise group increased the distance run on each successive occasion but the MPTP No-exercise and Vehicle groups did not. In the tests of spontaneous motor activity, running-wheel exercise over 4 days/week (30 min, Mon.–Thurs.) improved all three parameters of motor activity, locomotion, rearing, and total activity, in the activity test chamber during test weeks 2–7, compared to the MPTP group following the MPTP administration. In the L-Dopa-induced activity test, running-wheel exercise over 4 days/week enhanced locomotor and rearing but not total activity following the subthreshold dose of L-Dopa for the MPTP+Exercise group but not the MPTP No-exercise group. Running-wheel exercise over 4 days/week increased the DA concentrations in the striatum of MPTP+Exercise mice.

Keywords

Alleviation • Biomarkers • Dopamine • L-Dopa • Mice • MPTP • Parkinson's disease • Physical exercise • Spontaneous motor activity • Symptoms • Wheel running

1 Introduction

Physical exercise has been described as any and all activity that generates force through muscular activity that disrupts a homeostatic state (McArdle et al. 1971; Scheuer and Tipton 1977). Although daily physical activity holds benefits for general measures of function, quality of life, and physical strength, as well as increasing endurance (Dechamps et al. 2010a, b; Marks et al. 2009), much evidence presents the manifest advantages for cerebral integrity during aging (Kramer et al. 1999; Lustig et al. 2009; Marks et al. 2010). Any bodily activity that enhances or maintains physical fitness implies the involvement of regular and frequent exercise. Morris and Schoo (2004) have defined exercise as a planned, structured physical activity with the purpose of improving one or more aspects of physical fitness and functional capacity. Regular physical exercise/exertion promotes neuroimmune functioning and facilitates prevention of heart conditions, cardiovascular diseases, type II diabetes and obesity, and mental health improvements, e.g., depressiveness, all of which may exacerbate the PD condition (Nicolucci et al. 2012; Stampfer et al. 2000). Physical exercise offers a nonpharmacologic, noninvasive intervention that enhances brain health and plasticity (Cotman and Berchtold 2002). It has been characterized on the basis of type, intensity, frequency, and duration, with

either endurance or resistance as the training endpoint (Mougios 2010). Long-term exercise benefits brain functioning by increasing cerebral blood flow and oxygenation (Linkis et al. 1995), mobilizing growth factors and synaptic plasticity (Hunsberger et al. 2007) and facilitating performance through neurotransmitter release (Morishima et al. 2006; Hsu et al. 2011). Regular physical exercise holds particular benefits for older individuals, whether under conditions of normal aging or affected by neurodegenerative disorders (Archer 2011; Archer et al. 2011a, b).

Parkinson's disease (PD), a progressive brain disorder that impairs motor function, presents the cardinal symptoms of akinesia/hypokinesia, rigidity, and tremors as a result of the marked degeneration of the nigrostriatal dopamine (DA) pathway (Bernheimer et al. 1973; Goetz et al. 2001). This loss of DA in the basal ganglia is associated with deterioration in balance and postural control together with progressive reductions in the speed and amplitude of everyday movements (Morris et al. 2001; Smithson et al. 1998). The postural instability and disturbances in gait, which are major PD symptoms, are extremely disabling and lead to accidents, freezing, and involuntary movements, all of which worsen prognosis and elevate mortality risk (Adkin et al. 2003; Bloem et al. 2004; Wielinski et al. 2005). The progression of the disorder leads to reduced physical fitness and muscular capability (Canning et al. 1997) with psychosocial and economic implications for patients and caregivers (Schenkman et al. 2001). Fatigue presents a recurring symptom, described subjectively as "an overwhelming sense of tiredness, lack of energy, or feeling of exhaustion" (Krupp and Pollina 1996), that is linked to depressive symptoms and sleep disturbances with consequential loss of quality of life (Friedman and Friedman 2001; De Boer et al. 1996; Karlsen et al. 2000). Although there has been much advance in pharmacological and surgical interventions, postural instability, problems of gait, and instability of balance persist, thereby causing disablement (Grimbergen et al. 2004) and restriction of functional independence and quality of life (Martinez-Martin 1998; Schrag et al. 2000). Patients with PD may develop also a range of nonmotor conditions, including depression, apathy, sleep disturbances, constipation, and cognitive dysfunction, all of which together with the motor deficits may lead to adoption of a sedentary lifestyle (Fertl et al. 1993; van Nimwegen et al. 2011). Unsurprisingly, the prevalence of osteoporosis is high in patients with PD (63 % of PD women and 20 % of PD men compared with healthy age-matched control (29 % in women and 12 % in men) individuals (Invernizzi et al. 2009; Lorefält et al. 2007; Schuit et al. 2004), thereby increasing risk of falls (Sato et al. 2001).

It has been shown that physical exercise improves motor performance in PD patients (Bergen et al. 2002; Comella et al. 1994; Schenkman et al. 2008). In several controlled clinical studies, the implementation of continuous exercise for early-stage PD patients has provided improved daily activity, motor performance, ambulation, and overall functional independence (Baatile et al. 2000; Miyai et al. 2000; Reuter et al. 1999). In the laboratory, studies in healthy older rodents have shown that regular aerobic physical exercise promotes plasticity-related changes in the brain and CNS that include synaptogenesis neuronal arborization, enhanced

glucose utilization, angiogenesis, and neurogenesis (Hirsch and Farley 2009). It promotes also a healthy brain by reducing inflammation, suppressing oxidative stress, and stabilizing calcium homeostasis (Cotman et al. 2007). Several beneficial cerebral features of exercise have been observed from imaging studies: the volume and preservation of gray and white matters of sedentary individuals was enhanced through aerobic activity (Colcombe et al. 2006; Parker et al. 2011; Szabo et al. 2011). It has been suggested that in PD patients adaptive neuroplasticity may occur spontaneously at the level of both the basal ganglia and the cortex (Buhmann et al. 2005); these compensatory processes may be even initiated during the prodromal stage of disorder (van Nuenen et al. 2009). There is evidence that increased homocysteine (Hcy) levels might accelerate dopaminergic cell death in Parkinson's disease (PD) through neurotoxic effects (Gorgone et al. 2012; Lee et al. 2011; Zoccolella et al. 2010). Hyperhomocysteinemia presents a major risk factor for cerebral and peripheral vascular diseases as well as cortical and hippocampal injury, including an increased risk of dementia and cognitive impairment, and in PD patients treated with levodopa, elevated serum homocysteine (Hcy) concentrations are common. Nascimento et al. (2011) have found that even with regular levodopa therapy, Hcy concentrations in PD patients who had exercised regularly were significantly lower than in PD patients who had not exercised and were similar to the Hcy concentrations in the healthy controls. Taken together, evidence suggests that there is a strong tendency for PD patients toward sedentary lifestyle, whereas it is highly evident that a regime of regular physical exercise offers manifest benefits (Speelman et al. 2011; van Nimwegen et al. 2010).

Physical exercise is known to ameliorate both the symptoms and disability caused by PD (Bilowit 1956; Hurwitz 1989; Palmer et al. 1986). For example, the immediate effects of structured speed-dependent treadmill training, limited progressive treadmill training, conventional gait training, or a control intervention were compared in 17 early-stage PD patients. It was found that speed and stride length, the main gait disturbances, were improved markedly by speed-dependent training (Pohl et al. 2003). In another study, the effects of high-intensity training on the treadmill was compared with low- or no-intensity training with marked postexercise increases in gait speed, step, and stride by the former compared with the latter two conditions (Fisher et al. 2008). Treadmill physical activity has provided a variety of improvements for PD patients including motor performance (Schenkman et al. 2008), capacity for daily activities (Miyai et al. 2000, 2002), reduced incidence and lethality of disorder (Kuroda et al. 1992), strength, balance and gait speed (Toole et al. 2005), motor and postural flexibility (Kurtais et al. 2008), and neurocognitive performance (Ahlskog 2011; Gomez-Pinilla 2011). In addition to the cardinal symptoms, hypokinesia, gait and posture disturbance, tremors, and rigidity, several factors contribute negatively to reduced quality of life in PD patients: pain and fatigue, depressive symptoms and cognitive deficits, sleep problems, and detrimental social functioning with accompanying economical impact (Chrischilles et al. 2002; Whetten-Goldstein et al. 1997). Both functional mobility and quality-of-life variables have been improved by exercise programs of various different types (Herman et al. 2007; Kadivar et al. 2011; Rodrigues de Paula et al. 2006).

Despite being slower compared to age-matched healthy controls, PD patients show motor learning with repetitive practice (Jessop et al. 2006; Michel et al. 2009; Onla-or and Winstein 2008; Smiley-Oyen et al. 2006). Both short-term and extensive practices of movement gave benefits against the bradykinesia of PD patients (Smiley-Oyen et al. 2012).

Repeated administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to C57/BL6 mice induces selective and long-lasting lesions of DA in nigrostriatal regions of the brain (Jackson-Lewis et al. 1995; Jones-Humble et al. 1994). The neurotoxin, administered systemically, induces Parkinsonism in human and nonhuman primates (Langston 1985) that results in the loss of *substantia nigra* cells in the *pars compacta* of adult animals (Chiueh et al. 1985). It destroys selectively nigrostriatal neurons, thereby inducing acute, subacute, long-lasting, and even permanent effects that resemble certain features of PD, particularly the hypokinesic effects (Schultz et al. 1989). Systemic administration of MPTP (2 × 40 mg/kg, s.c.) caused L-Dopa reversible hypoactivity (Fredriksson et al. 1990; CR0105146 et al. 1990). A less rigorous dose regime, e.g., 2 × 20, or 25 or 30 mg/kg, of MPTP has been found not to reduce motility in the C57 black mice, although DA brain concentrations may indicate up to 50–80 % reductions (Heikkila et al. 1989; Sonsalla and Heikkila 1986), unless given much more repeatedly (cf. Kurz et al. 2007). The parameters of MPTP treatment neurotoxicity in mice are extremely long-lasting (up to and beyond 52 weeks after treatment) with strong correlations between the functional deficits, particularly hypokinesia, the main biomarker, severe DA depletions, and a dose- and time-dependent recovery of several parameters of motor behavior following treatment with the DA precursor, L-Dopa (Archer and Fredriksson 2003; Fredriksson and Archer 1994; Fredriksson et al. 1999).

In the unilateral 6-hydroxydopamine rat model of Parkinson's disease (PD), Tillerson et al. (2003) abolished the lesion-induced motor asymmetry by forcing the rats to use affected (contralateral) limb, whereas as forced nonuse exacerbated the injury (Tillerson et al. 2002a, b). Both dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were elevated markedly in "casted" 6-OHDA-treated rats (forced to use the contralateral limb) compared with "noncasted" rats (Cohen et al. 2003). Archer and Fredriksson (2010) found that daily running-wheel activity attenuated the hypokinesic effects of MPTP in both a concentrated (2 × 40 mg/kg, 24-h interval) and progressive (1 × 40 mg/kg, weekly doses over 4 weeks) schedule with regard to spontaneous motor behavior and activity following a subthreshold dose of L-Dopa. The loss of DA in each case was attenuated by exercise also (Experiment I, 61 % of control rather than 17 %, Experiment II, 24 % rather than 11 %). Using the progressive schedule of MPTP treatment and extending the exercise intervention from 7 to 14 weeks, it was shown that spontaneous motor activity after MPTP was close to restoration, whereas activity after subthreshold L-Dopa was completely recovered (Fredriksson et al. 2011); DA levels were restored from 17 % (non-exercised) to 64 % in the 14-week exercise intervention and levels of brain-derived neurotrophic factor were increased significantly. It was shown also that both the functional and DA deficits

by MPTP were attenuated even by delayed- introduction of exercise (Archer and Fredriksson 2012).

Brain-derived neurotrophic factor (BDNF) is a neurotrophin with widespread expression in the brain and is connected intimately with brain metabolism and homeostasis (Chaldakov 2011). It is associated with neurogenesis, neuronal survival, and neuroreparation in the brain and CNS (Cui 2006; Numakawa et al. 2010). Treatment interventions that enhance BDNF-related signaling have the potential to restore neural connectivity (Kaplan et al. 2010). Physical exercise induces improvements in motor ability and enhances BDNF expression (Macias et al. 2009). It is linked to elevated BDNF levels in the hippocampus (Neeper et al. 1996; Oliff et al. 1998). Voluntary running, as physical activity, amplifies the BDNF signal that augments neurogenesis through diverse molecular pathways (Stranahan et al. 2009). BDNF mediates several essential morphological changes at neuronal levels that include dendritic arborization (Imamura and Greer 2009; Zhou et al. 2008), axonal and dendritic remodeling (Menna et al. 2005), synaptogenesis (Liu et al. 2009; Tchantchou et al. 2009), and synaptic efficacy (Boulanger and Poo 1999; Sallert et al. 2009). Faherty et al. (2005) have shown that a combination of exercise, social interactions, and learning, or exercise alone during adulthood, gave total protection against MPTP-induced Parkinsonism. They found also that changes in mRNA expression suggested that increases in glia-derived neurotrophic factors, coupled with a decrease of dopamine-related transporters (e.g., dopamine transporter, DAT; vesicular monoamine transporter, VMAT2), contributed to the observed neuroprotection of dopamine neurons in the nigrostriatal system following MPTP exposure. Tajiri et al. (2010) observed that exercise induced behavioral recovery in an animal model of PD and caused increased BDNF and glial-derived neurotrophic factor (GDNF) in the striatum of 6-OHDA-treated rats.

In the pathophysiology of PD symptom profiles, not least with regard to the cardinal symptoms and fatigue, L-Dopa treatment, despite certain drawbacks, has been found to improve ventilator function (Vincken et al. 1989) and work endurance and efficiency without affecting exercise hemodynamics (LeWitt et al. 1994). As DA precursor, the compound, following acute administration, reduced cortisol concentrations in PD patients in resting positions that reduced stress conditions (Müller and Muhlack 2007; Müller et al. 2006); this effect has been related to alterations in serotonergic and dopaminergic transmission (Höglund et al. 2001; Khakimova et al. 2011; Lopez et al. 2001). Pickrell et al. (2011) have presented a mouse model that expresses a mitochondria-targeted restriction enzyme, **PstI** or **mito-PstI**, damaging mitochondrial DNA (mtDNA) in DA neurons by inducing double-strand breaks in the mtDNA, leading to an oxidative phosphorylation deficiency, mostly due to mtDNA depletion. Using a dopamine transporter (DAT) promoter-driven tetracycline transactivator protein (tTA), they expressed mito-PstI exclusively in DA neurons, thereby presenting a novel PD transgenic mouse model (PD-mito-PstI mouse). They have shown that the PD-mito-PstI mice present the major features of PD: possessing a motor phenotype that is reversible with L-Dopa treatment, a progressive neurodegeneration of the SN dopaminergic population, and striatal DA depletion. Their results showed also that behavioral phenotypes in

PD-mito-PstI mice were associated with striatal dysfunctions preceding SN loss of tyrosine hydroxylase-positive neurons and that other neurotransmitter systems [noradrenaline (NE) and serotonin (5-HT)] were increased after the disruption of DA neurons, potentially as a compensatory mechanism. The eventual effects of regular physical exercise upon the PD-mito-PstI mice are awaited. The administration of L-Dopa, despite development of tolerance, generally alleviates bradykinesia (Fredriksson and Archer 1994; Fredriksson et al. 1990, 1999), but clinically, the DA precursor by itself will not elevate movement unless assertive physical exertion in the early stages is provoked (Weiner and Singer 1989). It has been found that exercise induced a significantly faster stimulation of growth hormone release following L-Dopa release, in comparison with the rest condition, in patients with PD (Müller et al. 2007).

Although endurance training aerobic sessions enhance cortical release proportional to exercise intensity (Kanaley et al. 2001), intensive physical exercise sessions also reduced cortisol plasma concentrations (Kemmler et al. 2003). Forced exercise initially increased, and then reduced, adrenocorticotropic hormone (ACTH) in control, non-brain damaged animals, followed by marked increases in BDNF (Griesbach et al. 2012). Exercise effects upon Parkinsonian conditions are complex: it appears that PD patients treated with L-Dopa may report a decreased capacity for physical exercise (Reuter et al. 2000; Ziv et al. 1998). The influence of physical exercise as an intervention affecting the efficacy of L-Dopa upon Parkinson symptoms is a central issue: Müller and Muhlack (2008) reported that while endurance exercise did not affect the reduction in cortisol release by L-Dopa, grip strength increased only during rest. Although exercise seems not to affect the pharmacokinetics of L-Dopa in PD patients (Carter et al. 1992), there is an abundance of evidence showing that it impacts upon L-Dopa effects on motor parameters in PD (Muhlack et al. 2007; Reuter et al. 1999; Scandalis et al. 2001; Viliani et al. 1999). Frazzitta et al. (2012) have found that intensive rehabilitation treatment, that included treadmill and stabilometric platform training, reduced the daily medication dosage of L-Dopa in treated (exercised) patients, whereas it was significantly increased in control (non-exercised) patients. In addition, the beneficial effects of intensive rehabilitation treatment persisted over time with a second rehabilitation cycle administered after one year as effective as the initial treatment. Endurance exercise has the effect of increasing L-Dopa-induced growth hormone availability in PD patients (Keranen et al. 1996; Manetta et al. 2002): Müller et al. (2007) observed that during exercise the resulting higher blood pressure mobilized blood flow and thereby growth hormone-releasing hormone, secreted into the hypothalamic portal capillaries, toward the growth hormone-producing cells in the pituitary gland. In highlighting the unique importance of exercise in the disorder perspective, a recent review (Ahlskog 2011) made the following recommendations for clinicians: (1) ongoing vigorous exercise and physical fitness should be encouraged, (2) PD physical therapy programs ought to include structured, graduated fitness instruction and guidance for deconditioned patients with PD, and (3) levodopa and other forms of dopamine replenishment therapy should be utilized to achieve the maximum capability and motivation whereby patients maintain fitness.

The purpose of the present study was to examine whether or not physical exercise in a running wheel would ameliorate the functional and DA deficits induced by MPTP under a progressive dose regime of the neurotoxin.

2 Material and Methods

2.1 Animals

Male C57 Bl/6 mice were purchased from B&K, Sollentuna, Sweden, and were maintained, five to a cage, in plastic cages in a room at temperature of 22 ± 1 °C and a 12/12 h constant light/dark cycle (lights on between 06.00 and 18.00 h). They were placed and maintained in groups of 4–6 animals in a room maintained for male mice only following arrival at the laboratory for about 2 weeks in order to acclimatize. Free access to food and water was maintained throughout, except for the day previous to the initiation to running-wheel exercise which occurred at the end of the second week following arrival. They were housed in groups of six animals, running-wheel exercised and activity chamber tested only during the hours of light (08.00–15.00 h). All exercising and testing were performed in a normally lighted room. Half of the mice in the MPTP treatment condition (MPTP-Exercise) were given running-wheel exercise, whereas the other half were placed in a clean laboratory cage for the same period in a room in which the running wheels were placed. Motor activity was tested in a specially arranged test room. This test room, in which all 12 ADEA activity test chambers, each identical to the home cage, were placed, was well secluded and used only for this purpose. Each test chamber (i.e., motor activity test cage) was placed in a sound-proofed wooden box with 12 cm thick walls and front panels and a small double-glass window to allow observation; each box had a dimmed lighting.

Three weeks following arrival, three groups (n = 10) of DSP4-treated and two groups of vehicle-treated mice were administered either MPTP (2 × 40 mg/kg, s.c., 24 h between injections) or vehicle (0.9 % physiological saline injected s.c. in a volume of 2 ml/kg body weight). Milmed (see below for details of preparation) or vehicle were administered on one occasion per week (Experiment I) or twice weekly (Experiments II and III).

Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) after approval from the local ethical committee (Uppsala University and Agricultural Research Council) and by the Swedish Committee for Ethical Experiments on Laboratory Animals (license S93/92 and S77/94, Stockholm, Sweden).

2.2 Drugs

MPTP (Research Biomedical Inc., MA, USA; 2 × 20 mg/kg or 2 × 40 mg/kg, s.c., with a 24-h interval between injections in each case) was dissolved in saline and

administered s.c. in a volume of 2 ml/kg body weight. Melmid . . .. Saline was used as vehicle in each case.

3 Behavioral Measurements and Apparatus

Activity test chambers: An automated device, consisting of Makrolon rodent test cages (40 × 25 × 15 cm), each placed within two series of infrared beams (at two different heights, one low and one high, 2 and 8 cm, respectively, above the surface of the sawdust, 1 cm deep), was used to measure spontaneous motor activity (RAT-O-MATIC, ADEA Elektronik AB, Uppsala, Sweden). The distance between the infrared beams was as follows: the low-level beams were 73 mm apart lengthwise and 58 mm apart breadthwise in relation to the test chamber and the high-level beams, placed only along each long side of the test chamber, were 28 mm apart. According to the procedures described previously (Archer et al. 1986), the following parameters were measured: LOCOMOTION was measured by the low grid of infrared beams. Counts were registered only when the mouse is in the horizontal plane, ambulating around the test cage. REARING was registered throughout the time when at least one high-level beam was interrupted, i.e., the number of counts registered was proportional to the amount of time spent rearing. TOTAL ACTIVITY was measured by a sensor (a pickup similar to a gramophone needle, mounted on a lever with a counterweight) with which the test cage was constantly in contact. The sensor registered all types of vibration received from the test cage, such as those produced both by locomotion and rearing as well as shaking, tremors, scratching, and grooming. All three behavioral parameters were measured over three consecutive 20-min periods. The motor activity test room, in which all 12 ADEA activity test chambers, each identical to the home cage, were placed, was well secluded and used only for this purpose. Each test chamber (i.e., activity cage) was placed in a sound-proofed wooden box with 12 cm thick walls and front panels, and daylighting. Motor activity parameters were tested on one occasion only, over three consecutive 20-min periods, at the age of 3–4 months.

4 Design and Treatment

On Friday, week 1, mice were injected single weekly injections of MPTP (1 × 30 mg/kg, s.c.) or vehicle after a test of spontaneous motor activity, without access to wheel-running activity prior to the first administration of MPTP. Access to wheel-running activity was given on 1 day only for the Vehicle and MPTP groups (Wednesdays, 30 min) but on 4 days each week for the MPTP+Exercise (Mondays to Thursdays, 30 min), and this 1-day versus 4-day exercise regime was continued up to and during the 7th week. On Friday, weeks 2 and 3, mice were injected single weekly injections of MPTP (1 × 30 mg/kg, s.c.) or vehicle after a test of spontaneous motor activity. In each case, each mouse in each of the four groups received a 10-min access to the running wheel just prior to the 60-min spontaneous motor activity test (Table 1).

Table 1 The experimental design and treatment of mice administered either MPTP or Vehicle, with access to running wheel exercise either 1 day or 4 days each week

	Vehicle	MPTP	MPTP+Exer
Monday	Cage	Cage	Cage
	Cage	Cage	Cage
Week 1	Exer	Exer	Exer
	Cage	Cage	Cage
Friday: T◙	Test+sal	Test+MPTP	Test+MPTP
Måndag	Cage	Cage	Exer
	Cage	Cage	Exer
Weeks 2–3	Exer	Exer	Exer
	Cage	Cage	Exer
Fri: T2&3◙	Test+Sal	Test+MPTP	Test+MPTP
Måndag	Cage	Cage	Exer
	Cage	Cage	Exer
Weeks 4–7	Exer	Exer	Exer
	Cage	Cage	Exer
Fri. T4,5,6,7◙	Test	Test	Test
Fri.T*	Test	Test	Test
Week 8	Sacrifice and neurochemical analysis		

T◙ SMA only; T* SMA+L-Dopa
Vehicle = Saline (0.9% koksalt-lösning), Mptp = **30 mg/kg** OBS!!! subcutan injektion av 5 ml/kg Exer = 30 minuter i löphjul, Cage = 30 minuter i ensam bur Y = Yeast 10 ml/kg S = Saline 10 ml/kg, Test = Spontan aktivitet under 3 × 20 minuter, Inducerad aktivitet efter injektion av L-Dopa (5 mg/kg i en volym av 5 ml/kg)

4.1 Neurochemical Analysis

4.1.1 Dopamine

Mice were killed by cervical dislocation within 1–2 weeks of completion of behavioral testing. Determination of DA was performed using a high-performance liquid chromatography with electrochemical detection (HPLC-EC), according to Björk et al. (1991), as modified by Liu et al. (1995). Striatal regions were rapidly dissected out and stored at -80 °C until neurochemical analysis. DA concentration was measured as follows: the frozen tissue samples were weighed and homogenized in 1 ml of 0.1 M perchloric acid, and alpha-methyl-5-hydroxytryptophan was added as an internal standard. After centrifugation (12,000 rpm, i.e., 18,600 g, 4 °C, 10 min) and filtration, 20 µl of the supernatant was injected into the HPLC-EC to assay DA. The HPLC system consisted of a PM-48 pump (Bioanalytical Systems, BAS) with a CMA/240 autoinjector (injection volume: 20 µl), a precolumn (15 × 3.2 mm, RP-18 Newguard, 7 µm), a column (100 × 4.6 mm, SPHERI-5, RP-18, 5 µm), and an amperometric detector (LC-4B, BAS, equipped with an Ag/AgCl reference electrode and a MF-2000 cell) operating at a potential of +0.85V. The mobile phase, ph 2.69, consisted of K2HPO4 and citric acid buffer (pH 2.5),

10 % methanol, sodium octyl sulfate, 40 mg/l, and EDTA. The flow rate was 1 ml/min, and the temperature of the mobile phase was 35 °C.

4.2 Statistical Analysis

The locomotion, rearing, and total activity data over three consecutive 20-min periods in the activity test chambers from the spontaneous motor activity data were submitted to a split-plot ANOVA design (Kirk 1995). Brain (striatal) regional levels of dopamine, locomotion, rearing, and total activity over the full 60-min period following administration of apomorphine, each were submitted to a one-way ANOVA based on a completely randomized design (Kirk 1995). Pairwise testing between the different treatment groups was performed with the Tukey's HSD test (Kirk 1995). The 1 % level of significance was maintained throughout unless where otherwise stated.

4.2.1 Results

Distance/30 min: Wednesday Tests

Neither the MPTP nor the vehicle groups showed any increase in distance run over 30 min in the running wheels, whereas the MPTP+Exercise group increased significantly distance run over 30 min from test week 2 to test week 7. The former received only one 30-min exposure (Wed.) per week to the wheels, while the latter received four 30-min exposures (Mon.–Thurs.) per week. Split-plot ANOVA indicated a significant Groups x test week interaction effect $F(20, 251) = 25.83$, $p < 0.0001$, for distance during 30 min. Figure 1 presents the distance run by each group during 30 min in the running wheels on the Wednesday tests.

Pairwise testing with Tukey's HSD test indicated significant differences between the three groups over test weeks 2–7 and a significant increase by the MPTP+Exercise group over test weeks 2–7.

Distance/10 min: Friday Tests

Neither the MPTP nor the vehicle groups showed any increase in distance run over 10 min in the running wheels, whereas the MPTP+Exercise group increased significantly distance run over 10 min from test week 2 to test week 7. Split-plot ANOVA indicated a significant Groups x test week interaction effect $F(20, 251) = 47.36$, $p<0.0001$, for distance during 10 min. Figure 2 presents the distance run by each group during 10 min in the running wheels on the Friday tests.

Pairwise testing with Tukey's HSD test indicated significant differences between the three groups over test weeks 2–7 and a significant increase by the MPTP+Exercise group over test weeks 2–7.

Spontaneous Motor Activity: Friday Tests

Running-wheel exercise over 4 days/week (30 min, Mon.–Thurs.) improved all three parameters of motor activity: locomotion, rearing, and total activity, in the activity test chamber during test weeks 2–7, compared to the MPTP group

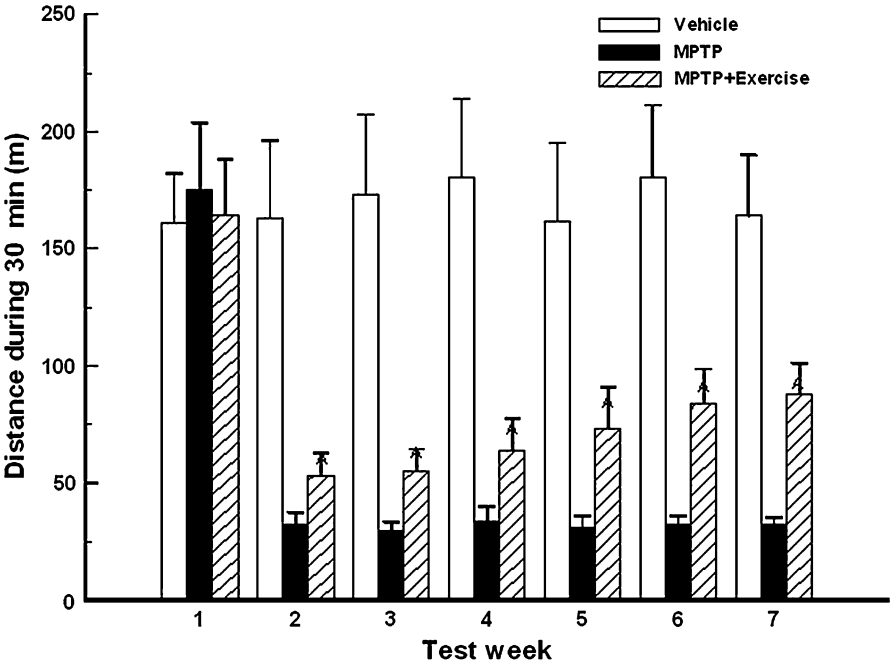

Fig. 1 Distance covered during the 30-min Wednesday test by the Vehicle, MPTP, and MPTP+Exercise groups

following the MPTP administration. Thus, split-plot ANOVA indicated a significant Groups x test week interaction effect for locomotion, rearing, and total activity: $F(20, 209) = 18.82$, $p<0.0001$; $F(20, 2309) = 15.36$, $p<0.001$; and $F(20, 209) = 7.44$, $p<0.0001$, respectively. Figure 3 presents the locomotion, rearing, and total activity by each group during 60 min in the activity test chambers on the Friday tests.

Pairwise testing with Tukey's HSD test indicated significant differences between the three groups over test weeks 2–7 and a significant increase by the MPTP+Exercise group over test weeks 2–7.

L-Dopa-Induced Activity: Friday Tests

Running-wheel exercise over 4 days/week enhanced locomotor and rearing but not total activity following the subthreshold dose of L-Dopa for the MPTP-Exercise group but not the MPTP No-exercise group. Thus, one-way ANOVA indicated a significant Groups effect for locomotion, rearing, and total activity: $F(2, 23) = 39.95$, $p<0.0001$; $F(2, 23) = 81.21$, $p<0.0001$; and $F(2, 23) = 4.77$, $p<0.02$, respectively. Figure 4 presents the locomotion, rearing, and total activity by each group during 180 min in the activity test chambers, following L-Dopa (5 mg/kg) on the Friday tests.

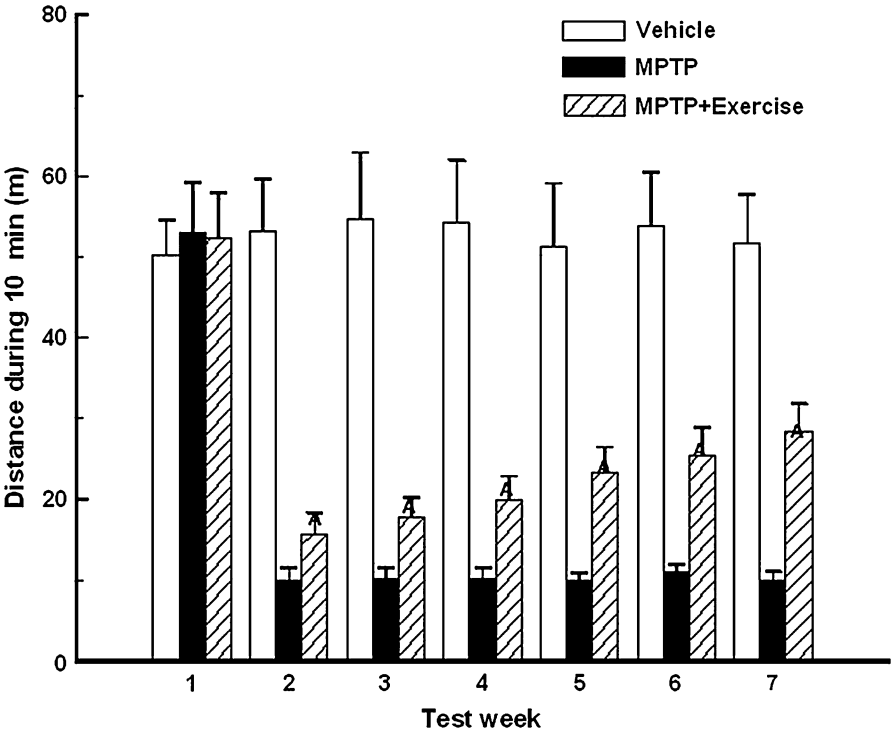

Fig. 2 Distance covered during the 10-min Friday test by the Vehicle, MPTP, and MPTP+Exercise groups

Pairwise testing with Tukey's HSD test indicated significant differences between the three groups over test weeks 2–7 and a significant increase by the MPTP+Exercise group over test weeks 2–7.

Dopamine Analysis

Running-wheel exercise over 4 days/week increased the DA concentrations in the striatum of MPTP+Exercise mice. Thus, one-way ANOVA indicated a significant Groups effect for DA concentration: $F(2, 17) = 121.03$, $p<0.0001$. Figure 5 presents the DA concentrations in the striatum of the Vehicle, MPTP, and MPTP+Exercise groups.

Percent of Vehicle control analyses indicated that striatal DA in the MPTP group was 11 % compared with 31 % in the MPTP-Exercise group.

5 Discussion

The present account of the utility of physical exercise (daily wheel-running activity, 4 days/week as opposed to 1 day/week) in alleviating the functional and

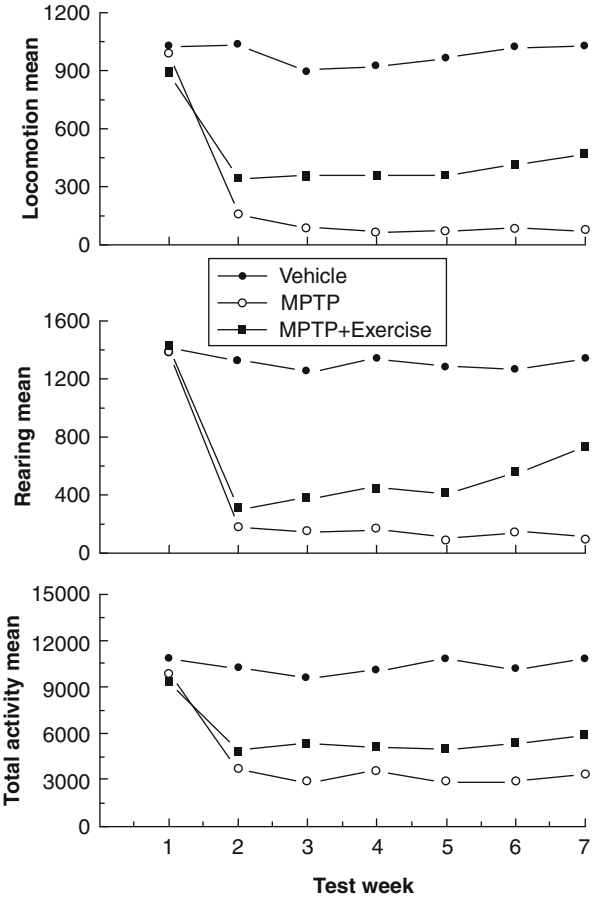

Fig. 3 Spontaneous motor activity by the Vehicle, MPTP, and MPTP+Exercise groups

neurochemical deficits induced by MPTP may be summarized as follows: (1) Only the MPTP+Exercise group, not the Vehicle and MPTP groups, showed significant increases in distance run over 30 min from test week 2 to test week 7 on the Wednesday tests. (2) Only the MPTP+Exercise group, not the Vehicle and MPTP groups, showed significant increases in distance run over 10 min from test week 2 to test week 7 on the Friday tests, given prior to the tests in the motor activity test chambers. (3) The tests of spontaneous motor activity indicated that running-wheel exercise over 4 days/week (30 min, Mon.–Thurs.) improved all three parameters of motor activity, locomotion, rearing, and total activity, in the activity test chamber during test weeks 2–7 (on Fridays), compared to the MPTP group following the MPTP administration. (4) The L-Dopa-induced activity tests indicated that running-wheel exercise over 4 days/week enhanced locomotor and rearing but not total activity following the subthreshold dose of L-Dopa administered on Fridays after the spontaneous motor activity tests. (5) Running-wheel exercise over 4 days/week

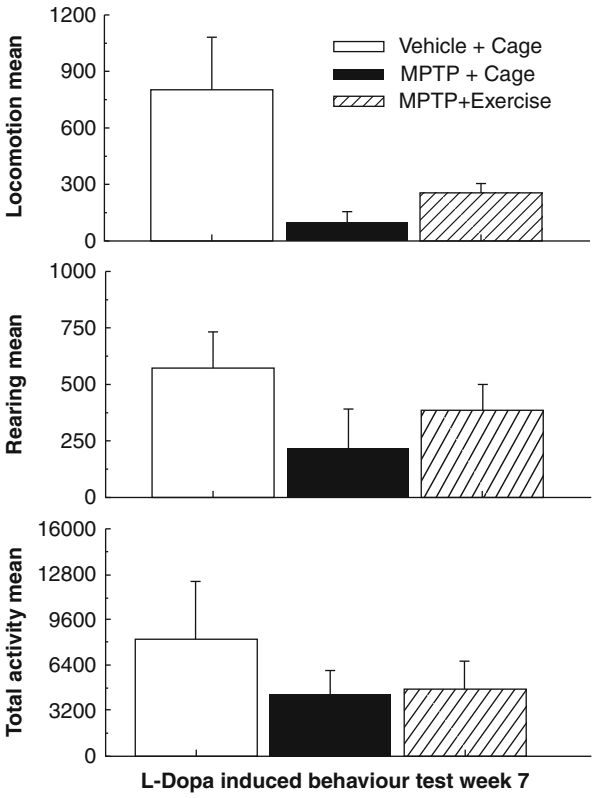

Fig. 4 Motor activity following L-Dopa (5 mg/kg) by the Vehicle, MPTP, and MPTP+Exercise groups

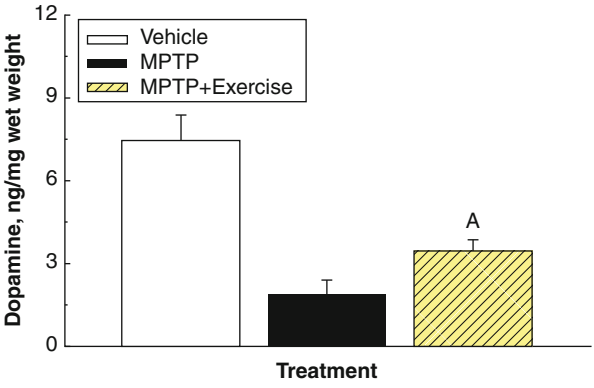

Fig. 5 Dopamine concentrations in the Vehicle, MPTP, and MPTP+Exercise groups

increased the DA concentrations in the striatum of MPTP+Exercise mice compared with the MPTP (one-day/week exposure to the running wheels only) group.

The present findings confirm and extend out previous observations (Archer and Fredriksson 2010, 2012; Fredriksson et al. 2011) in several respects. As observed

previously, physical exercise (30 min/day, 4 days/weeks) reduced the hypokinesic effects of MPTP and the DA loss induced by the neurotoxin under various conditions and parameters. However, none of the previous studies incorporated any method for establishing the exact distance run during each exercise bout by each mouse in its running wheel at any determined time interval, i.e., how much did each day of exercise. The present study incorporated also the condition that "non-exercised" groups in fact received one 30-min wheel-running exposure per week (Wednesdays) such that the exercise variable in fact involved one (non-exercise condition) as opposed to four (exercise condition). As the results (see Figs. 1 and 2, particularly) indicate, a single 30-min exercise session/week does nothing to affect exercise propensity, whereas four sessions/week produced a limited, but significant, and steady increase in distance run.

In the Archer and Fredriksson (2010) study, DA analyses indicated that after 4 × 40 mg/kg MPTP accompanied by 5 weeks of exercise (four 30-min/day sessions per week), the MPTP no-exercise group indicated 11 % of Vehicle control values, whereas the MPTP-Exercise group indicated 24 % of control values. In Experiment II of the Fredriksson et al. (2011) study applying the same 4 × 40 mg/kg MPTP administration regime but accompanied by a 4-week exercise schedule (four 30-min/day sessions per week), DA analyses indicated that the MPTP no-exercise showed 17 % of vehicle controls, whereas the MPTP-Exercise showed 64% of the vehicle controls. Taken together, a comparison of the results from these two studies suggests that longer periods of weekly exercise promote improved recovery from the MPTP-induced DA depletions. In the Archer and Fredriksson (2012) study, both experiments employed a 4 × 30 mg/kg MPTP administration regime accompanied by an 8-week exercise schedule (four 30-min/day sessions per week) in both Experiments I and II. For both experiments, exercise was introduced only after the second injection of MPTP. It was observed that DA levels in Experiment I were 20 % of control value for the MPTP no-exercise group and 39 % of control values for the MPTP-Exercise group; in Experiment II, DA concentrations were 23 % and 43% for the MPTP no-exercise and MPTP-Exercise groups, respectively. In the present experiment, a 3 × 30 mg/kg MPTP administration regime, with exercise introduced after the 1st MPTP injection (except for Wednesday) accompanied by a 6-week exercise schedule (four 30-min/day sessions per week). DA concentrations were 11 % of control values for the MPTP no-exercise group and 31 % for the MPTP-Exercise group. A plausible consensus from these MPTP experiments, employing single, weekly doses of MPTP, appears to be that independent of MPTP dose level or number of injections, the introduction of a multiple weekly exercise regime, rather than a single weekly session regime, significantly restores DA concentrations in the striatum as well as some restoration of motor activity.

The recovery from loss of DA presents an essential aspect of physical activity-mediated interventions in animal models of Parkinsonism and in particular those applying MPTP dose regimes. Petzinger et al. (2007) administered a series of 4 injections of MPTP (i.p.), or saline at 2-h intervals for a total of 80 mg/kg; physical exercise consisted of treadmill running sessions on an accelerating rotarod was initiated for half of the MPTP and half of the saline mice 5 days later. All the mice that were provided access to running-wheel exercise showed increased latencies to

fall off the accelerating rotarod, thereby demonstrating an improved balance, compared with the non-exercised mice. Kurz et al. (2007) administered male C57/BL6 mice with 10 injections of MPTP (25 mg/kg) and probenecid (250 mg/kg) over 5 weeks; the control group of mice received probenecid only, and all the mice were monitored on a motorized treadmill. They observed that the MPTP-treated mice showed significantly more variable stride length and less certain gait pattern than the control mice. In the Ahmad et al. (2009) study, the effects of physical exercise using a motorized rodent treadmill (speeds up to 15 m/min, 40 min/day, 5 days/week for 10 and 18 weeks) for mice given chronic treatment with MPTP (12.5 mg/kg × 10, with a 3.5-day interval between doses, and in combination with probenecid, 250 mg/kg) were examined. This chronic MPTP treatment regime induces a 52 % loss of neurons in the ventral tegmental area (VTA), including decrease in cell volume and irregular or disparaging axonal and dendritic projections that express deficits in dendritic arborization morphology in animals that remain sedentary (German et al. 1996). Positron emission tomography (PET)-imaging studies applying DA-D2R radiotracers have provided the prospect of performing longitudinal studies of the effects of exercise in both humans and animals. Nevertheless, no alteration in DA release or alteration in the binding of [^{11}C]raclopride as a result of aerobic exercise was observed although DA release was triggered (Wang et al. 2000). Vuckovic et al. (2010) found that in MPTP mice high-intensity treadmill exercise, using PET imaging with [^{18}F]fallypride, induced an increase in striatal DA-D2R expression.

In PD patients treated with L-Dopa, elevated serum homocysteine (Hcy) concentrations are commonly observed (Müller 2011: Müller and Muhlack 2010; Sui and Zhang 2010). Nascimento et al. (2011) have showed that Hcy concentrations in PD patients who exercised regularly were significantly lower than in sedentary patients but were similar in healthy controls. Regular exercise improves other aspects of brain health and integrity: for example, endurance exercise promoted cardiorespiratory rehabilitation in MPTP mice with severe neurodegeneration (Al-Jarrah et al. 2007). PD results in significant decreases in blood vessel density (Geny et al. 1994). Al-Jarrah et al. (2010) showed that four weeks of treadmill exercise training induced an increased striatal blood vessel density in chronic Parkinsonian mice that had received 10 administrations of MPTP (25 mg/kg) and probenecid (250 mg/kg) over 5 weeks. They found also that exercise induced an increase in striatal blood vessel density in the control mice although this effect did not attain significance. Smith et al. (2011) measured recovery of gait performance and amount of spontaneous physical activity using a "parallel rod activity chamber" (PRAC) in MPTP-treated and control mice provided with treadmill running (1h/day at 18 cm/s, 5 days/week) or not. They observed that the treadmill exercise program improved gait performance and increased physical activity as well as promoting increased protein expression of striatal dopamine transporter and tyrosine hydroxylase in both MPTP and control mice, but most markedly in the former.

Also applying the chronic MPTP mouse model of PD with moderate neurodegeneration, Lau et al. (2011) observed that exercise improved mitochondrial function and produced increases in region-specific levels of brain-derived and glial cell line-derived neurotrophic factors (BDNF and GDNF). They have demonstrated the impact of exercise upon protection against nigrostriatal neuronal,

mitochondrial, and locomotor deficits in the moderate chronic mouse model of PD. Tajiri et al. (2010) examined the effects of compulsive exercise on Parkinson's disease model of rats. Bromodeoxyuridine (BrdU) was injected to label proliferating cells prior to 6-hydroxydopamine (6-OHDA, 20 μg) lesion into the right striatum of female SD rats. Later, at 24 h after the lesion, the rats were forced to run on the treadmill (5 days/week, 30 min/day, 11 m/min). For behavioral analysis, cylinder tests were performed at 1, 2, 3, and 4 weeks and amphetamine-induced rotational test was performed at 2 and 4 weeks followed by sacrifice for immunohistochemical investigations. The exercise group showed better behavioral recovery in cylinder test and significant decrease in the number of amphetamine-induced rotations, compared to the non-exercise group. Correspondingly, significant preservation of tyrosine hydroxylase (TH)-positive fibers in the striatum and TH-positive neurons in the substantia nigra pars compacta (SNc) was demonstrated, compared to the non-exercise group. In addition, the number of migrated BrdU- and doublecortin-positive cells toward the lesioned striatum was increased in the exercise group. BDNF and GDNF were increased in the striatum by exercise. Their observations suggest that exercise exerts neuroprotective effects or enhances the neuronal differentiation in Parkinson's disease model of rats with subsequent improvement in deteriorated motor function. Finally, Patki and Lau (2011) have demonstrated that long-term exercise (over 18 weeks) attenuated both cytochrome c release and elevated levels of p53; both proteins are associated with mitochondrial dysfunction in the chronic MPTP mouse model. Cytochrome *c* is released by the mitochondria in response to proapoptotic stimuli. The p53 pathway responds to stresses that can disrupt the fidelity of DNA replication and cell division. A stress signal is transmitted to the p53 protein by posttranslational modifications that causes the activation of the p53 protein as a transcription factor that initiates a program of cell cycle arrest, cellular senescence, or apoptosis (e.g., Culmsee and Mattson 2005; Mihara et al. 2003).

6 Conclusion

Taken together, the present findings and the accumulation of laboratory and human studies reinforce the contention that physical exercise may be highly beneficial in conditions of mild to moderate Parkinsonism.

References

Adkin, A. L., Frank, J. S., & Jog, M. S. (2003). Fear of falling and postural control in Parkinson's disease. *Movement Disorders, 18*, 496–502.

Ahlskog, J. E. (2011). Does vigorous exercise have a neuroprotective effect in Parkinson disease? *Neurology, 77*, 288–294.

Ahmad, A. O., Park, J.-H., Stenho-Bittel, L., & Lau, Y. S. (2009). Effects of endurance exercise on ventral tegmental area neurons in the chronic 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine and probenecid-treated. *Neuroscience Letters, 450*, 102–105.

Al-Jarrah, M., Pothakos, K., Novikova, L., Smirnova, I. V., Kurz, M. J., Stehno-Bittel, L., & Lau, Y. S. (2007). Endurance exercise promotes cardiorespiratory rehabilitation without neurorestoration in the chronic mouse model of parkinsonism with severe neurodegeneration. *Neuroscience, 149*, 28–37.

Al-Jarrah, M., Jamous, M., Al Zailaey, K., & Bweir, S. O. (2010). Endurance exercise training promotes angiogenesis in the brain of chronic/progressive mouse model of Parkinson's disease. *Neuro Rehabilitation, 26*, 369–373.

Archer, T. (2011). Physical exercise alleviates debilities of normal aging and Alzheimer's disease. *Acta Neurologica Scandinavica, 123*, 221–239.

Archer, T., & Fredriksson, A. (2003). An antihypokinesic action of α-2-adrenoceptors upon MPTP-induced behaviour deficits in mice. *Journal of Neural Transmission*, *110*, 183–200.

Archer, T., & Fredriksson, A. (2010). Physical exercise attenuates MPTP-induced deficits in mice. *Neurotoxicity Research*, *18*, 313–327.

Archer, T., & Fredriksson, A. (2012). Delayed exercise-induced functional and neurochemical partial restoration following MPTP. *Neurotoxicity Research, 21*(2), 210–221. doi:10.1007/s12640-011-92617-z.

Archer, T., Fredriksson, A., & Johansson, B. (2011a). Exercise alleviates Parkinsonism: Clinical and laboratory evidence. *Acta Neurologica Scandinavica*. doi:10.1111/j.1600-0404.2010.01360.x.

Archer, T., Fredriksson, A., Jonsson, G., Lewander, T., Mohammed, A. K., Ross, S. B., & Soderberg, U. (1986). Central nor adrenaline depletion antagonises aspects of d-amphetamine-induced hyperactivity in the rats. *Psychopharmacology*, *88*, 141–146.

Archer, T., Fredriksson, A., Schütz, E., & Kostrzewa, R. M. (2011b). Influence of physical exercise on neuroimmune functioning and health: aging and stress. *Neurotoxicity Research, 20*, 69–83.

Baatile, J., Langbein, W. E., Weaver, F., Maloney, C., & Jost, M. B. (2000). Effect of exercise upon perceived quality of life of individuals with Parkinson's disease.*The Journal of Rehabilitation Research and Development, 37*, 529–534.

Bergen, J. L., Toole, T., Elliott, R. G. R., Wallace, B., Robinson, K., & Maitland, C. G. (2002). Aerobic exercise intervention improves aerobic capacity and movement initiation in Parkinson's disease patients. *NeuroRehabilitation, 17*, 161–168.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *The Journal of the Neurological Sciences, 20*, 415–455.

Bilowit, D. S. (1956). Establishing physical objectives in the rehabilitation of patients with Parkinson's disease: Gymnasium activities. *Physical Therapy Review, 36*, 176–178.

Björk, L., Lindgren, S., Hacksell, U., & Lewander, T. (1991). (s)-UH-301 antogonises 8-OH-DPAT-induced cardiovascular effects in the rat. *European Journal of Pharmacology*, *199*, 367–370.

Bloem, B. R., Hausdorff, J. M., Visser, J. E., & Giladi, N. (2004). Falls and freezing of gait in Parkinson's disease: a review of two interconnected, episodic phenomena. *Movement Disorders, 19*, 871–884.

Boulanger, L., & Poo, M. M. (1999). Gating of BDNF-induced synaptic potentiation by cAMP. *Science*, *284*, 1982–1984.

Buhmann, C., Binkofski, F., Klein, C., Büchel, C., van Eimeren, T., Erdmann, C., Hedrich, K., Kasten, M., Hagenah, J., Deuschl, G., Pramstaller, P. P., & Siebner, H. R. (2005). Motor reorganization in asymptomatic carriers of a single mutant *Parkin* allele: A human model for presymptomatic parkinsonism. *Brain, 128*, 2281–2290.

Canning, C. G., Alison, J. A., Allen, N. E., & Groeller, H. (1997). Parkinson's disease: an investigation of exercise capacity, respiratory function, and gait. *Archives of Physical Medicine and Rehabilitation, 78*, 199–207.

Carter, J. H., Nutt, J. G., & Woodward, W. R. (1992). The effect of exercise on levodopa absorption. *Neurology, 42*, 2042–2045.

Chaldakov, G. (2011). The metabotrophic NGF and BDNF: An emerging concept. *Archives of Italian Biology*, *149*, 257–263.

Chiueh, C.C., Burns, R.S., Markey, S. P., Jacobowitz, D. M., Kopin, J. J. (1985). Private model of Parkinsonism: Selective lesion of nigrostriatal neurons by 1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine produces an extra pyramidal syndrome in rhesus monkeys. *Life Sciences*, *36*, 213–218.

Chrischilles, E. A., Rubenstein, L. M., Voelker, M. D., Wallace, R. B., & Rodnitzky, R. L. (2002). Linking clinical variables to health-related quality of life in Parkinson's disease. *Parkinsonism and Related Disorders, 8*, 199–209.

Cohen, A. D., Tillerson, J. L., Smith, A. D., Schallert, T., & Zigmond, M. J. (2003). Neuroprotective effects of prior limbuse in 6-hydroxydepamine-treated rats; possible role of GDNF. *Journal of Neurochemistry*, *85*, 299–305.

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., Elavsky, S., Marquez, D. X., Hu, L., & Kramer, A. F. (2006). Aerobic training increases brain volume in aging humans. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 61*, 1166–1170.

Comella CL, Stebbins GT, Brown-Toms N, Goetz CG (1994) Physical therapy and Parkinson's disease: a controlled clinical trial. Neurology 44[3 Part I], 376–378.

Cotman, C. W., & Berchtold, N. C. (2002). Exercise: A behavioural intervention to enhance brain health and plasticity. *Trends in Neurosciences, 25*, 295–301.

Cotman, C. W., Berchtold, N. C., & Christie, L. A. (2007). Exercise builds brain health: Key rolls of growth factor cascades and inflammation. *Trends in Neurosciences, 30*, 464–472.

Culmsee, C., & Mattson, M. P. (2005). p53 in neuronal apoptosis. *Biochemical and Biophysical Research Communications, 331*, 761–777.

De Boer, A. G., Wijker, W., Speelman, J. D., & De Haes, J. C. (1996). Quality of life in patients with Parkinson's disease: Development of a questionnaire. *Journal of Neurology Neurosurgery Psychiatry, 61*, 70–74.

Dechamps, A., Diolez, P., Thiaudière, E., Tulon, A., Onifade, C., Vuong, T., Helmer, C., & Bourdel-Marchasson, I. (2010a). Effects of exercise programs to prevent decline in health-related quality of life in highly deconditioned institutionalized elderly persons: a randomized controlled trial. *Archives of Internal Medicine, 170*(2), 162–9.

Dechamps, A., Alban, R., Jen, J., Decamps, A., Traissac, T., & Dehail, P. (2010b). Individualized cognition-action intervention to prevent behavioral disturbances and functional decline in institutionalized older adults: A randomized pilot trial. *International Journal of Geriatric Psychiatry, 25*(8), 850–60.

Faherty, C. J., Raviie Shepherd, K., Herasimtschuk, A., & Smeyne, R. J. (2005). Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism. *Brain Research Molecular Brain Research*, *134*, 170–179.

Fertl, E., Doppelbauer, A., & Auff, E. (1993). Physical activity and sports in patients suffering from Parkinson's disease in comparison with healthy seniors. *Journal of Neural Transmission Parkinson's Disease and Dementia Section, 5*, 157–161.

Fisher, B. E., Wu, A. D., Salem, G. J., et al. (2008). The effect of exercise training in improving motor performance and corticomotor excitability in people with early Parkinson's disease. *Archives of Physical Medicine and Rehabilitation, 89*, 1221–1229.

Frazzitta, G., Bertotti, G., Riboldazzi, G., Turla, M., Uccellini, D., Boveri, N., Guaglio, G., Perini, M., Comi, C., Balbi, P., & Maestri, R. (2012). Effectiveness of intensive inpatient rehabilitation treatment on disease progression in Parkinsonian patients: A randomized controlled trial with 1-year follow-up. *Neurorehabilitation and Neural Repair, 26*, 144–150.

Fredriksson, A., & Archer, T. (1994). MPTP-induced behavioural and biochemical deficits: A parametric analysis. *Journal of Neural Transmission. Parkinson's Disease and Dementia Section, 7*, 123–132.

Fredriksson, A., Plaznik, A., CR0105146, E., Jonsson, G., & Archer, T. (1990). MPTP-induced hypoactivity in mice: Reversal by L-Dopa. *Pharmacology and Toxicology, 67*, 295–301.

Fredriksson, A., Palomo, T., Chase, T. N., & Archer, T. (1999). Tolerance to a suprathreshold dose of L-Dopa in MPTP mice: Effects of glutamate antagonists. *Journal of Neural Transmission, 106*, 283–300.

Fredriksson, A., Stigsdotter, I. M., Hurtig, A., Ewalds-Kvist, B., & Archer, T. (2011). Running Wheel activity restores MPTP-induced functional deficits. *Journal of Neural Transmission*, *18*, 407–420.

Friedman, J. H., & Friedman, H. (2001). Fatigue in Parkinson's disease: A nine-year follow-up. *Movement Disorders, 16*, 1120–1122.

Geny, C., Naimi-Sadaoui, S., Jeny, R., Belkadi, A. M., Juliano, S. L., & Peschanski, M. (1994). Long-term delayed vascularization of human neural transplants to the rat brain. *Journal of Neuroscience, 14*, 7553–62.

German, D. C., Nelson, E. L., Liang, C. L., Speciale, S. G., Sinton, C. M., & Sonsalla, P. K. (1996). The neurotoxin MPTP causes degeneration of specific nucleus A8, A9 and A10 dopaminergic neurons in the mouse. *Neurodegeneration, 5*, 299–312.

Goetz, C. G., Thelen, J. A., MacLeod, C. M., Carvey, P. M., Bartley, E. A., & Stebbins, G. T. (2001). Blood levodopa levels and unified Parkinson's disease rating scale function: With and without exercise. *Neurology, 43*, 1040–1042.

Gomez-Pinilla, F. (2011). Collaborative effects of diet and exercise on cognitive enhancement. *Nutrition and Health, 20*, 165–169.

Gorgone, G., Currò, M., Ferlazzo, N., Parisi, G., Parnetti, L., Belcastro, V., Tambasco, N., Rossi, A., Pisani, F., Calabresi, P., Ientile, R, Caccamo D (2012) Coenzyme Q10, hyperhomocysteinemia and MTHFR C677T polymorphism in levodopa-treated Parkinson's disease patients. *Neuromolecular Medicine, 14*(1), 84–90. PMID: 22354693.

Griesbach, G. S., Tio, D. L., Vincelli, J., McArthur, D. L., Taylor, A. N. (2012). Differential effects of voluntary and forced exercise after traumatic brain injury on stress responses. *Journal of Neurotrauma, 29*(7), 1426–1433. PMID: 22233388.

Grimbergen, Y. A., Munneke, M., & Bloem, B. R. (2004). Falls in Parkinson's disease. *Current Opinion in Neurology, 17*, 405–415.

Heikkila, R. E., Sieber, B.-A., Manzino, L., & Sonsalla, P. K. (1989). Some features of the nigrostriatal dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the mouse. *Molecular and Chemical Neuropathology*, *10*, 171–183.

Herman, T., Giladi, N., Gruendlinger, L., & Hausdorff, J. M. (2007). Six weeks of intensive treadmill training improves gait and quality of life in patients with Parkinson's disease: A pilot study. *Archives of Physical Medicine and Rehabilitation, 88*, 1154–1158.

Hirsch, M. A., & Farley, B. G. (2009). Exercise and neuroplasticity in persons living with Parkinson's disease. *European Journal of Physical and Rehabilitation Medicine, 45*, 215–229.

Höglund, E., Kolm, N., & Winberg, S. (2001). Stress-induced changes in brain serotonergic activity, plasma cortisol and aggressive behavior in the Arctic charr (*Salvelinus alpinus*) is counteracted by L-DOPA. *Physiology and Behavior, 74*, 381–389.

Hsu, Y. C., Chen, H. I., Kuo, Y. M., Yu, L., Huang, T. Y., Chen, S. J., Chuang, J. I., Wu, F. S., & Jen, C. J. (2011). Chronic treadmill running in normotensive rats resets the resting blood pressure to lower levels by upregulating the hypothalamic GABAergic system. *Journal of Hypertension, 29*(12), 2339–2348.

Hunsberger, J. G., Newton, S. S., Bennett, A. H., Duman, C. H., Russell, D. S., Salton, S. R., & Duman, R. S. (2007). Antidepressant actions of the exercise-regulated gene VGF. *Nature Medicine, 13*(12), 1476–1482.

Hurwitz, A. (1989). The benefit of a home exercise regime for ambulatory Parkinson's disease patients. *Journal of Neuroscience Nursing, 21*, 180–184.

Imamura, F., & Greer, C. A. (2009). Dendritic branching of olfactory bulb mitral and tufted cells: Regulation by TrkB. *PLoS One*, *4*, e6729.

Invernizzi, M., Carda, S., Viscontini, G. S., & Cisari, C. (2009). Osteoporosis in Parkinson's disease. *Parkinsonism and Related Disorders, 15*, 339–346.

Jackson-Lewis, V., Jakowec, M., Burke, R. E., & Przed Borski, S. (1995). Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurodegeneration*, *4*, 257–269.

Jessop, R. T., Horowicz, C., & Dibble, L. E. (2006). Motor learning and Parkinson disease: Refinement of movement velocity and endpoint excursion in a limits of stability balance task. *Neurorehabil Neural Repair, 20*, 459–467.

Jones-Humble, S. A., Morgan, P. F., & Cooper, B. R. (1994) The novel anticonvulsant lamotrigine prevents dopamine depletion in C57 black mice in the MPTP animal model of Parkinson's disease. *Life Sciences*, *54*, 245–252.

Kadivar, Z., Corcos, D. M., Foto, J., & Hondzinski, J. M. (2011). Effect of step training and rhythmic auditory stimulation on functional performance in Parkinson patients. *Neurorehabilitation and Neural Repair, 25*, 626–635.

Kanaley, J. A., Weltman, J. Y., Pieper, K. S., Weltman, A., & Hartman, M. L. (2001). Cortisol and growth hormone responses to exercise at different times of day. *Journal of Clinical Endocrinology and Metabolism, 86*, 2881–2889.

Kaplan, G. B., Vasterling, J. J., & Vedak, P.C. (2010). Brain-derived neurotrophic factor in traumatic brain injury, post traumatic stress disorder, and their comorbid conditions: role in pathogenesis and treatment. *Behavioural Pharmacology*, *21*, 427–437.

Karlsen, K. H., Tandberg, E., Arsland, D., & Larsen, J. P. (2000). Health related quality of life in Parkinson's disease: A prospective longitudinal study. *Journal of Neurology, Neurosurgery, and Psychiatry, 69*, 584–589.

Kemmler, W., Wildt, L., Engelke, K., et al. (2003). Acute hormonal responses of a high impact physical exercise session in early menopausal women. *European Journal of Applied Physiology, 90*, 199–209.

Keranen, T., Gordin, A., Scheinin, M., Antila, S., Sundberg, S., & Wikberg, T. (1996). COMT inhibition by entacapone does not affect growth hormone or prolactin secretion in healthy volunteers. *Journal of Neural Transmission, 103*, 729–736.

Khakimova, G. R., Kozina, E. A., Sapronova, A. Y., & Ugrumov, M. V. (2011). Dopamine synthesis in the nigrostriatal system at the presymptomatic and early symptomatic stages in Parkinsonian mice. *Doklady Biological Sciences, 440*, 284–286.

Kirk, R (1995). Experimental design: Procedures for the Behavioral Sciences. Brooks/cole, Belmont.

Kramer, A. F., Hahn, S., Cohen, N. J., Banich, M. T., McAuley, E., Harrison, C. R., Chason, J., Vakil, E., Bardell, L., Boileau, R. A., & Colcombe, A. (1999). Ageing, fitness and neurocognitive function. *Nature, 400*, 418–419.

Krupp, L. B., & Pollina, D. A. (1996). Mechanisms and management of fatigue in progressive neurological disorders. *Current Opinion in Neurology, 9*, 456–460.

Kuroda, K., Tatara, K., Takatorige, T., & Shinsho, F. (1992). Effect of physical exercise on mortality in patients with Parkinson's disease. *Acta Neurologica Scandinavica, 86*, 55–59.

Kurtais, Y., Kutlay, S., Tur, B. S., Gok, H., & Akbostanci, C. (2008). Does treadmill training improve lower-extremity tasks in Parkinson disease? A randomized control trial. *Clinical Journal of Sport Medicine, 18*, 289–291.

Kurz, M. J., Pothakos, K., Jamaluddin, S., Scott-Pandort, M., Arellane, C., & Lau, Y.S. (2007) A chronic mouse model of Parkinson's disease has a reduced gait pattern certainty. *Neuroscience Letters*, *429*, 39–42.

Langston, J. W. (1985). MPTP neurotoxicity: an overview and characterization of phases of toxicity. *Life Sciences*, *36*, 201–206.

Lau, Y.-S., Patki, G., Das-Panja, K., Le, W.-D., & Ahmad, S. O. (2011). Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. *European Journal of Neuroscience, 33*, 1264–1274.

Lee, P. H., Kim, H. S., Lee, J. E., Choi, Y., Hong, J. Y., Nam, H. S., Sohn, Y. H., & Kim, H. O. (2011). Comparison of endothelial progenitor cells in Parkinson's disease patients treated with levodopa and levodopa/COMT inhibitor. *PLoS One, 6*, e21536.

LeWitt, P. A., Bharucha, A., Chitrit, I., et al. (1994). Perceived exertion and muscle efficiency in Parkinson's disease: L-DOPA effects. *Neuropharmacology, 17*, 454–459.

Linkis, P., Jørgensen, L. G., Olesen, H. L., Madsen, P. L., Lassen, N. A., & Secher, N. H. (1995). Dynamic exercise enhances regional cerebral artery mean flow velocity. *Journal of Applied Physiology, 78*(1), 12–16.

Liu, Y., Yu, H., Mohell, N., Nordvall, G., Lewander, T., & Hacksell, U. (1995). Derivatives of Cis-2-amino-8-hydroxy-1-methyltetralin: Mixed 5-HTIA-receptor agonists and dopamine D2-receptor antagonists. *Journal of Medicinal Chemistry*, *30*, 150–160.

Lopez, A., Munoz, A., Guerra, M. J., & Labandeira-Garcia, J. L. (2001). Mechanisms of the effects of exogenous levodopa on the dopamine-enervated striatum. *Neuroscience, 103*, 639–651.

Lorefält, B., Toss, G., & Granérus, A. K. (2007). Bone mass in elderly patients with Parkinson's disease. *Acta Neurologica Scandinavica, 116*, 248–254.

Lustig, C., Shah, P., Seidler, R., & Reuter-Lorenz, P. A. (2009). Aging, training, and the brain: A review and future directions. *Neuropsychology Review, 19*, 504–522.

Manetta, J., Brun, J. F., Maimoun, L., Callis, A., Prefaut, C., & Mercier, J. (2002). Effect of training on the GH/IGF-I axis during exercise in middle-aged men: Relationship to glucose homeostasis. *American Journal of Physiology-Endocrinology and Metabolism, 283*, E929–E936.

Marks, B. L., Katz, L. M., & Smith, J. K. (2009). Exercise and the aging mind: Buffing the baby boomer's body and brain. *Physical Sports Medicine, 37*, 119–125.

Marks, B. L., Katz, L. M., Styner, M., & Smith, J. K. (2010). Aerobic fitness and obesity: Relationship to cerebral white matter integrity in the brain of active and sedentary older adults. *British Journal of Sports Medicine, 45*, 1208–1215.

Martinez-Martin, P. (1998). An introduction to the concept of "quality of life in Parkinson's disease". *Journal of Neurology, 245*(Suppl. 1), S2–S6.

McArdle, W. D., Glaser, R. M., & Magel, J. R. (1971). Metabolic and cardiorespiratory response during free swimming and treadmill walking. *Journal of Applied Physiology, 30*(5), 733–738.

Menna, E., Cenni, M. C., Naska, S., & Maffei, L. (2005). The anterogradely transported BDNF promotes retinal axon remodeling during eye specific segregation within the LGN. *Molecular Cellular Neuroscience*, *24*, 972–983.

Michel, J., Benninger, D., Dietz, V., & van Hedel, H. J. (2009). Obstacle stepping in patients with Parkinson's disease: Complexity does influence performance. *Journal of Neurology, 256*, 457–463.

Mihara, M., Erster, S., Zaika, A., Petrenko, O., Chittenden, T., Pancoska, P., & Moll, U. M. (2003). p53 has a direct apoptogenic role at the mitochondria. *Molecular Cell, 11*, 577–590.

Miyai, I., Fujimoto, Y., Ueda, Y., Yamamoto, H., Nozaki, S., Saito, T., & Kang, J. (2000). Treadmill training with body weight support: its effect on Parkinson's disease. *Archives of Physical Medicine and Rehabilitation, 81*, 849–852.

Miyai, I., Fujimoto, Y., Yamamoto, H., et al. (2002). Long-term effect of body weight-supported treadmill training in Parkinson's disease: A randomized control trial. *Archives of Physical Medicine and Rehabilitation, 83*, 1370–1373.

Morishima, M., Harada, N., Hara, S., Sano, A., Seno, H., Takahashi, A., Morita, Y., & Nakaya, Y. (2006). Monoamine oxidase A activity and norepinephrine level in hippocampus determine hyperwheel running in SPORTS rats. *Neuropsychopharmacology, 31*(12), 2627–2638.

Morris, M., & Schoo, A. (2004). *Optimizing exercise and physical activity in older adults*. Edinburgh: Butterworth Heinemann.

Morris, M. E., Huxham, F., McGinley, J., Dodd, K., & Iansek, R. (2001). The biomechanics and motor control of gait in Parkinson disease. *Clinical biomechanics, 16*, 459–470.

Mougios, V. (2010). Exercise metabolism. In M. S. Bahrke (Ed.), *Exercise biochemistry* (p. 122). Champaign: Human Kinetics.

Muhlack, S., Welnic, J., Woitalla, D., & Müller, T. (2007). Exercise improves efficacy of levodopa in patients with Parkinson's disease. *Movement Disorders, 22*, 427–430.

Müller, T. (2011). Motor complications, levodopa metabolism and progression of Parkinson's disease. *Expert Opinion on Drug Metabolism and Toxicology, 7*(7), 847–855.

Müller, T., & Muhlack, S. (2007). Acute levodopa intake and associated cortisol decrease in patients with Parkinson disease. *Clinical Neuropharmacology, 30*, 101–106.

Müller, T., & Muhlack, S. (2008). Impact of endurance exercise upon levodopa-associated cortisol release and force increase in patients with Parkinson's disease. *Journal of Neural Transmission, 115*, 851–855.

Müller, T., & Muhlack, S. (2010). Acute homocysteine rise after repeated levodopa application in patients with Parkinson's disease. *Parkinsonism & Related Disorders, 16*(10), 688–689.

Müller, T., Erdmann, C., Bremen, D., et al. (2006). Impact of gastric emptying on levodopa pharmacokinetics in Parkinson disease patients. *Clinical Neuropharmacology, 29*, 61–67.

Müller, T., Welnic, J., Woitalla, D., & Muhlack, S. (2007). Endurance exercise modulates levodopa induced growth hormone release in patients with Parkinson's disease. *Neuroscience Letters, 422*, 119–122.

Nascimento, C. M., Stella, F., Garlipp, C. R., Santos, R. F., Gobbi, S., & Gobbi, L. T. (2011). Serum homocysteine and physical exercise in patients with Parkinson's disease. *Psychogeriatrics, 11*, 105–112.

Nicolucci, A., Balducci, S., Cardelli, P., Cavallo, S., Fallucca, S., Bazuro, A., Simonelli, P Iacobini, C., Zanuso, S., Pugliese, G.; for the Italian Diabetes Exercise Study (IDES) Investigators. (2012). Relationship of exercise volume to improvements of quality of life with supervised exercise training in patients with type 2 diabetes in a randomised controlled trial: The Italian Diabetes and Exercise Study (IDES). *Diabetologia, 55*, 579–588.

Onla-or, S., & Winstein, C. J. (2008). Determining the optimal challenge point for motor skill learning in adults with moderately severe Parkinson's disease. *Neurorehabilitation and Neural Repair, 22*, 385–395.

Palmer, S. S., Mortimer, J. A., Webster, D. D., Bistevins, R., & Dickinson, G. L. (1986). Exercise therapy for Parkinson's disease. *Archives of Physical Medicine and Rehabilitation, 67*, 741–745.

Parker, B. A., Thompson, P. D., Jordan, K. C., Grimaldi, A. S., Assaf, M., Jagannathan, K., & Pearlson, G. D. (2011). Effect of exercise training on hippocampal volume in humans: A pilot study. *Research Quarterly for Exercise and Sport, 82*, 585–591.

Patki, G., & Lau, Y.-S. (2011). Impact of exercise on mitochondrial transcription factor expression and damage in the striatum of a chronic mouse model of Parkinson's disease. *Neuroscience Letters, 505*, 268–272.

Pickrell, A. M., Pinto, M., Hida, A., & Moraes, C. T. (2011). Striatal dysfunctions associated with mitochondrial DNA damage in dopaminergic neurons in a mouse model of Parkinson's disease. *Journal of Neuroscience, 31*, 17649–17658.

Pohl, M., Rockstroh, G., Ruckreim, S., Mrass, G., & Mehrholz, J. (2003). Immediate effects of speed-dependent treadmill training on gait parameters in early Parkinson's disease. *Archives of Physical Medicine and Rehabilitation, 84*, 1760–1766.

Reuter, I., Engelhardt, M., Freiwaldt, J., & Baas, H. (1999). Exercise test in Parkinson's disease. *Clinical Autonomic Research, 9*, 129–134.

Reuter, I., Harder, S., Engelhardt, M., & Baas, H. (2000). The effect of exercise on pharmacokinetics and pharmacodynamics of levodopa. *Movement Disorders, 15*, 862–868.

Rodrigues De Paula, F., Teixeira-Satmetla, L. F., Coelho De Morais Faria, C. D., Rocha De Brito, P., & Cardoso, F. (2006). Impact of an exercise program on physical, emotional and social aspects of quality of life of individuals with Parkinson's disease. *Movement Disorders, 21*, 1073–1077.

Sato, Y., Kaji, M., Tsuru, T., & Oizumi, K. (2001). Risk factors for hip fracture among elderly patients with Parkinson's disease. *Journal of Neurological Sciences, 182*, 89–93.

Scandalis, T. A., Bosak, A., Berliner, J. C., Helman, L. L., & Wells, M. R. (2001). Resistance training and gait function in patients with Parkinson's disease. *American Journal of Physical Medicine & Rehabilitation, 80*, 38–43.

Schenkman, M. L., Clark, K., Xie, T., Kuchibhatla, M., Shinberg, M., & Ray, L. (2001). Spinal movement and performance of a standing reach task in participants with and without Parkinson disease. *Physical Therapy, 81*, 1400–1411.

Schenkman, M. L., Hall, D., Kumar, R., & Kohrt, W. M. (2008). Endurance training to improve economy of movement of people with Parkinson's disease: Three case reports. *Physical Therapy, 88*, 63–76.

Scheuer, J., & Tipton, C. M. (1977). Cardiovascular adaptations to physical training. *Annual Review of Physiology, 39*, 221–251.

Schrag, A., Jahanshahi, M., & Quinn, N. (2000). How does Parkinson's disease affect quality of life? A comparison with quality of life in the general population. *Movement Disorders, 15*, 1112–1118.

Schuit, S. C., van der Klift, M., Weel, A. E., de Laet, C. E., Burger, H., Seeman, E., Hofman, A., Uitterlinden, A. G., van Leeuwen, J. P., Pols, H. A. (2004). Fracture incidence and association with bone mineral density in elderly men and women: The Rotterdam Study. *Bone, 34*(1), 195–202. Erratum in: *Bone*, 2006; *38*(4), 603.

Smiley-Oyen, A. L., Lowry, K. A., & Emerson, Q. R. (2006). Learning and retention of movement sequences in Parkinson's disease. *Movement Disorders, 21*, 1078–1087.

Smiley-Oyen, A. L., Hall, S. A., Lowry, K. A., & Kerr, J. P. (2012). Effects of extensive practice on bradykinesia in Parkinson's disease: Improvement, retention and transfer. *Motor Control, 16*, 1–18.

Smith, B. A., Goldberg, N. R. S., & Meshul, C. K. (2011). Effects of treadmill exercise on behavioral recovery and neural changes in the substantia nigra and striatum of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse. *Brain Research, 1386*, 70–80.

Smithson, F., Morris, M. E., & Iansek, R. (1998). Performance on clinical tests of balance in Parkinson's disease. *Physical Therapy, 78*, 577–592.

Sonsalla, P. K., & Heikkila, R. E. (1986). Neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine (MPTP) and methamphetamine in several strains of mice. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *12*, 345–354.

Speelman, A. D., van de Warrenburg, B. P., van Wimwegen, M., Petzinger, G. M., Munneke, M., & Bloem, R. (2011). How might physical activity benefit patients with Parkinson's disease. *Nature Reviews. Neurology, 7*, 528–534.

Stampfer, M., Hu, F., Manson, J., Rimm, E., & Willett, W. (2000). Primary prevention of coronary heart disease in women through diet and lifestyle. *The New England Journal of Medicine, 343*, 16–23.

Sui, R., & Zhang, L. (2010). Depression, Parkinson disease, Alzheimer disease. The homocysteine hypothesis. *Neurosciences (Riyadh), 15*, 211–213.

Sundström, E., Fredriksson, A., & Archer, T. (1990). Chronic neurochemical and behavioral changes in MPTP-lesioned C57BL/6 mice: A model for Parkinson's disease. *Brain Research*, *528*, 181–188.

Szabo, A. N., McAuley, E., Erickson, K. I., Voss, M., Prakash, R. S., Mailey, E. L., Wójcicki, T. R., White, S. M., Gothe, N., Olson, E. A., & Kramer, A. F. (2011). Cardiorespiratory fitness, hippocampal volume, and frequency of forgetting in older adults. *Neuropsychology, 25*, 545–553.

Tajiri, N., Yasuhara, T., Shingo, T., Kondo, A., Yuan, W., Kadota, T., Wang, F., Baba, T., Tayra, J. T., Morimoto, T., Jing, M., Kikuchi, Y., Kuramoto, S., Agari, T., Miyoshi, Y., Fujino, H., Obata, F., Takeda, I., Furuta, T., & Date, I. (2010). Effects exerts neuroprotective effects on Parkinson's disease model of rats. *Brain Research, 1310*, 200–207.

van Nimwegen, M., Speelman, A. D., Smulders, K., Overeem, S., Borm, G. F., Backx, F. J., Bloem, B. R., Munneke, M.; ParkFit Study Group. (2010). Design and baseline characteristics of the ParkFit study, a randomized controlled trial evaluating the effectiveness of a multifaceted behavioral program to increase physical activity in Parkinson patients. *BMC Neurology 10*, 70–79.

van Nimwegen, M., Speelman, A. D., Hofman-van Rossum, E. J., Overeem, S., Deeg, D. J., Borm, G. F., van der Horst, M. H., Bloem, B. R., & Munneke, M. (2011). Physical inactivity in Parkinson's disease. *Journal of Neurology, 258*, 2214–2221.

van Nuenen, B. F., Weiss, M. M., Bloem, B. R., Reetz, K., van Eimeren, T., Lohmann, K., Hagenah, J., Pramstaller, P. P., Binkofski, F., Klein, C., & Siebner, H. R. (2009). Heterozygous

carriers of a *Parkin* or *PINK1* mutation share a common functional endophenotype. *Neurology, 72*, 1041–1047.

Viliani, T., Pasquetti, P., Magnolfi, S., et al. (1999). Effects of physical training on straightening-up processes in patients with Parkinson's disease. *Disability and Rehabilitation, 21*, 68–73.

Vincken, W. G., Darauay, C. M., & Cosio, M. G. (1989). Reversibility of upper airway obstruction after levodopa therapy in Parkinson's disease. *Chest, 96*, 210–212.

Vučković, M. G., Li, Q., Fisher, B., Nacca, A., Leahy, R. M., Walsh, J. P., Mukherjee, J., Williams, C., Jakowec, M. W., & Petzinger, G. M. (2010). Exercise elevates dopamine D2 receptor in a mouse model of Parkinson's disease: In vivo imaging with [^{18}F]fallypride. *Movement Disorders, 25*, 2777–2784.

Wang, G. J., Volkow, N. D., Fowler, J. S., Franceschi, D., Logan, J., Pappas, N. R., Wong, C. T., & Netusil, N. (2000). PET studies of the effects of aerobic exercise on brain striatal dopamine release. *Journal of Nuclear Medicine, 41*, 1352–1356.

Weiner, W. J., & Singer, C. (1989). Parkinson's disease and nonpharmacologic treatment programs. *Journal of American Geriatrics Society, 37*, 359–363.

Whetten-Goldstein, K., Sloan, F., Kulas, E., Cutson, T., & Schenkman, M. (1997). The burden of Parkinson's disease on society, family, and the individual. *Journal of American Geriatrics Society, 45*, 844–849.

Wielinski, C. L., Erickson-Davis, C., Wichmann, R., Walde-Douglas, M., & Parashos, S. A. (2005). Falls and injuries resulting from falls among patients with Parkinson's disease and other Parkinsonian syndromes. *Movement Disorders, 20*, 410–415.

Ziv, I., Avraham, M., Michaelov, Y., et al. (1998). Enhanced fatigue during motor performance in patients with Parkinson's disease. *Neurology, 51*, 1583–1586.

Zoccolella, S., Lamberti, S. V., Iliceto, G., Santamato, A., Lamberti, P., & Logroscino, G. (2010). Hyperhomocysteinemia in L-dopa treated patients with Parkinson's disease: Potential implications in cognitive dysfunction and dementia? *Current Medicinal Chemistry, 17*, 3253–3261.

Protective Agents in Parkinson's Disease: Caffeine and Adenosine A2A Receptor Antagonists

Nicola Simola, Annalisa Pinna, Lucia Frau, and Micaela Morelli

Contents

Abstract

The pharmacologic management of Parkinson's disease is based on drugs that act on the motor symptoms, whereas there are currently no drugs available that can alter the progressive neurodegeneration of dopaminergic neurons. Based on recent findings suggesting that the adenosinergic system is one of the most interesting in the field of neuroprotection in Parkinson's disease, this chapter describes the functions of adenosine and its receptors in the central nervous system, with particular emphasis on their role in neurotoxicity/neuroprotection. Results of epidemiologic surveys demonstrating that intake of caffeine, an adenosine A_1/A_{2A} receptor antagonist, is inversely correlated with Parkinson's

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disease are summarized. Moreover, evidence originating from preclinical studies showing that the antagonism of the adenosine A_{2A} receptor is responsible for the neuroprotective effects of caffeine is also presented. This chapter therefore provides a comprehensive analysis of the current literature concerning the adenosinergic-based neuroprotective intervention strategy for Parkinson's disease.

1 Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, with an occurrence that tends to rise dramatically with increasing age, thereby amplifying the social relevance of the disease as life expectancy increases (de Rijk et al. 2000). The degeneration of dopaminergic neurons of the substantia nigra pars compacta (SNc) that project to the striatum, the hallmark of PD, provokes functional modifications within the basal ganglia (BG) circuitry causing the typical motor symptoms (tremor, rigidity, and bradykinesia). PD is a classic movement disorder; however, the motor symptoms, which appear when the dopaminergic neuron degeneration has reached at least 70–80 %, are preceded by numerous non-motor symptoms, including autonomic dysfunction, sleep disorders, psychiatric symptoms, olfactory deficits, and gastrointestinal and cognitive dysfunctions (Poewe 2008). Idiopathic PD is a complex disorder characterized by the involvement of selected neuronal populations throughout the central and peripheral nervous systems; however, neurotransmitters other than dopamine (DA) are involved in the disease, including noradrenaline, serotonin, and acetylcholine (Jellinger 1991).

The primary cause of the degenerative process underlying PD remains unknown; however, several contributing factors have so far been identified for the idiopathic form of the disease. The most important of these include mitochondrial defects, oxidative damage, anomalous protein aggregation, and neuroinflammation (Schapira 2006). These processes, once initiated, continue to produce dopaminergic neuron liability contributing to the loss of efficacy of DA-replacement therapy.

The main therapeutic developments in the field of PD have focused, first, on improvement of DA-replacement therapies in order to better manage or prevent the onset of motor complications and, second, on the discovery of compounds that could modify the course of neurodegeneration. Among the various therapeutic approaches aimed at addressing these objectives, the manipulation of adenosine neurotransmission is one of the most valuable.

The first suggestion that manipulating adenosine neurotransmission might have a beneficial effect on PD onset or progression came from epidemiologic evidence showing that consumption of caffeine, a nonselective A_1/A_{2A} receptor antagonist, reduced the risk of developing PD (Ascherio et al. 2001; Costa et al. 2010) (see Sect. 5). Furthermore, preclinical studies investigating the adenosine receptor type involved in these effects suggested that the blockade of the A_{2A} receptor subtype provides the best neuroprotective effect (Schwarzschild et al. 2006).

2 Neuromodulatory Role of Adenosine

Adenosine is an endogenous purine nucleoside constitutively present in mammalian tissues, where it plays a regulatory role in a variety of important physiologic processes by acting as a homeostatic modulator. Adenosine is produced as a result of hydrolysis of adenosine monophosphate (AMP) by means of an action of ecto-5'-nucleotidase (Fredholm et al. 2005); thus, its formation depends upon the metabolism of adenosine triphosphate (ATP). In the extracellular compartment, the level of adenosine also depends on the rate of hydrolysis of ATP, which is released from either neurons or glial cells, and on the adenosine carrier, which keeps adenosine at a stable level. The actions of adenosine are mediated by specific G-protein-coupled receptors. To date, four adenosine receptors have been cloned and characterized: A_1, A_{2A}, A_{2B}, and A_3 (Fredholm et al. 2005).

Adenosine plays different roles in normal physiology, which include promoting and/or maintaining sleep, regulating the general state of arousal, and coupling cerebral blood flow to energy demand (Dunwiddie and Masino 2001). Many of the effects of adenosine that are observed in normal conditions are modified during pathologic events, and the multiple roles of adenosine may produce dual neuroprotective/neurotoxic effects based on the type of insult and cellular condition (Cunha 2001).

Endogenous adenosine released during hypoxia, ischemia, electrical activity, hypoglycemia, or aglycemia reduces the subsequent damage to the neuronal tissue. The neuroprotection offered by adenosine is also effective against other kinds of damage that are not as directly related to energy metabolism, such as mechanical cell injury (Mitchell et al. 1995), methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA)-induced neuroinflammation, and neurotoxicity (Delle Donne and Sonsalla 1994; Khairnar et al. 2010). In these conditions, the neuroprotective actions of adenosine are mediated primarily via A_1 receptor activation, whereas it seems that the action on the A_{2A} receptor could result in a neurotoxic effect. Caffeine and other A_{2A} antagonists, in fact, have induced neuroprotection and reduced glial cell activation in animal models of dopaminergic neurotoxicity utilizing 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) (Schwarzschild et al. 2006; Carta et al. 2009; Frau et al. 2011).

3 Adenosine A_{2A} Receptors

Among the different adenosine receptors, of particular interest for PD are the A_{2A} receptors, specifically located in the BG circuit (Fig. 1). The adenosine A_{2A} receptors interact functionally and are co-expressed with dopaminergic D_2 receptors on the striatal GABAergic neurons of the "indirect" BG pathway projecting from the striatum to the globus pallidus (Hettinger et al. 2001). In the BG circuit, A_{2A} receptors are prevalently localized in the axons, soma, dendrites, and dendritic

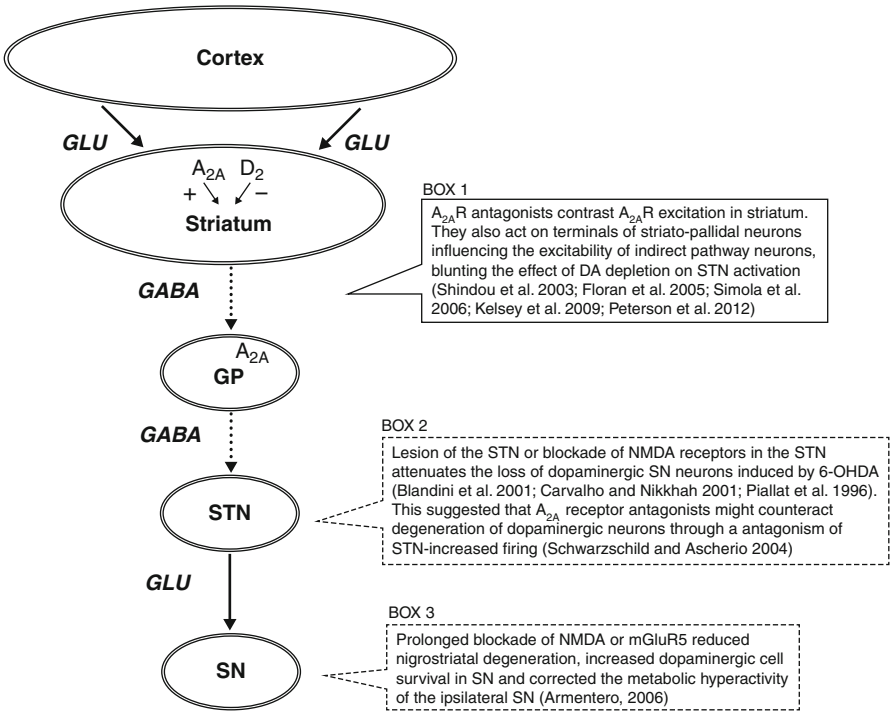

Fig. 1 Proposed actions of A_{2A} receptors on the indirect striatonigral pathway. Classical view of BG functions demonstrating that dopaminergic neuron degeneration provokes an increase in glutamatergic input from the cortex to the striatum and an increase in GABAergic indirect output from the striatum to the GP, leading to an increase in STN activity. In turn, an increase in STN glutamatergic activity contributes to excitotoxic dopaminergic neuron degeneration in the SN. As shown in *Box 1*, several reports indicate that A_{2A} receptors control excitability of the striatopallidal GABAergic pathway and that A_{2A} receptor antagonism counteracts the effects of dopaminergic neuron degeneration. As a result, the GABAergic control from the GP to the STN is increased, leading to a decrease in STN activity. As shown in *Box 2*, a decrease in STN activity and glutamatergic output from the STN to the SN counteracts excitotoxic degeneration of the dopaminergic neurons in the SN. As shown in *Box 3*, blockade of glutamatergic receptors in the SN reduces excitotoxic degeneration of dopaminergic neurons. Therefore, one of the mechanisms of neuroprotection by A_{2A} receptor antagonists may be the indirect inhibition of STN activity. *Box 1* (*continuous line*) represents a direct action of A_{2A} receptor antagonist; *Boxes 2* and *3* (*dashed line*) represent an indirect action of A_{2A} receptor antagonist on neurotransmitter release. Abbreviations: *BG* basal ganglia, *DA* dopamine, *GLU* glutamate, *GP* globus pallidus, *mGluR5* metabotropic glutamate receptor 5, *SN* substantia nigra, *STN* subthalamic nucleus, *6-OHDA* 6-hydroxydopamine

spines of the striatal GABAergic neurons of the indirect pathway. In contrast, very few striatonigral neurons of the "direct" pathway, containing dopaminergic D_1 receptors, express A_{2A} receptors (Schiffmann and Vanderhaeghen 1993) (Fig. 1). However, activation or blockade of A_{2A} receptors in the indirect striatopallidal pathway impairs or facilitates dopaminergic D_1-mediated responses as well (Ferre et al. 1997).

In light of the neuromodulatory role of adenosine, A_{2A} receptors have been described to interact directly or indirectly with several receptors, such as dopaminergic D_2 and D_3 receptors, NMDA, metabotropic glutamate receptor types 4 ($mGLUR_4$) and 5 ($mGLUR_5$), cannabinoid receptor type 1 (CB1), and 5-hydroxytryptamine receptor type 1A ($5\text{-}HT_{1A}$), and to form heteromeric complexes with some of them (Kurokawa et al. 1996; Gerevich et al. 2002; Łukasiewicz et al. 2007; Armentero et al. 2006, 2011; Bogenpohl et al. 2012; Jones et al. 2012).

Besides the presence of A_{2A} receptors in neurons, their existence has been described in astrocytes: specifically, about 3 % of A_{2A} receptors were found in glial cells of the striatum (Hettinger et al. 2001).

4 Caffeine Properties

Caffeine is a natural xanthine alkaloid, contained in several popular dietary sources, such as coffee, tea, chocolate, and soft drinks. Its ability to elicit psychostimulatory effects, that are usually not accompanied by harmful unwanted consequences, renders caffeine the most consumed psychoactive substance (Fredholm et al. 1999).

At doses in the range of normal consumption, caffeine induces its effects by blocking the central adenosine A_1 and A_{2A} receptors (Fredholm et al. 1999). Caffeine-induced psychostimulant effects appear to be influenced by the dopaminergic transmission since enhancement of caffeine-elicited psychomotor activation is observed in experimental rodents following administration of dopaminergic agonists (Cauli and Morelli 2005), whereas an attenuation of the effects of caffeine is present after administration of dopaminergic antagonists (Green and Schenk 2002). The influence that caffeine exerts on dopaminergic transmission is of great interest, considering the critical involvement of DA in the modulation of important functions, such as movement, attention, goal-directed behavior, and associative learning (Robbins and Roberts 2007; Phillips et al. 2008).

The existence of negative correlations between PD and dietary factors, such as caffeine, is of particular interest, in the light of caffeine's safety and widespread consumption (see Sect. 5). In this regard, the results from preclinical studies demonstrating that caffeine can exert protective effects in different models of neurotoxicity add further importance to the finding (Table 1).

Caffeine administration has been shown to have a neuroprotective effect in rodent and primate models of dopaminergic neuron degeneration, such as the MPTP and 6-OHDA paradigms (Table 1). Caffeine neuroprotection is particularly important since it is afforded by a substance with negligible toxic or adverse effects. Often, substances proposed to be neuroprotective impair neuronal functions essential for correct cellular homeostasis or alter the levels of neurotransmitters, such as glutamate, which are important for cognitive functions. Caffeine, in contrast, is a safe substance used worldwide with no unwanted effects in the broad population.

Table 1 Overview of the most common paradigms of toxin-induced neurodegeneration used to investigate neuronal damage and neuroprotection in PD

Model	Type	Toxic effects observed	Main features of the model	Effect of caffeine administered before or concomitantly to the toxin
Cell cultures	Acute Can use different cells (e.g., neuroblastoma or mesencephalic cells) and toxins (e.g., 6-OHDA, MPP^+, rotenone)	Decrease in cell viability	Allows to rapidly identify putative neuroprotective agents and to study their molecular mechanisms Can be used as a complement of in vivo models	Cytoprotection[a; b]
6-OHDA in rats	Acute Employs the direct intracerebral infusion of the toxin	Degeneration of DA neurons in the SNc Reduction in the striatal content of DA and its metabolites	The degeneration is rapid and reaches a near terminal stage few days after infusion Oxidative stress is the major mechanism of neurotoxicity Allows to selectively target different brain regions according to the site of toxin infusion	Attenuation in the demise of SNc neurons[c] Restoration of the levels of DA and its metabolites in the striatum[c]
Lipopolysaccharide in rats and mice	Acute or chronic Can use either intracerebral infusion or systemic administration	Loss of DA neurons in the SNc Activation of glial cells Elevation in the levels of neuroinflammatory mediators	Induces a selective degeneration of DA neurons in SNc, while sparing other DA neurons Degeneration of DA neurons is delayed, progressive, and irreversible Neuroinflammation is the main mechanism underlying neuronal death	Attenuation of neuroinflammation[d]
Paraquat or Paraquat + maneb in mice	Chronic	Degeneration of DA neurons in the SNc Loss of DA terminals in the striatum	This model may yield variable results and significant mortality The toxins used are clinically relevant, since they have been suggested as a potential causative factor of PD	Attenuation in the degeneration of DA neurons[i]

MPTP in mice	Acute or acute-repeated with multiple administration at a fixed interval The toxin is administered systemically	Degeneration of DA neurons in the SNc Loss of DA terminals in the striatum Reduction in the striatal levels of DA and its metabolites Glial activation	The severity of DA lesion can be adjusted by varying the protocol of administration It is still questioned whether the lesion is progressive The model can be used to study both neurotoxicity and neuroinflammation The toxin acts chiefly on mitochondrial function The toxin is clinically relevant, as MPTP can induce PD in humans	Attenuation in the degeneration of SNc neurons[e,] Restoration of the levels of DA and its metabolites in the striatum[e,f,g] Counteraction of neuroinflammation[h]
Rotenone in rats and mice	Chronic The toxin is administered by osmotic minipumps or parenteral injection	Degeneration of SNc DA neurons Loss of striatal DA terminals accompanied by reduction in striatal DA content	DA neuron degeneration is often accompanied by other nonspecific brain damages Systemic toxicity and high mortality is often associated with, or precedes, neurodegeneration There is a high variability in the results obtained with the use of this model Differently from other commonly used models, chronic rotenone can generate proteinaceous inclusions in DA neurons resembling those observed in human PD The toxin used is clinically relevant, since exposure to rotenone has been suggested as a possible causative factor of PD	Not tested

The table describes some of the most common toxin-based experimental models of parkinsonian-like neuron degeneration. The main characteristics of each model are reported, together with the effects of caffeine on the degenerative process, when available

Abbreviations: *DA* dopamine, *MPP^+* 1-methyl-4-phenylpyridinium, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *PD* Parkinson's disease, *SNc* substantia nigra pars compacta, *6-OHDA* 6-hydroxydopamine

[a]Nakaso et al. 2008; [b]Nobre et al. 2010; [c]Aguiar et al. 2006; [d]Brothers et al. 2010; [e]Schwarzschild et al. 2003a; [f]Chen et al. 2001; [g]Xu et al. 2010; [h]Carta et al. 2009; [i]Kachroo et al. 2010

5 Epidemiologic Studies on the Neuroprotective Role of Caffeine in Parkinson's Disease

Numerous findings suggest that the two decades before the manifestation of motor impairments in PD are fundamental in the onset of the progressive demise of dopaminergic neurons. Therefore, epidemiologic studies investigating habits, health status, and lifestyle of individuals who developed PD are very important in order to obtain new information about PD development and to identify new approaches to prevent neurodegeneration in PD.

One of the most interesting results that has emerged from these epidemiologic studies is the inverse correlation between caffeine intake and risk of developing PD. The three main epidemiologic studies, that provided consistent suggestions regarding this relationship, are the Honolulu Heart Program (HHP, 30 years of follow-up of ~8,000 Japanese-American men), the Health Professionals Follow-up Study (HPFS, 10 years of follow-up of ~47,000 men), and the Nurses' Health Study (NHS, 16 years of follow-up of ~88,500 women) (Ross et al. 2000; Ascherio et al. 2001, 2003). In these surveys, besides information of lifestyles and health status of individuals, nutritional behaviors, including data about consumption of coffee and/or other caffeinated or decaffeinated beverages, were collected. The results of the investigation demonstrated that individuals consuming medium to high quantities of caffeine have a decreased risk of developing PD (Ross et al. 2000; Ascherio et al. 2001, 2003). Importantly, this finding was dose dependent and was adjusted for age, smoking status, alcohol use, other nutrients, or potential confounding variables (Ascherio et al. 2001, 2003). The studies described that individuals consuming caffeine (including tea and all caffeine sources) had a lower risk of developing PD compared with non-regular caffeine drinkers (less than one cup a day), whereas no association was found with consumption of decaffeinated coffee. Moreover, a 50 % risk reduction of PD was observed among men consuming less than one cup a day compared with male non-coffee drinkers (Ross et al. 2000; Ascherio et al. 2001). Notably, this inverse relationship between caffeine intake and PD risk was not so evident in women (Ascherio et al. 2001, 2003). Indeed, a clear gender difference was demonstrated by Ascherio and coworkers (2001), regarding the association between caffeine intake and risk of PD in women in the NHS. Similar findings have been reported by Benedetti and coworkers (2000). Since gender difference with regard to the effect of caffeine suggested a possible hormonal cause, in the women reported in the NHS, the correlation between use of postmenopausal estrogens, caffeine intake, and risk of PD was examined. Generally, use of postmenopausal estrogens was not correlated with risk of developing PD. On the other hand, among estrogens users, women consuming more than five cups of coffee per day were found to have a higher risk of PD compared with women who never drink coffee. In contrast, similar to men, women who were coffee drinkers and had never taken postmenopausal estrogens had a lower risk of PD than non-coffee drinkers (Ascherio et al. 2001). These data were confirmed by two separate prospective studies involving men and women which provided evidence that caffeine may decrease risk of PD only in men and in women who did not take postmenopausal

estrogens, but not among estrogen consumers (Ascherio et al. 2003, 2004). Furthermore, the potential beneficial effect of caffeine might be prevented by estrogen therapy (Ascherio et al. 2003, 2004). Conversely, two prospective studies of Finnish individuals reported no gender differences in the opposite correlation between coffee intake and PD risk (Hu et al. 2007; Sääksjärvi et al. 2008). However, in the study by Saaksjarvi and coworkers (2008), the percentage of women was only about 5 % of the cohorts; therefore, the effect of estrogen therapy could not be examined for this small number of women.

Recently, an experimental study in the MPTP mouse model of PD investigated the biologic basis of the interaction between estrogen and caffeine consumption on risk of PD. This study showed that caffeine attenuated, dose dependently, the MPTP-induced striatal DA loss in both male and ovariectomized female compared with female non-ovariectomized mice (Xu et al. 2006). Moreover, chronic estrogen treatment prevented the neuroprotection induced by caffeine in male and in female ovariectomized mice, confirming that the hormonal therapy might prevent the neuroprotective effect of caffeine in this model of PD (Xu et al. 2006). Metabolic or pharmacokinetic interactions between caffeine and estrogens have been supposed, but it is not clear whether this interaction is fundamental in the reduced neuroprotective effect observed when estrogen therapy and caffeine are combined (Xu et al. 2006).

A recent statistical study by Costa and coworkers (2010) collected and statistically analyzed the majority of epidemiologic studies, which better evaluate the effect of caffeine intake and the incidence of PD, up to September 2009. This statistical review, which includes 26 studies, unequivocally confirms the inverse association between caffeine exposure and the risk of developing PD (Costa et al. 2010).

In spite of the epidemiologic and experimental findings strongly supporting the concept that dietary caffeine decreases the possibility of developing PD, very little is known about the correlation between caffeine intake and the degree of disease progression in PD patients. Two clinical studies, in which the progression of the disease and the consumption of several caffeinate beverages were evaluated by the modified Unified Parkinson's Disease Rating Scale (UPDRS), did not demonstrate clear evidence of the neuroprotective role of caffeine with respect to degree of PD progression (Schwarzschild et al. 2003b; Simon et al. 2008). Therefore, further larger investigations are required to fully assess the neuroprotective role of caffeine on disease progression in PD patients.

6 Mechanisms of Dopaminergic Neuroprotection by Caffeine and A_{2A} Receptors Blockade

Studies of neuroprotection in PD should carefully consider a number of factors with regard to the features of neurodegeneration, as well as to the experimental model in which the study is performed. In recent years, PD has been increasingly envisaged as a multifactorial pathology, and several factors have been proposed to be involved in the degeneration of dopaminergic neurons (Schapira 2006). Therefore, it is

hypothesized that neuroprotective drugs should act by means of multiple mechanisms rather than a single mechanism. Moreover, it is worth mentioning that most of the experimental models of PD in use employ toxins according to an acute, or acute-repeated, protocol of administration (Table 1). This may significantly differ from idiopathic PD, with respect to time course of degeneration and mechanisms involved in neuronal demise. Therefore, the choice of experimental model is a critical step when addressing the mechanisms of neuroprotective agents.

Several studies in rodent models of PD demonstrate that A_{2A} receptor antagonists counteract both the demise of dopaminergic neurons in the SNc and the drop of DA levels in the striatum (Schwarzschild et al. 2003a; Morelli et al. 2010). However, A_{2A} antagonists exert beneficial effects not only in models of PD neurodegeneration but also in paradigms of Alzheimer's and Huntington's disease-like neurotoxicity (Stone et al. 2009). This evidence suggests that A_{2A} receptor antagonists counteract PD-like neurodegeneration by means of mechanisms which are not selective towards dopaminergic neurons but are part of a broader neuroprotective action. In the light of these observations, the two major hypotheses proposed to explain the neuroprotective effects of A_{2A} antagonists on dopaminergic neurons concern the modulation of glutamate-induced excitotoxicity and neuroinflammation, two mechanisms involved in several neurodegenerative pathologies.

Glutamate-mediated excitotoxicity has long been envisaged as an important player in PD neurodegeneration. Thus, dopaminergic neurons are vulnerable to changes in glutamate extracellular concentrations, on the one hand, while a dysregulation of glutamate transmission has been described in PD, on the other (Lancelot and Beal 1998; Greenamyre 2001). Remarkably, the stimulation of adenosine A_{2A} receptors can elevate the extracellular concentrations of glutamate, while blockade of A_{2A} receptors decreases glutamate extracellular levels which, in turn, may relieve the excitotoxic insult and afford neuroprotection towards dopaminergic neurons (Popoli et al. 1995). Recent evidence supports a role for nonneuronal cells in the modulation of glutamate release by A_{2A} receptor blockade (Melani et al. 2003; Yu et al. 2008), which is in line with the localization of these receptors on glial cells (see Sect. 3).

Several reports have shown that A_{2A} receptor antagonists modulate the activity of the indirect pathway by acting on the terminals of striatopallidal medium spiny neurons or by influencing the intrinsic excitability of indirect pathway spiny projection neurons, blunting the effects of DA depletion (Shindou et al. 2003; Floran et al. 2005; Simola et al. 2006; Kelsey et al 2009; Peterson et al. 2012) (Fig. 1). Moreover, independent investigations have shown that both lesion of the subthalamic nucleus (STN) and blockade of the NMDA receptors in the STN attenuate the loss of dopaminergic neurons induced by 6-OHDA (Piallat et al. 1996; Blandini et al. 2001; Carvalho and Nikkhah 2001). Based on these considerations, release of glutamate from the STN can be envisioned as an important mechanism in dopaminergic neuron degeneration. Moreover, it has to be considered that the overactivation of the STN progresses in the course of PD, and this can eventually exacerbate the loss of dopaminergic neurons over time. Therefore, it is

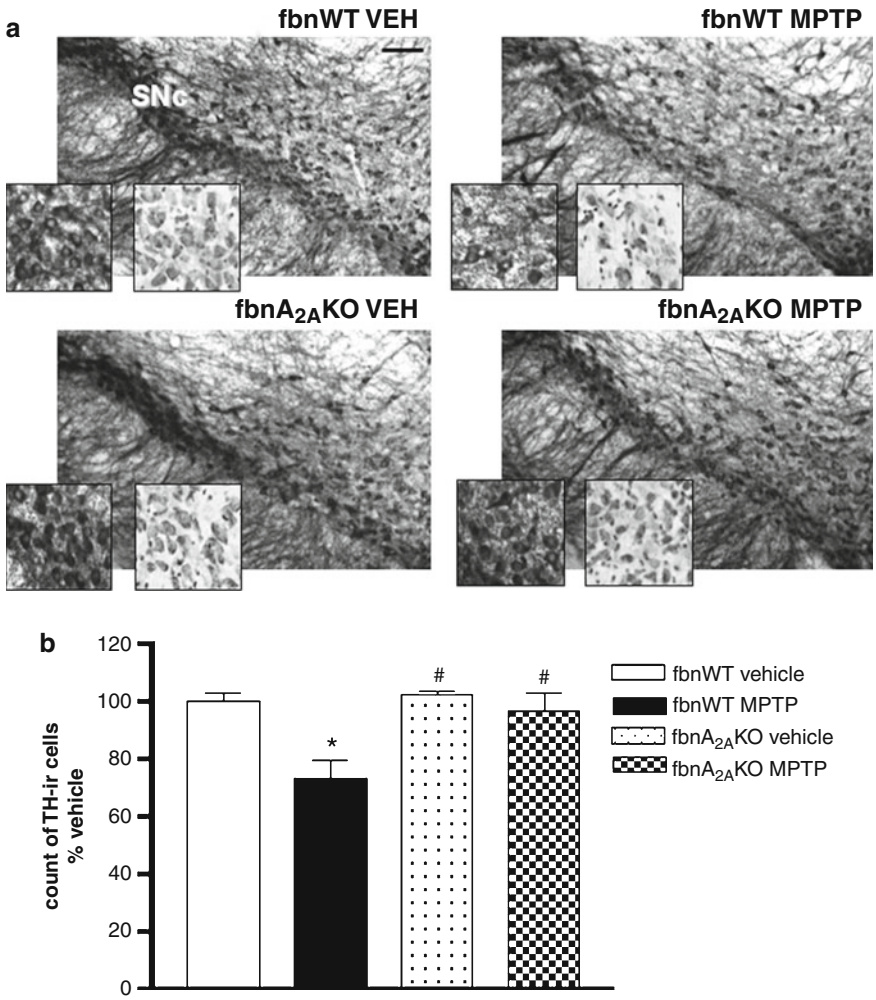

Fig. 2 $FbnA_{2A}$ KO mice are protected against MPTP-induced loss of dopaminergic cells in the SNc. (**a**) Representative sections from SNc immunostained for TH. Insets show higher magnification of TH-labeled (*left*) and cresyl violet-labeled (*right*) cells. Mice were treated with MPTP (20 mg/kg once a day for 4 days) or vehicle. (**b**) Graph shows analysis of TH immunostaining in $fbnA_{2A}$ KO mice, reported as a percentage of TH-positive cells compared with vehicle-treated mice. *$p < 0.05$ versus WT vehicle-treated group; #$p < 0.05$ versus WT MPTP-treated group, by Tukey's post hoc test. Scale bar, 50 μm. Abbreviations: *Fbn* forebrain neurons, *KO* knock-out, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *SNc* substantia nigra pars compacta, *TH* tyrosine hydroxylase, *WT* wild type. (Reproduced from Carta et al. 2009)

conceivable that A_{2A} antagonists, by regulating the activity of the striatopallidal pathway, besides counteracting motor disability, may also attenuate degeneration of the dopaminergic neurons through a modulation of STN firing (Schwarzschild and Ascherio 2004) (Fig. 1).

An important mechanism involved in the neurodegenerative process that characterizes PD is neuroinflammation, as suggested by preclinical and epidemiologic evidence (Hirsch and Hunot 2009; Halliday and Stevens 2011). Neuroinflammation gives rise to a complex cascade of events in the brain, resulting in the activation of glial cells (microglia, astrocytes, and oligodendrocytes) and the subsequent generation of inflammatory mediators (e.g., cytokines, interferons, interleukins) (Reale et al. 2009). Even though transient neuroinflammation can be beneficial, as it may help the brain in coping with noxious insults, protracted neuroinflammation is detrimental and may lead to neuronal damage. It has been recently suggested that glial activation plays an important role in the initiation of early response and in the progression of degenerative diseases, such as PD, in which α-synuclein accumulation in astrocytes causes recruitment of phagocytic microglia that attack selected neurons causing neuronal degeneration (Halliday and Stevens 2011). Furthermore, not only can neuroinflammation promote neurodegeneration, but neuronal death itself can trigger inflammatory responses, which may aggravate the ongoing neuronal damage (Litteljohn et al. 2010).

Both microglia and astrocytes express A_{2A} receptors, the stimulation of which promotes the recruitment of these cells in neuroinflammatory responses (Lopes et al. 2011). Conversely, blockade of A_{2A} receptors suppresses the activation of microglia and astrocytes, as well as the stimulation of the proinflammatory signal cascade (Armentero et al. 2011). Interestingly, recent evidence obtained in the mouse MPTP model of PD demonstrates that the increased survival of dopaminergic neurons mediated by A_{2A} antagonists is accompanied by a significant attenuation in microgliosis and astrogliosis (Yu et al. 2008; Carta et al. 2009; Frau et al. 2011).

Based on these premises and on the current knowledge of A_{2A} antagonists and neuroprotection, it is feasible that attenuation of glutamate-mediated excitotoxicity and neuroinflammation may participate in the protective effects of A_{2A} antagonists on dopaminergic neurons. However, it is important to consider that in experimental models of PD, adenosine A_{2A} receptor antagonists counteract the degeneration of dopaminergic neurons at doses lower than those required to elicit motor stimulation (Yu et al. 2008; Carta et al. 2009). It is therefore hypothesized that excitotoxicity and neuroinflammation are phenomena clearly distinct from regulation of motor function, as for the mechanisms involved. Furthermore, excitotoxicity and neuroinflammation have been envisaged as causative factors in neurodegenerative disorders other than PD (Glass et al. 2010). Therefore, an attenuation, or regulation, of these noxious phenomena by A_{2A} antagonists, besides justifying the protective effect observed in PD models, would be in agreement with the data demonstrating that these drugs can protect neurons from a wide array of insults.

Even though the hypotheses on excitotoxicity and neuroinflammation are intriguing, caution should be taken when addressing the mechanisms underlying the neuroprotection mediated by A_{2A} antagonists in models of PD. In this connection, it is interesting to point out that astrocytes, the activation of which has been found to be increased in the MPTP model of PD (Carta et al. 2009), regulate not only neuroinflammation but also glutamate extracellular levels and, accordingly, excitotoxicity (Melani et al. 2003). Therefore, astrocytes could be a crucial point at

which A_{2A} antagonists may promote the survival of dopaminergic neurons, perhaps by attenuating both excitotoxicity and neuroinflammation. Even though the evidence supporting the involvement of glial elements in the dopaminergic neuroprotection by A_{2A} antagonists is increasingly growing, neuronal mechanisms cannot be disregarded. Thus, recent evidence demonstrates that the inactivation of neuronal forebrain A_{2A} receptors completely counteracts the degeneration of dopaminergic neurons in the MPTP model of PD (Carta et al. 2009) (Fig. 2). Furthermore, it has to be considered that a number of factors have been proposed to promote and/or sustain the degeneration of dopaminergic neurons in PD, besides excitotoxicity and neuroinflammation. These include, for example, oxidative stress, proteolytic damage, and mitochondrial defects (Schapira 2006). Therefore, it is conceivable that several different mechanisms may participate in the rescue of dopaminergic transmission by A_{2A} antagonists. In this connection, it is interesting to note that A_{2A} antagonists have been reported to protect dopaminergic neurons in a model of mitochondrial dysfunction (Alfinito et al. 2003). In addition, it has been suggested that the different causative factors of PD neurotoxicity may engender a sort of vicious cycle, with some triggering initial neuronal damage and others perpetuating it until neuronal death is reached. Therefore, A_{2A} antagonists could intervene at different steps of this cycle and, by interrupting it, afford neuroprotection.

To date, blockade of adenosine A_{2A} receptors has been demonstrated to efficiently restore motor function in PD, without exacerbating dyskinesia, both in animal models and in clinical trials of PD patients (Hauser and Schwarzschild 2005; Schwarzschild et al. 2006; Morelli et al. 2010). Therefore, their role as disease-modifying drugs in PD is of specific relevance since A_{2A} antagonists may unequivocally affect PD with regard to both symptoms and neuroprotection.

7 Conclusion

Caffeine and adenosine A_{2A} receptor antagonists have given new impetus to the research on PD in the latest 10 years. In the absence of effective neuroprotective treatments for PD, epidemiologic studies investigating dietary factors, such as caffeine, that may allow individuals to lower the risk of PD became compelling. Remarkably, preclinical studies have shown that the neuroprotective potential of caffeine extends to chronic administration of pesticides (Kachroo et al. 2010), suggesting that caffeine can prevent degeneration of dopaminergic neurons induced by a broad range of agents, including environmental toxins, in addition to pathophysiologic mechanisms.

Moreover, the preclinical and clinical investigations suggesting that A_{2A} receptor antagonists represent a class of drugs that might counteract motor deficits symptomatically and, at the same time, delay or halt the progression of dopaminergic neuron degeneration are very important for future delineation of effective therapies for this disease. It should, however, be underlined that the

discovery of neuroprotective strategies in chronic neurodegenerative diseases, such as PD, largely depends on the possibility of monitoring the progression of the neurodegeneration. The availability of biomarkers associated with disease progression therefore becomes crucial for the advancement of this strategy. Although the research on biomarkers is very promising (Halperin et al. 2009), none of them can predict PD with 100 % confidence nor provide a clear definition of subgroups at risk. Therefore, the future of research in PD clearly depends on the possibility of developing reliable and affordable biomarkers in order to facilitate and render the clinical trials of new molecules consistent.

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References

Aguiar, L. M., Nobre, H. V., Jr., Macêdo, D. S., Oliveira, A. A., Freitas, R. M., Vasconcelos, S. M., Cunha, G. M., Sousa, F. C., & Viana, G. S. (2006). Neuroprotective effects of caffeine in the model of 6-hydroxydopamine lesion in rats. *Pharmacology, Biochemistry, and Behavior, 84*, 415–419.

Alfinito, P. D., Wang, S. P., Manzino, L., Rijhsinghani, S., Zeevalk, G. D., & Sonsalla, P. K. (2003). Adenosinergic protection of dopaminergic and GABAergic neurons against mitochondrial inhibition through receptors located in the substantia nigra and striatum, respectively. *Journal of Neuroscience, 23*, 10982–10987.

Armentero, M. T., Fancellu, R., Nappi, G., Bramanti, P., & Blandini, F. (2006). Prolonged blockade of NMDA or mGluR5 glutamate receptors reduces nigrostriatal degeneration while inducing selective metabolic changes in the basal ganglia circuitry in a rodent model of Parkinson's disease. *Neurobiology of Disease, 22*, 1–9.

Armentero, M. T., Pinna, A., Ferré, S., Lanciego, J. L., Müller, C. E., & Franco, R. (2011). Past, present and future of A(2A) adenosine receptor antagonists in the therapy of Parkinson's disease. *Pharmacology and Therapeutics, 132*, 280–299.

Ascherio, A., Zhang, S. M., Hernán, M. A., Kawachi, I., Colditz, G. A., Speizer, F. E., & Willett, W. C. (2001). Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. *Annals of Neurology, 50*, 56–63.

Ascherio, A., Chen, H., Schwarzschild, M. A., Zhang, S. M., Colditz, G. A., & Speizer, F. E. (2003). Caffeine, postmenopausal estrogen, and risk of Parkinson's disease. *Neurology, 60*, 790–795.

Ascherio, A., Weisskopf, M. G., O'Reilly, E. J., McCullough, M. L., Calle, E. E., Rodriguez, C., & Thun, M. J. (2004). Coffee consumption, gender, and Parkinson's disease mortality in the cancer prevention study II cohort: The modifying effects of estrogen. *American Journal of Epidemiology, 160*, 977–984.

Benedetti, M. D., Bower, J. H., Maraganore, D. M., McDonnell, S. K., Peterson, B. J., Ahlskog, J. E., Schaid, D. J., & Rocca, W. A. (2000). Smoking, alcohol, and coffee consumption preceding Parkinson's disease: A case–control study. *Neurology, 55*, 1350–1358.

Blandini, F., Nappi, G., & Greenamyre, J. T. (2001). Subthalamic infusion of an NMDA antagonist prevents basal ganglia metabolic changes and nigral degeneration in a rodent model of Parkinson's disease. *Annals of Neurology, 49*, 525–529.

Bogenpohl, J. W., Ritter, S. L., Hall, R. A., & Smith, Y. (2012). Adenosine A(2A) receptor in the monkey basal ganglia: Ultrastructural localization and colocalization with the metabotropic glutamate receptor 5 in the striatum. *The Journal of Comparative Neurology, 520*, 570–589.

Brothers, H. M., Marchalant, Y., & Wenk, G. L. (2010). Caffeine attenuates lipopolysaccharide-induced neuroinflammation. *Neuroscience Letters, 480*, 97–100.

Carta, A. R., Kachroo, A., Schintu, N., Xu, K., Schwarzschild, M. A., Wardas, J., & Morelli, M. (2009). Inactivation of neuronal forebrain a receptors protects dopaminergic neurons in a mouse model of Parkinson's disease. *Journal of Neurochemistry, 111*, 1478–1489.

Carvalho, G. A., & Nikkhah, G. (2001). Subthalamic nucleus lesions are neuroprotective against terminal 6-OHDA-induced striatal lesions and restore postural balancing reactions. *Experimental Neurology, 171*, 405–417.

Cauli, O., & Morelli, M. (2005). Caffeine and the dopaminergic system. *Behavioural Pharmacology, 16*, 63–77.

Chen, J. F., Xu, K., Petzer, J. P., Staal, R., Xu, Y. H., Beilstein, M., Sonsalla, P. K., Castagnoli, K., Castagnoli, N., Jr., & Schwarzschild, M. A. (2001). Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson's disease. *Journal of Neuroscience, 21*, RC143.

Costa, J., Lunet, N., Santos, C., Santos, J., & Vaz-Carneiro, A. (2010). Caffeine exposure and the risk of Parkinson's disease: A systematic review and meta-analysis of observational studies. *Journal of Alzheimer's Disease, 20*, S221–S238.

Cunha, R. A. (2001). Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: Different roles, different sources and different receptors. *Neurochemistry International, 38*, 107–125.

de Rijk, M. C., Launer, L. J., Berger, K., Breteler, M. M., Dartigues, J. F., Baldereschi, M., Fratiglioni, L., Lobo, A., Martinez-Lage, J., Trenkwalder, C., & Hofman, A. (2000). Prevalence of Parkinson's disease in Europe: A collaborative study of population-based cohorts. Neurologic diseases in the elderly research group. *Neurology, 54*, S21–S23.

Delle Donne, K. T., & Sonsalla, P. K. (1994). Protection against methamphetamine-induced neurotoxicity to neostriatal dopaminergic neurons by adenosine receptor activation. *Journal of Pharmacology and Experimental Therapeutics, 271*, 1320–1326.

Dunwiddie, T. V., & Masino, S. A. (2001). The role and regulation of adenosine in the central nervous system. *Annual Review of Neuroscience, 24*, 31–55.

Ferre, S., Fredholm, B. B., Morelli, M., Popoli, P., & Fuxe, K. (1997). Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. *Trends in Neurosciences, 20*, 482–487.

Floran, B., Gonzalez, B., Florán, L., Erlij, D., & Aceves, J. (2005). Interactions between adenosine A(2A) and dopamine D2 receptors in the control of [(3)H]GABA release in the globus pallidus of the rat. *European Journal of Pharmacology, 520*, 43–50.

Frau, L., Borsini, F., Wardas, J., Khairnar, A. S., Schintu, N., & Morelli, M. (2011). Neuroprotective and anti-inflammatory effects of the adenosine A(2A) receptor antagonist ST1535 in a MPTP mouse model of Parkinson's disease. *Synapse, 65*, 181–188.

Fredholm, B. B., Bättig, K., Holmén, J., Nehlig, A., & Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews, 51*, 83–133.

Fredholm, B. B., Chen, J. F., Cunha, R. A., Svenningsson, P., & Vaugeois, J. M. (2005). Adenosine and brain function. *International Review of Neurobiology, 63*, 191–270.

Gerevich, Z., Wirkner, K., & Illes, P. (2002). Adenosine A2A receptors inhibit the N-methyl-D-aspartate component of excitatory synaptic currents in rat striatal neurons. *European Journal of Pharmacology, 451*, 161–164.

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., & Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell, 140*, 918–934.

Green, T. A., & Schenk, S. (2002). Dopaminergic mechanism for caffeine-produced cocaine seeking in rats. *Neuropsychopharmacology, 26*, 422–430.

Greenamyre, J. T. (2001). Glutamatergic influences on the basal ganglia. *Clinical Neuropharmacology, 24*, 65–70.

Halliday, G. M., & Stevens, C. H. (2011). Glia: Initiators and progressors of pathology in Parkinson's disease. *Movement Disorders, 26*, 6–17.

Halperin, I., Morelli, M., Korczyn, A. D., Youdim, M. B., & Mandel, S. A. (2009). Biomarkers for evaluation of clinical efficacy of multipotential neuroprotective drugs for Alzheimer's and Parkinson's diseases. *Neurotherapeutics, 6*, 128–140.

Hauser, R. A., & Schwarzschild, M. A. (2005). Adenosine A(2A) receptor antagonists for Parkinson's disease: Rationale, therapeutic potential and clinical experience. *Drugs & Aging, 22*, 471–482.

Hettinger, B. D., Lee, A., Linden, J., & Rosin, D. L. (2001). Ultrastructural localization of adenosine A2A receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. *The Journal of Comparative Neurology, 431*, 331–346.

Hirsch, E. C., & Hunot, S. (2009). Neuroinflammation in Parkinson's disease: A target for neuroprotection? *Lancet Neurology, 8*, 382–397.

Hu, G., Bidel, S., Jousilahti, P., Antikainen, R., & Tuomilehto, J. (2007). Coffee and tea consumption and the risk of Parkinson's disease. *Movement Disorders, 22*, 2242–2248.

Jellinger, K. A. (1991). Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. *Molecular and Chemical Neuropathology, 14*, 153–197.

Jones, C. K., Bubser, M., Thompson, A. D., Dickerson, J. W., Turle-Lorenzo, N., Amalric, M., Blobaum, A. L., Bridges, T. M., Morrison, R. D., Jadhav, S., Engers, D. W., Italiano, K., Bode, J., Daniels, J. S., Lindsley, C. W., Hopkins, C. R., Conn, P. J., & Niswender, C. M. (2012). The metabotropic glutamate receptor 4-positive allosteric modulator VU0364770 produces efficacy alone and in combination with L-DOPA or an adenosine 2A antagonist in preclinical rodent models of Parkinson's disease. *Journal of Pharmacology and Experimental Therapeutics, 340*, 404–421.

Kachroo, A., Irizarry, M. C., & Schwarzschild, M. A. (2010). Caffeine protects against combined paraquat and maneb-induced dopaminergic neuron degeneration. *Experimental Neurology, 223*, 657–661.

Kelsey, J. E., Langelier, N. A., Oriel, B. S., & Reedy, C. (2009). The effects of systemic, intrastriatal, and intrapallidal injections of caffeine and systemic injections of A(2A) and A(1) antagonists on forepaw stepping in the unilateral 6-OHDA-lesioned rat. *Psychopharmacology, 201*, 529–539.

Khairnar, A., Plumitallo, A., Frau, L., Schintu, N., & Morelli, M. (2010). Caffeine enhances astroglia and microglia reactivity induced by 3,4-methylenedioxymethamphetamine ('ecstasy') in mouse brain. *Neurotoxicity Research, 17*, 435–439.

Kurokawa, M., Koga, K., Kase, H., Nakamura, J., & Kuwana, Y. (1996). Adenosine A(2A) receptor-mediated modulation of striatal acetylcholine release in vivo. *Journal of Neurochemistry, 66*, 1882–1888.

Lancelot, E., & Beal, M. F. (1998). Glutamate toxicity in chronic neurodegenerative disease. *Progress in Brain Research, 116*, 331–347.

Litteljohn, D., Mangano, E., Clarke, M., Bobyn, J., Moloney, K., & Hayley, S. (2010). Inflammatory mechanisms of neurodegeneration in toxin-based models of Parkinson's disease. *Parkinsons Dis. 30*, 2011:713517.

Lopes, L. V., Sebastião, A. M., & Ribeiro, J. A. (2011). Adenosine and related drugs in brain diseases: Present and future in clinical trials. *Current Topics in Medicinal Chemistry, 11*, 1087–1101.

Łukasiewicz, S., Błasiak, E., Faron-Górecka, A., Polit, A., Tworzydło, M., Górecki, A., Wasylewski, Z., & Dziedzicka-Wasylewska, M. (2007). Fluorescence studies of homooligomerization of adenosine A(2A) and serotonin 5-HT(1A) receptors reveal the specificity of receptor interactions in the plasma membrane. *Pharmacological Reports, 59*, 379–392.

Melani, A., Pantoni, L., Bordoni, F., Gianfriddo, M., Bianchi, L., Vannucchi, M. G., Bertorelli, R., Monopoli, A., & Pedata, F. (2003). The selective A(2A) receptor antagonist SCH 58261

reduces striatal transmitter outflow, turning behavior and ischemic brain damage induced by permanent focal ischemia in the rat. *Brain Research, 959*, 243–250.

Mitchell, H. L., Frisella, W. A., Brooker, R. W., & Yoon, K. W. (1995). Attenuation of traumatic cell death by an adenosine A(1) agonist in rat hippocampal cells. *Neurosurgery, 36*, 1003–1008.

Morelli, M., Carta, A. R., Kachroo, A., & Schwarzschild, M. A. (2010). Pathophysiological roles for purines: Adenosine, caffeine and urate. *Progress in Brain Research, 183*, 183–208.

Nakaso, K., Ito, S., & Nakashima, K. (2008). Caffeine activates the PI3K/Akt pathway and prevents apoptotic cell death in a Parkinson's disease model of SH-SY5Y cells. *Neuroscience Letters, 432*, 146–150.

Nobre, H. V., Jr., Cunha, G. M., de Vasconcelos, L. M., Magalhães, H. I., Oliveira Neto, R. N., Maia, F. D., de Moraes, M. O., Leal, L. K., & Viana, G. S. (2010). Caffeine and CSC, adenosine A(2A) antagonists, offer neuroprotection against 6-OHDA-induced neurotoxicity in rat mesencephalic cells. *Neurochemistry International, 56*, 51–58.

Peterson, J. D., Goldberg, J. A., & Surmeier, D. J. (2012). Adenosine A(2A) receptor antagonists attenuate striatal adaptations following dopamine depletion. *Neurobiology of Disease, 45*, 409–416.

Phillips, A. G., Vacca, G., & Ahn, S. (2008). A top-down perspective on dopamine, motivation and memory. *Pharmacology, Biochemistry, and Behavior, 90*, 236–249.

Piallat, B., Benazzouz, A., & Benabid, A. L. (1996). Subthalamic nucleus lesion in rats prevents dopaminergic nigral neuron degeneration after striatal 6-OHDA injection: Behavioural and immunohistochemical studies. *European Journal of Neuroscience, 8*, 1408–1414.

Poewe, W. (2008). Non-motor symptoms in Parkinson's disease. *European Journal of Neurology, 15*, 14–20.

Popoli, P., Betto, P., Reggio, R., & Ricciarello, G. (1995). Adenosine A(2A) receptor stimulation enhances striatal extracellular glutamate levels in rats. *European Journal of Pharmacology, 287*, 215–217.

Reale, M., Iarlori, C., Thomas, A., Gambi, D., Perfetti, B., Di Nicola, M., & Onofrj, M. (2009). Peripheral cytokines profile in Parkinson's disease. *Brain, Behavior, and Immunity, 23*, 55–63.

Robbins, T. W., & Roberts, A. C. (2007). Differential regulation of fronto-executive function by the monoamines and acetylcholine. *Cerebral Cortex, 17*, 151–160.

Ross, G. W., Abbott, R. D., Petrovitch, H., Morens, D. M., Grandinetti, A., Tung, K. H., Tanner, C. M., Masaki, K. H., Blanchette, P. L., Curb, J. D., Popper, J. S., & White, L. R. (2000). Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA : The Journal of the American Medical Association, 283*, 2674–2679.

Sääksjärvi, K., Knekt, P., Rissanen, H., Laaksonen, M. A., Reunanen, A., & Männistö, S. (2008). Prospective study of coffee consumption and risk of Parkinson's disease. *European Journal of Clinical Nutrition, 62*, 908–915.

Schapira, A. H. (2006). Etiology of Parkinson's disease. *Neurology, 66*, S10–S23.

Schiffmann, S. N., & Vanderhaeghen, J. J. (1993). Adenosine A(2) receptors regulate the gene expression of striatopallidal and striatonigral neurons. *Journal of Neuroscience, 13*, 1080–1087.

Schwarzschild, M. A., & Ascherio, A. (2004). Caffeine and Parkinson's disease. In A. Nehlig (Ed.), *Coffee, tea, chocolate and the brain* (pp. 147–163). Boca Raton: CRC Press.

Schwarzschild, M. A., Chen, J. F., Tennis, M., Messing, S., Kamp, C., Ascherio, A., Holloway, R. G., Marek, K., Tanner, C. M., McDermott, M., Lang, A. E., & The Parkinson Study Group. (2003a). Relating caffeine consumption to Parkinson's disease progression and dyskinesias development. *Movement Disorders, 18*, 1082–1083.

Schwarzschild, M. A., Xu, K., Oztas, E., Petzer, J. P., Castagnoli, K., Castagnoli, N., Jr., & Chen, J. F. (2003b). Neuroprotection by caffeine and more specific A(2A) receptor antagonists in animal models of Parkinson's disease. *Neurology, 61*, S55–S61.

Schwarzschild, M. A., Agnati, L., Fuxe, K., Chen, J. F., & Morelli, M. (2006). Targeting adenosine A(2A) receptors in Parkinson's disease. *Trends in Neurosciences, 29*, 647–654.

Shindou, T., Richardson, P. J., Mori, A., Kase, H., & Ichimura, M. (2003). Adenosine modulates the striatal GABAergic inputs to the globus pallidus via adenosine A(2A) receptors in rats. *Neuroscience Letters, 352*, 167–170.

Simola, N., Fenu, S., Baraldi, P. G., Tabrizi, M. A., & Morelli, M. (2006). Involvement of globus pallidus in the antiparkinsonian effects of adenosine A(2A) receptor antagonists. *Experimental Neurology, 202*, 255–257.

Simon, D. K., Swearingen, C. J., Hauser, R. A., Trugman, J. M., Aminoff, M. J., Singer, C., Truong, D., Tilley, B. C., & Investigators, N. E. T.-D. (2008). Caffeine and progression of Parkinson disease. *Clinical Neuropharmacology, 31*, 189–196.

Stone, T. W., Ceruti, S., & Abbracchio, M. P. (2009). Adenosine receptors and neurological disease: Neuroprotection and neurodegeneration. *Handbook of Experimental Pharmacology, 193*, 535–587.

Xu, K., Xu, Y., Brown-Jermyn, D., Chen, J. F., Ascherio, A., Dluzen, D. E., & Schwarzschild, M. A. (2006). Estrogen prevents neuroprotection by caffeine in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *Journal of Neuroscience, 26*, 535–541.

Xu, K., Xu, Y. H., Chen, J. F., & Schwarzschild, M. A. (2010). Neuroprotection by caffeine: Time course and role of its metabolites in the MPTP model of Parkinson's disease. *Neuroscience, 167*, 475–481.

Yu, L., Shen, H. Y., Coelho, J. E., Araújo, I. M., Huang, Q. Y., Day, Y. J., Rebola, N., Canas, P. M., Rapp, E. K., Ferrara, J., Taylor, D., Müller, C. E., Linden, J., Cunha, R. A., & Chen, J. F. (2008). Adenosine A(2A) receptor antagonists exert motor and neuroprotective effects by distinct cellular mechanisms. *Annals of Neurology, 63*, 338–346.

Stem Cell Therapies for Age Associated Neurodegeneration

Stephanie Merchant, Sarah Stegeman, and Kiminobu Sugaya

Contents

Abstract

Regenerative medicine is the newly emerging field of this decade, namely, stem cell therapeutics. Interestingly, the field of stem cell therapeutics has been around for decades, such as the treatment of Leukemia using bone marrow transplantations; however, little momentum has been generated in the field until recently. During this predominantly stationary period of the regenerative movement, many issues have been debated regarding stem cell therapeutics. Many questions to these past issues are now starting to rise to the forefront due to breakthrough research being conducted in the regenerative field: adult versus embryonic stem cell populations, ethics, ease of access to these populations, and, more importantly, what types of diseased states can these stem cell populations be effective in? In order to answer this last question, it is imperative to

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understand not only basic stem cell pathologies but also the pathological conditions of the diseased state to be treated. Perhaps one of the most challenging to overcome is Alzheimer's disease (AD), a neurodegenerative condition characterized by a predominant loss of adult stem cell populations. What makes AD a challenge is the need to overcome both the natural effects of aging as well as the caustic environmental conditions. In order to introduce new neuronal networking through stem cell therapeutics, a better understanding of the pathological environment is needed. With new and upcoming technology, such as induced pluripotent stem cells, used to produce neural cells from somatic cells, and small molecular compounds which increase endogenous stem cell populations, it will be possible to perform autologous regenerative therapies, thus providing potential cures for age-related diseases such as AD.

List of Abbreviations

AD	Alzheimer's disease
APOE4	Apolipoprotein E-e4
APP	Amyloid precursor protein
AS	Adult stem cells
β-amyloid	Beta amyloid
BACE	Beta-site APP-cleaving enzyme
BBB	Blood–brain barrier
bFGF	Basic fibroblast growth factor
BMPs	Bone morphogenetic protein
CCg	Glycosylated form of cysteine C
CDC	Center for Disease Control
ChEIs	Cholinesterase inhibitors
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
DNA	Deoxyribonucleic acid
DS	Down syndrome
EGF	Epidermal growth factor
eNS	Endogenous neural stem cells
ES	Embryonic stem
FDA	Food and Drug Administration
GFAP	Glial fibrillary acidic protein
gp130	Glycoprotein 130
hNSC	Human neural stem cells
IGF-1	Insulin-like growth factor
IL-6	Interluekin-6
iPS	Induced pluripotent stem
JAK	Janus kinase
Klf4	Krüppel-like family of transcription factors
LIF	Leukemia inhibitory factor
MAPK	Mitogen-activated protein kinase
MS	Mesenchymal stem

NFT	Intracellular neurofibrillary tangles
Ngn-1, Ngn-2	Neurogenin 1 and 2
NP	Neural progenitor
NS	Neural stem
Oct 3/4	Octamer-binding transcription factor 3/4
PS-1, PS-2	Presenilin 1 and 2
RNA	Ribonucleic acid
sAPP	Secreted amyloid protein precursor
SCI	Spinal cord injury
Sox 2	Sex-determining region Y-box 2
STAT3	Signal transducer and activator of transcription 3
SVZ	Sub-ventricular zone
TUNEL assay	Terminal deoxynucleotidyl transferase dUTP nick-end labeling

1 Introduction

Aging is something that all living things must endure; however, how we approach aging as individuals can be vastly disparate due to genetics or environmental conditions. Each time cellular division occurs, telomeres, the protective noncoding deoxyribonucleic acid (DNA) sequences found at the ends of chromosomes on the 3' end, are shortened due to the replication mechanism of the lagging strand. Each cell can undergo a predetermined number of replications before telomeres have been exhausted, at which point the cell receives signaling to become senescent and die. This continual process of telomere shortening and cell death is commonly known as the process of aging.

In the 1980s, an enzyme was discovered that allowed cells to bypass cellular aging and divide without exhausting their telomeres. For the past two decades, researchers have toiled away at unlocking the secrets of this enzyme, telomerase, thought to be the modern-day fountain of youth. Telomerase is a reverse transcriptase containing its own template ribonucleic acid (RNA) molecule, allowing it to add additional telomeres to the ends of chromosomes. Somatic human cells have been found to be severely lacking in telomerase and continue to experience telomere shortening at each division, causing DNA damage and cell death. In contrast, stem cells, cells that are described as any cell that has the ability to renew itself and differentiate into different types of cells, were found to be rich in telomerase.

Due to their high levels of telomerase activity, stem cells are capable of dividing without experiencing telomere shortening. These unique properties allow the body to continue to thrive, as stem cells replace damaged or exhausted somatic cells throughout the body. However, not even stem cells are invincible to the aging process. As the body ages, stem cells become less active, and the rate of cellular turnover decreases. It is the current belief that if the stem cell population could be increased or reactivated, it would be possible to slow the effects of aging and even mitigate many age-related and degenerative diseases.

The limitless possibilities for the use of stem cells have sparked a new era in the field of regenerative medicine and have led the scientific community to recognize stem cell therapeutics as the key to unlocking future treatments for many disabling and currently incurable diseases. Companies and medical institutions around the globe have poured billions into the development of stem cell technologies and are now offering various stem cell-related products and therapies. Many of these technologies are currently in the process of clinical trials to seek approvals from officials, making it possible for treatments to be available in the European Union and United States in the near future.

2 Embryonic versus Adult Stem Cell Therapeutics

There are two prominent types of stem cells: embryonic stem (ES) cells, isolated from blastocysts, and adult stem (AS) cells, referring to more specialized stem cells present throughout the body after embryonic development. AS cells have been found in tissues and organs such as brain, skin, heart, liver, gut, testis, blood, and bone marrow. Over the past few decades, it has been widely debated as to which of these sources is the most effective for the development of safe and successful treatments.

ES cells are rapidly self-renewing cells capable of giving rise to any of the three germ layers. Their ability to be kept in culture indefinitely also makes them a valuable research and treatment tool. However, ES cells are only available 4–5 days postfertilization up until the end of embryogenesis (Thomson et al. 1998). The limited time for collection, as well as the availability of willing donors, increases both the difficulty and cost of production. In addition to challenges regarding the availability of this cell population, the use of ES cells has sparked many political debates regarding the ethics pertaining to the use of embryonic tissue, which some view to be the morally adverse destruction of human life. Furthermore, there are two very serious scientific concerns: the bodies' ability to recognize foreign materials – immunorejection can be just as lethal as the potential cure – as well as the presence of tumorigenesis, which occurs when ES cells are transplanted (Wiles and Johansson 1999; Yu et al. 2007).

In contrast, AS cells are more ethically accepted than ES cells since they are isolated from tissues, which does not involve termination of embryogenesis. They are readily available and can be isolated from sources such as umbilical cord, bone marrow, and peripheral blood, thus increasing cost-effectiveness. Also, it allows us to use a patient's own cells to for treatment, known as autologous therapy, which eliminates the concern of immunorejection. Since AS cells are already committed to becoming certain types of cells, there is no issue of tumorigenesis upon transplantation. However, their limited potency also poses a challenge in that easily available AS cells (bone marrow, blood, umbilical cord, etc.) are limited to the treatment of diseases within the scope of the tissue of origin, and cannot be used in instances such as neurological disorders. Nonetheless, many researchers feel that

the above concerns regarding the use of ES cells outweigh the benefits and that AS cells may be a much more viable choice for potential therapeutics.

Embryonic stem cells versus adult stem cells			
Embryonic stem cells		Adult stem cells	
Pro	Con	Pro	Con
Can give rise to all three germ layers	Available only from human blastocysts	Source of cells is more ethically accepted	Limited in its ability to differentiate into cell types
Fast self-renewal	Immunorejection	Easy maintenance	Slow self-renewal
Embryonic stem cells can be kept in culture indefinitely	Ethics issue on destruction of human life	Can be isolated from patients at any time	Limited expansion in culture
	Tumorigenesis	Easy access to the source of cells	
	Limiting funding for R&D	Autologous use of cells to eliminate immunorejection	
	Patent protection on hES cell lines in the USA by the University of the Wisconsin Alumni Research Foundation		

In order to bypass potency limitations of AS cells, researchers have developed induced pluripotent stem (iPS) cells, which are cells that have been reprogrammed to an embryonic-like state. In 2006, researchers used a retroviral vector to transfect mouse fibroblasts with four factors: Oct-3/4, Sox2, Klf4, and with the oncogene Myc. The results from this study showed that these cells represented the morphology of ES cells, as well as expressed ES marker genes, and showed that iPS cells can be directly generated from mouse fibroblasts in culture (Yamanaka 2008), (Meissner et al. 2007; Nakagawa et al. 2008; Okita et al. 2007; Takahashi and Yamanaka 2006). These results were replicated by several independent groups using human somatic cells (Takahashi and Yamanaka 2006; Park et al. 2008) and were also repeated using combinations of Nanog, Oct-4, Sox2, and Lin28 (Yu et al. 2007).

Although these studies represent a major breakthrough in the scientific community for the creation of patient- and disease-specific stem cells, the use of viral vectors and oncogenes in human cells has raised safety concerns. Also, the starting material used for these studies, skin blasts, is highly differentiated with a high level of DNA methylation. This may prevent certain gene expression and stop cells from becoming fully functional. In order to overcome this problem, alternative starting material with less DNA methylation, such as adult stem cells, which have less methylation and may be more flexible, needs to be explored. One such study used mesenchymal stem (MS) cells as the starting material for the induction of pluripotency. Not only was this study successful in creating MS cell-derived iPS

cells, but it was done using a single factor, Nanog, as well as a nonviral transfection method (Alvarez et al. 2010). In addition, this study went on to show that the generated iPS cells were capable of developing into cells exhibiting neural cell-like characteristics, demonstrating their ability to transdifferentiate (Alvarez et al. 2010).

These promising studies show incredible potential for the development of treatments for a limitless number of diseases, and with momentum continually building in the field of iPS research, it is not unreasonable to predict that iPS cells will soon be implemented in clinical therapeutic studies.

3 Alzheimer's Disease

Alzheimer's disease (AD) is a neurological disorder effecting the central nervous system characterized by the progressive loss of memory and intellectual functioning, typically becoming apparent after age 65 (Cruts and Van Broeckhoven 1998). AD is the most common type of dementia associated with aging, which is now the sixth leading cause of death for those 18 years of age and above and the fifth for those 65 and above. It is expected that 2.6–5.2 million Americans will be diagnosed with AD; if this current trend continues we will have over 16 million Americans diagnosed with AD by the year of 2050. The economic impact is projected to increase from 183 billion for 2011 to 1.1 trillion in 2050 (an executive summary progress report on the CDC Healthy Brain Initiative 2006–2011). As an incurable, progressive, and terminal disease with limited treatment options, this changes the status of what was once considered a rare disorder to a major medical disease that impacts a vast amount of both families and patients (Brookmeyer et al. 2011).

4 Alzheimer's Disease Pathology

As AD progresses, cell-to-cell communication is disrupted, ultimately leading to cell death and loss in brain mass. This devastating reduction of cells affects nearly all brain functions. Symptoms of AD may include memory loss which disrupts daily life, challenges in problem solving and planning, difficulty completing common daily tasks, confusion with time or place, difficulty interpreting spatial relationships and visual images, problems with speaking and writing, misplacing objects without the ability to retrace one's steps, poor judgment, withdrawal from social activities or work, and changes in mood and personality.

The mechanism behind AD is still greatly researched and debated. The most widely recognized theory for the pathology of this disease is the amyloid cascade hypothesis, which describes the hallmark formation of plaques. In this model, plaques are caused by the accumulation of beta amyloid (β-amyloid) between neurons. β-amyloid is a normally occurring, sticky protein fragment produced in the brain when it is snipped from amyloid precursor protein (APP) via sequential scission, the β-APP-cleaving enzyme (BACE) (Hussain et al. 1999; Lin et al. 2000;

Sinha et al. 1999; Vassar et al. 1999; Yan et al. 1999; Karran et al. 2011); however, in the AD brain, it is undigested and gathers into hard, insoluble plaques. Another widely studied hallmark of AD is the formation of tangles. Intracellular neurofibrillary tangles (NFT) occur when the tau protein, a highly soluble microtubule-associated protein, which aids in the formation of microtubules (Cleveland et al. 1977; Obulesu et al. 2011), is hyperphosphorylated. Hyperphosphorylated tau is incapable of binding to and stabilizing microtubules, and they therefore collapse, thus creating insoluble twisted fibers inside brain cells (Obulesu et al. 2011; Mandelkow et al. 2007; Larbig et al. 2007; Metcalfe and Figueiredo-Pereira 2010). Some researchers argue that these two hallmarks, plaques and tangles, may occur at the late-stage of the disease, and that they do not address the initial biochemical mechanisms of AD. Other researchers have focused on what drives the loss of connections between brain cells, disruption in cells signaling pathways, and inflammation as alternative possible pathways for AD.

Both risk and deterministic genes have been identified in AD. Four genes have been found to play a crucial role in the onset of Alzheimer's disease: amyloid precursor protein (APP), presenilin-1 (PS-1), presenilin-2 (PS-2), and apolipoprotein E-e4 (APOE4). One crucial pathological condition of AD is the amyloid beta deposition, plaques, in the brain. This occurs through both excess production and miscutting of amyloid beta (A4) precursor protein (APP).

5 Current Therapies for Alzheimer's Disease

There is currently no cure for Alzheimer's Disease; however, some strides have been made in slowing down the progression of the disease in the hopes that a viable treatment is on the horizon. Memory loss and confusion, the most debilitating symptoms of AD, are presently being treated using (drugs approved by the FDA) cholinesterase inhibitors (Aricept, Exelon, Razadyne, Cognex, and Namenda). Donepezil (brand name Aricept) is approved for all stages of AD and can present with side effects such as vomiting, nausea, increased bowel movement, and loss of appetite. Galantamine (brand name Razadyne), rivastigmine (brand name Exelon), and tacrine (brand name Cognex) have all been approved for AD presenting as mild to moderate progression. Symptoms for these vary from nausea, vomiting, increased bowel movement, loss of appetite, and possible liver damage. Memantine (brand name Namenda) is for AD presenting as moderate to severe in progression and has side effects such as headache, constipation, confusion, and dizziness. Even though these treatments help in slowing the progression of AD, it tends to equate to putting a Band-Aid on a gashing wound.

6 Stem Cell Replacement Strategies for Alzheimer's Disease

Studies conducted using neural stem (NS) cells isolated from both fetal and adult mammalian cells, (Doetsch et al. 1999; Gould et al. 1999) located within the central

nervous system (Johansson et al. 1999) (CNS), were proliferated in vitro using a variety of culture medium systems (Svendsen et al. 1998) then transplanted in already progressive AD environments with hopes of creating new neuronal connections. These culture systems are crucial in that they contain certain factors that are necessary for successful integration into the host CNS. There are currently established methods of maintaining and expanding NS cells in culture in a serum-free defined medium that contains basic fibroblast growth factor (bFGF) and the epidermal growth factor (EGF) (Brannen and Sugaya 2000), (Fricker et al. 1999). However, there are issues related to NS cells regarding their much shorter telomeres and that they express almost no telomerase after a certain number of passages (Ostenfeld et al. 2000). Other researchers have been successful in keeping these cells in culture long term while maintaining the ability to differentiate into cells that stain positive for beta III tubulin, a neuronal marker, and glial fibrillary acidic protein (GFAP), which is a marker for astrocytes, in neurobasal medium without the addition of exogenous factors. This suggests that these cells, once in the appropriate environment, are able to produce factors on their own. With these studies in mind we are able to expand NS cells in vitro in order to produce a well-defined source of transplantable material to continue investigations into stem cell replacement strategies.

One such study used NS cells that were expanded without exogenous mitogenic factors using serum-free media (Brannen and Sugaya 2000) then injected into the lateral ventricle of both mature (6-month-old) and aged (24-month-old) rats. Cognitive capabilities were tested using a Morris water maze 4 weeks posttransplantation. Results demonstrated a markedly significant increase in cognitive function of memory-impaired rats to that of a level of memory unimpaired or to a level considered normal for the animal's age. Postmortem evaluation by immunohistochemistry showed positive for both beta III tubulin and GFAP. Positively stained neurons were found in the bilateral cingulate, in the parietal cortexes, and in the hippocampus. Morphological identities associate with these locations indicate that the aged brain is still able to guide migration and differentiation patterns. Other researchers have tried to pre-differentiate cells into specific neuronal types in vitro and then transplant to a direct location; however, these efforts showed no host integration or increased cognition (Clarkson 2001). This could be due to the fact that fully differentiated cells are no longer able to migrate and, therefore, cannot successfully integrate into the system. Another issue could be the destruction of tissue produced by injecting cells into a direct location, which could cause an immune response resulting in the destruction of transplanted tissue. These discoveries promote that it is a key element to deliver undifferentiated versus differentiated cells into the brain ventricle instead of using a direct location and overall use of NS cells poses promising opportunities as a stem cell replacement strategy.

Another group used NS cell transplantation in order to reduce cerebral ischemia within the brain. Adult rats were given intracerebral hemorrhages by administering bacterial collagenase intrastriatally. In the study, the NS cell groups showed better function on the rotarod test – a performance test used to evaluate balance and coordination – two weeks posttransplantation and improvement 5 weeks

posttransplantation with the modified limb-placing test assessing sensorimotor integration of the limbs. These results suggest that NS cell transplantation can be a viable treatment format for the restoration of neurological defects (Jeong et al. 2003).

Chemicon, a company that was recently acquired by Millipore, developed two immortalized human neural progenitor cell lines, ReNcell VM and CX. ReNcell VM was derived from the ventral mesencephalon of the fetal brain, whereas CX comes from the cortical region of the fetal brain. Both cell lines share the ability to differentiate into neurons and glial cells along with the ability to continually reproduce themselves. The company further showed that the cells differentiated into high-level neurons and maintained their electrophysiology. With this in mind, these cells have the potential to become a therapeutic treatment for AD (Donato et al. 2007).

Other recent findings promote stroke-based therapies generated through self-repair of neurons. Induced middle cerebral artery occlusions led to the death of striatal neurons, which then triggered an increase in NS cell production through activation of the dormant NS cells already located in the sub-ventricular zone (SVZ). The newly premature neurons were able to migrate to the location of the lesions and repair it through the production of new neurons (Lindvall et al. 2004). The area of spinal cord injury (SCI) is another topic of interest in the research arena. One such group using isolated human fetal central nervous system (CNS)-derived stem cells grown as neurospheres were transplanted into immunodeficient (scid) mice. The cells were able to survive the transplantation, migrate, and differentiate with minimal GFAP-positive expression. Engraftment into the host was demonstrated through improved locomotor functioning. This provides promising results for hNSCs as a potential treatment for spinal cord injuries (Cummings et al. 2006).

As demonstrated by the abovementioned studies, it is possible the NS cells could provide a possible therapeutic approach to many diseases that currently have no productive or readily available cure. However, these studies are still in their infancy and will need much more elucidation before becoming an acceptable treatment choice and many challenges lay in its way.

7 Pharmacological Applications as a Neuroreplacement Strategy

In brain development, production of new nervous system cells occurs in early childhood and fetal stages; however, the adult brain also contains deposits of stem cells all the way into advanced years (Galli et al. 2003). Neurogenesis was first documented in the SVZ (Gould et al. 1999) and the dentate gyrus of the hippocampus (Eriksson et al. 1998; Kuhn et al. 1996; Gage 2000). Specific signaling induces mitogenic factors, such as EGF and bFGF, to direct the division of endogenous neural stem (eNS) cells located in these areas and move them towards differentiation and migration to their final destination. Plasticity of adult eNS cells has also been reported by numerous groups (Eriksson et al. 1998; van Praag et al. 2002).

In the normal brain, the rate of production of proliferating NS cells appears to be balanced by apoptosis, allowing for controlled modeling and maintenance of brain structures. Aging and stress may decrease the proliferation of NS cells (Kuhn et al. 1996), while neuronal production is upregulated in response to brain injury and ischemic diseases (Felling and Levison 2003), suggesting that eNS cells may play a role in repair as well as normal adaptive responses. When the mechanism of self-renewal and repair is no longer able to cope with the loss of neuronal mass, progression of neurodegenerative diseases occurs, leaving patients to suffer functional brain deterioration and behavior patterns ultimately leading to a decline in the quality of life. The rate of neurogenesis from eNS cells tends to rise or fall in response to physiological conditions, such as aging, diseases, and stress (Kuhn et al. 1996; Felling and Levison 2003; McEwen 2001; Jin et al. 2004), indicating that eNS cell proliferation may be controlled by certain intrinsic factors. Many researchers have isolated factors from the conditioned media of neural stem cells and tested these factors in various combinations. Fibroblast growth factor (FGF) and epidermal growth factor (EGF) have been implicated in neural migration, axon navigation, and synaptogenesis (Guillemot and Zimmer 2011) and have the ability to increase proliferation of neuronal precursor cells (Arsenijevic et al. 2001).

It stands to reason that the development of similar compounds that mimic these conditions would be a valuable tool.

Therefore, these factors have become a major pharmacological target. In one study, researchers purified an autocrine/paracrine cofactor, a glycosylated form of cystatin C (CCg), and combined it with the trophic factor, basic FGF (FGF-2), both in vitro and in vivo. The addition of both of these factors showed stimulated neurogenesis in the dentate gyrus, thus suggesting that neurogenesis may be controlled by both trophic factors and autocrine/paracrine cofactors, namely, CCg (Taupin et al. 2000). Similarly, another study showed that FGF or EGF alone cannot stimulate neurogenesis. In this study, researchers considered the early appearance of insulin-like growth factor (IGF-I) receptors during mouse striatum development and its role in NS cell regulation. Combinations of these three factors were used in order to study the action of IGF-I on striatal NS cell proliferation. The results demonstrated that proliferation was not induced in the presence of neither EGF nor FGF-2 alone; however, the addition of IGF-I resulted in large proliferative clusters of multipotent spheres. This data suggests that IGF-I plays a crucial role in the regulation of NSC activation in conjunction with FGF-2 and EGF (Arsenijevic et al. 2001).

Although these factors seem to play a pivotal role in neurogenesis, there is a major pitfall associated with this treatment course. Since factors are incapable of penetrating the blood–brain barrier (BBB), these types of pharmaceutical would need to be injected directly to the brain. An alternative method of treatment would involve the use of small molecules capable of crossing the BBB, such as antidepressants, shown to increase stem cell proliferation. In one study, human hippocampal cells from untreated subjects with major depressive disorder were used to test the effect of selective serotonin reuptake inhibitors and tricyclic antidepressants on increasing neural progenitor (NP) cells and proliferation in the human dentate gyrus. Results showed that these antidepressants were capable of increasing NP

cells in the anterior human dentate gyrus (Boldrini et al. 2009). Another study confirms these findings and reports that chronic treatment with antidepressants increased cell proliferation in the hippocampus of a rodent model (Malberg et al. 2000). Although these finding may be significant for the treatment of depression, the increase of cell proliferation will most likely not be significant enough for the treatment of neurodegenerative diseases such as AD.

One such small molecular compound, a pyrrolopyrimidine (MS-818) (Itoh et al. 1999), may be a promising target for increasing stem cell proliferation. This compound was initially identified for its ability to promote neurite outgrowth in isolated neurons maintained in vitro (Awaya et al. 1993). In another cell culture study using neurons isolated from rodent cortex, MS-818 was reported to be active primarily in the presence of various growth factors, including bFGF, nerve growth factor (NGF), EGF, and insulin-like growth factor (IGF-1) (Sanjo et al. 1998; Torigoe and Awaya 1998; Sugiyama et al. 2002; Watanabe et al. 1998). The mechanism of action of MS-818 is not known, although some evidence implies the activation of the MAPK (mitogen-activated protein kinase) pathway, a cascade that is also activated by peptide growth factors, may play a key role (Sanjo et al. 1998). In the same report, it was suggested that MS-818 promoted the survival of rodent cortical neurons by reducing the rate of apoptosis, as measured by TUNEL assays. MS-818 has been tested in isolated animal models, axon growth in mice (Torigoe and Awaya 1998), and muscle regeneration in rats (Sugiyama et al. 2002), with evidence of but no mechanistic details relating to function.

Recent studies have shown that MS-818 induces the proliferation of endogenous NSCs, which may explain mechanism of this compound's function (Sugaya 2005; Sugaya and Merchant 2008). In these studies, they have shown that MS-818 increased NS cell population 600 % in the cortex of aged rodent models. It was also found that MS-818 treatment improved behavior of Parkinson's disease model in rotarod test with association of increased proliferation of NS cells. Although toxicity studies using MS-818 have not been reported, the compound was not found to have obvious side effects in rodents in these studies, and even at the high dose, it may be a good candidate for a pharmacological treatment of AD (Sugaya et al. 2007, 2006; Kwak et al. 2006a).

8 Obstacles for Neuroregeneration Therapy for Alzheimer's Disease

The ability to increase eNS cells populations via a small molecular compound, MS-818, as well as transplanted NS cells in order to recover cognitive function in aged rats has been demonstrated. However, we may need to consider that AD pathologies may affect NS cells and reduce the efficacy of these neuroregenerative treatments. Over the past decade, scientists have uncovered much about AD pathologies such as the APP and tau defects, along with other related gene mutations, but how these modify the AD environment needs to be elucidated in order to the successful stem cell therapy of this disease.

One such example of the challenges associated with the diseased environment of the AD brain is induction of glial differentiation of NS cells as a result of high concentration of APP in the environment. In a recent study, NS cells were transplanted in the brain of APP transgenic mice where human APP is highly expressed to find whether NS cell can be used to treat AD. The hope of this study was that the transplanted cells would differentiate into functional neurons. However, this research lead to the conclusion that the high concentration of APP induced glial differentiation of NS cells instead of the much needed neuronal differentiation (Marutle et al. 2007; Kwak et al. 2011, 2010, 2006b, 2006c; Keilani and Sugaya 2008). It has shown that recombinant secreted amyloid precursor protein (sAPP) dose dependently differentiated NS cells into astrocytes.

A further study was conducted in order to elucidate the pathway of APP induced glial differentiation through the activation of interleukin-6 (IL-6) and glycoprotein (gp130) signaling pathways, which are associated with inflammation. Gliogenesis, the formation of glial populations derived from multipotent neural stem cells, is modulated through certain external factors: leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), bone morphogenetic protein (BMPs), and Notch along with integral factors neurogenin 1 (Ngn1), neurogenin (Ngn2), and deoxyribonucleic acid (DNA) methylation. It has been revealed that all of these above factors are responsible for glial differentiation through the activation of the IL-6/gp 130 signaling pathway. Ligands, LIF and CNTF, bind to receptors and subsequently phosphorylates gp130, Janus kinase (JAK), and signal transducer and activator of transcription 3 (STAT3) pathways. STAT3 then translocates into the nucleus in order to stimulate gene expression of the target gene such as glial fibrillary acidic protein (GFAP). The IL-6/gp130 signaling pathway is modulated by an autoregulatory loop which eventually coordinates with other pathways related to glial differentiation, such as Notch and BMP {Kwak, 2010 #1990}. This helps to confirm previous studies showing that sAPPα in high concentrations works as a gliogenic factor in stem cell differentiation.

The APP gene is located on chromosome 21. Trisomy of chromosome 21 is responsible for most cases of Down syndrome (DS). DS patients have shown AD symptoms as early as 40 years of age (Schnabel 2011); this may be due to the extra copy of chromosome 21, which produces an extra copy of APP gene also. Svendsen (Svendsen et al. 2001) found that DS patient's do not produce normal level of neurons and even those that are produced, the morphology is altered (Sugaya et al. 2007; Schnabel 2011; Svendsen et al. 2001; Svendsen and Caldwell 2000). When APP genes are overexpressed in the normal NS cells, they show exactly the same, less production of neurons and altered morphology.

One of the symptoms in aging and AD is the loss of AS cells, for it is this cell population that will ultimately be needed to replace neuronal functioning within the AD brain. In a healthy individual, AS cells will remain quiescent within the body in a nondividing state until they are called upon to perform duties for the purpose of tissue maintenance, disease-related repairs, or when injury has occurred. The slow exhaustion of these cells over an extended time course may lead to the natural symptomatic aging process; however, under diseased conditions such as AD, the number of AS cell may decline more rapidly than normal aging. Abovementioned

APP functions to induce glial differentiation of NS cells and induce premature differentiation of NS cell population in the AD or DC. Thus, regulation of APP level in the AD may be a useful prevention therapy as well.

9 (+)-Phenserine's Ability to Overcome Pathological Effect of Alzheimer's Disease

Before treatment for AD can begin, modification must be made to the environment which will allow for the differentiation of neuronal cells. The challenge is in discovering how to go about this without disrupting other normal functioning. There are several cholinesterase inhibitors (ChEIs) currently available for the treatment of mild to moderate AD (e.g., Donepezil, Galantamine, and rivastigmine). These ChEIs have been found to improve memory, thinking, language, and judgment through the increase of acetylcholine levels in the brain. It is believed that this increase of acetylcholine may increase cognition by compensating for loss of functioning brain cells as stated by an Alzheimer's Association report regarding FDA-approved treatments for Alzheimer's disease. Additionally, ChEIs have been found to regulate APP levels. (−)-Phenserine is one such inhibitor known to decrease levels of APP. However, the dosing of (−)-phenserine has presented a challenge. In order to suppress the appropriate amount of APP and Aβ levels, the concentration of (−)-phenserine must be significantly increased. At such high concentrations, (−)-phenserine has been known to produce severe side effects, such as increased production of acetylcholine, resulting in the autonomic nervous system to become unbalanced. In order to efficiently regulate APP levels and bypass the side effects associated with (−)-phenserine, an isoform, (+)-phenserine, was developed. When using this isoform, it is possible to give the drug at the required doses without eliciting the associated side effects (Marutle et al. 2007).

10 Conclusion

Aging is the highest risk factor for AD. Although the role of stem cells in the adult remains unclear, reduction of the stem cell population associated with aging may significantly affect brain function. If the other factors are combined, such as a high concentration of APP or inflammation in the brain, the reduction of NS cells during the aging could be accelerated. Thus, augmentation of the stem cell population or transplantation of stem cells may help to find a cure for AD. However, we have to always consider the effect of the pathological condition of AD on stem cell biology as the mature neurons may not be able to incorporate into the host brain as functional cells. We have iPS cell technology to produce neural cells from somatic cells and small molecular compound which increases endogenous stem cell population, which allow us to perform autologous regenerative therapies. As our knowledge increases in clear understanding the pathological changes in AD brain, we will be able to fight against aging and AD.

References

Alvarez, A., et al. (2010). Nanog overexpression allows human mesenchymal stem cells to differentiate into neural cells. *Neuroscience & Medicine, 1*(1), 1–13.

Arsenijevic, Y., et al. (2001). Insulin-like growth factor-I is necessary for neural stem cell proliferation and demonstrates distinct actions of epidermal growth factor and fibroblast growth factor-2. *The Journal of Neuroscience, 21*(18), 7194–7202.

Awaya, A., et al. (1993). Neurotropic pyrimidine heterocyclic compounds. I. The newly synthesized pyrimidine compounds promote neurite outgrowth of GOTO and neuro 2a neuroblastoma cell lines, and potentiate nerve growth factor (NGF)-induced neurite sprouting of PC 12 cells. *Biological & Pharmaceutical Bulletin, 16*(3), 248–253.

Boldrini, M., et al. (2009). Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology, 34*(11), 2376–2389.

Brannen, C. L., & Sugaya, K. (2000). In vitro differentiation of multipotent human neural progenitors in serum-free medium. *Neuroreport, 11*(5), 1123–1128.

Brookmeyer, R., et al. (2011). National estimates of the prevalence of Alzheimer's disease in the United States. *Alzheimer's & Dementia, 7*(1), 61–73.

Clarkson, E. D. (2001). Fetal tissue transplantation for patients with Parkinson's disease: A database of published clinical results. *Drugs & Aging, 18*(10), 773–785.

Cleveland, D. W., Hwo, S. Y., & Kirschner, M. W. (1977). Physical and chemical properties of purified tau factor and the role of tau in microtubule assembly. *Journal of Molecular Biology, 116*(2), 227–247.

Cruts, M., & Van Broeckhoven, C. (1998). Presenilin mutations in Alzheimer's disease. *Human Mutation, 11*(3), 183–190.

Cummings, B. J., et al. (2006). Human neural stem cell differentiation following transplantation into spinal cord injured mice: Association with recovery of locomotor function. *Neurological Research, 28*(5), 474–481.

Doetsch, F., et al. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell, 97*(6), 703–716.

Donato, R., et al. (2007). Differential development of neuronal physiological responsiveness in two human neural stem cell lines. *BMC Neuroscience, 8*, 36.

Eriksson, P. S., et al. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine, 4*(11), 1313–1317.

Felling, R. J., & Levison, S. W. (2003). Enhanced neurogenesis following stroke. *Journal of Neuroscience Research, 73*(3), 277–283.

Fricker, R. A., et al. (1999). Site-specific migration and neuronal differentiation of human neural progenitor cells after transplantation in the adult rat brain. *The Journal of Neuroscience, 19*(14), 5990–6005.

Gage, F. H. (2000). Mammalian neural stem cells. *Science, 287*(5457), 1433–1438.

Galli, R., et al. (2003). Neural stem cells: An overview. *Circulation Research, 92*(6), 598–608.

Gould, E., et al. (1999). Hippocampal neurogenesis in adult old world primates. *Proceedings of the National Academy of Sciences of the United States of America, 96*(9), 5263–5267.

Guillemot, F., & Zimmer, C. (2011). From cradle to grave: The multiple roles of fibroblast growth factors in neural development. *Neuron, 71*(4), 574–588.

Hussain, I., et al. (1999). Identification of a novel aspartic protease (Asp 2) as beta-secretase. *Molecular and Cellular Neurosciences, 14*(6), 419–427.

Itoh, S., et al. (1999). The effect of neurotrophic pyrimidine heterocyclic compounds, MS-818 and MS-430, on the regeneration of injured peripheral nerves. *Restorative Neurology and Neuroscience, 14*(4), 265–273.

Jeong, S. W., et al. (2003). Human neural stem cell transplantation promotes functional recovery in rats with experimental intracerebral hemorrhage. *Stroke, 34*(9), 2258–2263.

Jin, K., et al. (2004). Increased hippocampal neurogenesis in Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America, 101*(1), 343–347.

Johansson, C. B., et al. (1999). Identification of a neural stem cell in the adult mammalian central nervous system. *Cell, 96*(1), 25–34.

Karran, E., Mercken, M., & De Strooper, B. (2011). The amyloid cascade hypothesis for Alzheimer's disease: An appraisal for the development of therapeutics. *Nature Reviews. Drug Discovery, 10*(9), 698–712.

Kuhn, H. G., Dickinson-Anson, H., & Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *The Journal of Neuroscience, 16*(6), 2027–2033.

Kwak, J. W., Kim, H. K., & Chae, C. B. (2006a). Potential lead for an Alzheimer drug: A peptide that blocks intermolecular interaction and amyloid beta protein-induced cytotoxicity. *Journal of Medicinal Chemistry, 49*(16), 4813–4817.

Kwak, Y. D., et al. (2006b). Amyloid precursor protein regulates differentiation of human neural stem cells. *Stem Cells and Development, 15*(3), 381–389.

Kwak, Y. D., Choumkina, E., & Sugaya, K. (2006c). Amyloid precursor protein is involved in staurosporine induced glial differentiation of neural progenitor cells. *Biochemical and Biophysical Research Communications, 344*(1), 431–437.

Kwak, Y. D., et al. (2011). Involvement of notch signaling pathway in amyloid precursor protein induced glial differentiation. *European Journal of Pharmacology, 650*(1), 18–27.

Kwak, Y. D., et al. (2010). Amyloid-beta precursor protein induces glial differentiation of neural progenitor cells by activation of the IL-6/gp130 signaling pathway. *Neurotoxicity Research, 18*(3–4), 328–338.

Keilani, S., & Sugaya, K. (2008). Reelin induces a radial glial phenotype in human neural progenitor cells by activation of notch-1. *BMC Developmental Biology, 8*, 69.

Larbig, G., et al. (2007). Screening for inhibitors of tau protein aggregation into Alzheimer paired helical filaments: A ligand based approach results in successful scaffold hopping. *Current Alzheimer Research, 4*(3), 315–323.

Lin, X., et al. (2000). Human aspartic protease memapsin 2 cleaves the beta-secretase site of beta-amyloid precursor protein. *Proceedings of the National Academy of Sciences of the United States of America, 97*(4), 1456–1460.

Lindvall, O., Kokaia, Z., & Martinez-Serrano, A. (2004). Stem cell therapy for human neurodegenerative disorders-how to make it work. *Nature Medicine, 10*(Suppl), S42–S50.

Malberg, J. E., et al. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *The Journal of Neuroscience, 20*(24), 9104–9110.

Mandelkow, E., et al. (2007). Structural principles of tau and the paired helical filaments of Alzheimer's disease. *Brain Pathology, 17*(1), 83–90.

Marutle, A., et al. (2007). Modulation of human neural stem cell differentiation in Alzheimer (APP23) transgenic mice by phenserine. *Proceedings of the National Academy of Sciences of the United States of America, 104*(30), 12506–12511.

McEwen, B. S. (2001). Plasticity of the hippocampus: Adaptation to chronic stress and allostatic load. *Annals of the New York Academy of Sciences, 933*, 265–277.

Meissner, A., Wernig, M., & Jaenisch, R. (2007). Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nature Biotechnology, 25*(10), 1177–1181.

Metcalfe, M. J., & Figueiredo-Pereira, M. E. (2010). Relationship between tau pathology and neuroinflammation in Alzheimer's disease. *The Mount Sinai Journal of Medicine, 77*(1), 50–58.

Nakagawa, M., et al. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nature Biotechnology, 26*(1), 101–106.

Obulesu, M., Venu, R., & Somashekhar, R. (2011). Tau mediated neurodegeneration: An insight into Alzheimer's disease pathology. *Neurochemical Research, 36*(8), 1329–1335.

Okita, K., Ichisaka, T., & Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. *Nature, 448*(7151), 313–317.

Ostenfeld, T., et al. (2000). Human neural precursor cells express low levels of telomerase in vitro and show diminishing cell proliferation with extensive axonal outgrowth following transplantation. *Experimental Neurology, 164*(1), 215–226.

Park, I. H., et al. (2008). Reprogramming of human somatic cells to pluripotency with defined factors. *Nature, 451*(7175), 141–146.

Sanjo, N., et al. (1998). A novel neurotrophic pyrimidine compound MS-818 enhances neurotrophic effects of basic fibroblast growth factor. *Journal of Neuroscience Research, 54*(5), 604–612.

Schnabel, J. (2011). Amyloid: Little proteins, big clues. *Nature, 475*(7355), S12–S14.

Sinha, S., et al. (1999). Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature, 402*(6761), 537–540.

Sugaya, K. (2005). Possible use of autologous stem cell therapies for Alzheimer's disease. *Current Alzheimer Research, 2*(3), 367–376.

Sugaya, K., et al. (2006). Stem cell strategies for Alzheimer's disease therapy. *Panminerva Medica, 48*(2), 87–96.

Sugaya, K., et al. (2007). Practical issues in stem cell therapy for Alzheimer's disease. *Current Alzheimer Research, 4*(4), 370–377.

Sugaya, K., & Merchant, S. (2008). How to approach Alzheimer's disease therapy using stem cell technologies. *Journal of Alzheimer's Disease, 15*(2), 241–254.

Sugiyama, N., et al. (2002). Acceleration by MS-818 of early muscle regeneration and enhanced muscle recovery after surgical transection. *Muscle & Nerve, 25*(2), 218–229.

Svendsen, C. N., et al. (1998). A new method for the rapid and long term growth of human neural precursor cells. *Journal of Neuroscience Methods, 85*(2), 141–152.

Svendsen, C. N., Bhattacharyya, A., & Tai, Y. T. (2001). Neurons from stem cells: Preventing an identity crisis. *Nature Reviews. Neuroscience, 2*(11), 831–834.

Svendsen, C. N., & Caldwell, M. A. (2000). Neural stem cells in the developing central nervous system: Implications for cell therapy through transplantation. *Progress in Brain Research, 127*, 13–34.

Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell, 126*(4), 663–676.

Taupin, P., et al. (2000). FGF-2-responsive neural stem cell proliferation requires CCg, a novel autocrine/paracrine cofactor. *Neuron, 28*(2), 385–397.

Thomson, J. A., et al. (1998). Embryonic stem cell lines derived from human blastocysts. *Science, 282*(5391), 1145–1147.

Torigoe, K., & Awaya, A. (1998). A newly synthesized neurotropic pyrimidine compound, MS-818, may activate migratory Schwann cells in peripheral nerve regeneration. *Brain Research, 787*(2), 337–340.

van Praag, H., et al. (2002). Functional neurogenesis in the adult hippocampus. *Nature, 415*(6875), 1030–1034.

Vassar, R., et al. (1999). Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science, 286*(5440), 735–741.

Yamanaka, S. (2008). Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. *Cell Proliferation, 41*(Suppl 1), 51–56.

Yan, R., et al. (1999). Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature, 402*(6761), 533–537.

Yu, J., et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science, 318*(5858), 1917–1920.

Watanabe, S., et al. (1998). A neurotrophic pyrimidine compound, MS-818, enhances EGF-induced restoration of gastric epithelial wounds in vitro. *Journal of Clinical Gastroenterology, 27*(Suppl 1), S105–S109.

Wiles, M. V., & Johansson, B. M. (1999). Embryonic stem cell development in a chemically defined medium. *Experimental Cell Research, 247*(1), 241–248.

Tardive Dyskinesia: Outcome of Antipsychotic Treatment and Brain Damage?

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Abstract

Tardive dyskinesia (TD), marked by abnormal involuntary movements and frequently expressed as perioral activity, represents an adverse outcome of prolonged antipsychotic therapy, occurring in approximately 5 % of patients per treatment year. Although neuronal mechanisms underlying TD are largely unknown, more recent experimental studies in animal models of TD are providing insight into the neuronal mechanisms associated with TD and implicating newer treatment approaches. It is now evident that a predominance in the ratio of dopamine (DA) D_1:D_2 receptor (R) activation accounts for induction of perioral movements in rodent models of TD, in nonhuman primate models of TD, and in humans with TD. Experimentally, TD is produced in animal models of TD, in a manner analogous to that by which TD is produced in humans – by continuous and prolonged administration of a DA D_2R antagonist (i.e., an antipsychotic drug). More recently, in a rodent model of TD, it has been shown that a lesion of dopaminergic – mainly nigroneostriatal – neurons reduces the time latency for occurrence of TD, also increases the severity of perioral activity, and results in permanence of TD after complete removal of D_2R antagonist treatment. The induction of perioral activity is related to DAR supersensitivity but unrelated to numbers of D_1R and D_2R in the neostriatum, a brain region associated with perioral activity. More apropos, serotoninergic systems are now recognized as having a greater role in effecting perioral activity, and it appears that 5-HT_{2C} receptor antagonists are most effective in abating perioral activity in a rodent model of TD. These processes and mechanisms, topics addressed in this chapter, highlight a newer understanding of mechanisms underlying TD and provide insight into new approaches towards treatment of TD in humans.

Keywords

5-HT_2 receptor • 5-Hydroxytryptamine • 5,7-Dihydroxytryptamine • 6-Hydroxydopamine • D_1 receptor • D_2 receptor • Dopamine • Haloperidol • Perioral movements • Receptor supersensitivity • Serotonin • Tardive dyskinesia • Vacuous chewing

1 Introduction

On the website of the National Institute of Neurological Disorders and Stroke (NINDS) (http://www.ninds.nih.gov/disorders/tardive/tardive.htm), tardive dyskinesia (TD) is classified as an extrapyramidal syndrome arising from long-term use of neuroleptic agents (i.e., antipsychotics). TD is characterized by repetitive involuntary purposeless movements of specific muscle groups which may include fingers, arms, legs, and trunk but generally presents as oral dyskinesia

(typically, chewing movements) which may include lip smacking and tongue protrusion. A person with TD may appear to be chewing gum.

TD arises in humans, nonhuman primates (Blanchet et al. 2012), and rodents by chronic (exclusive) treatment with a dopamine (DA) D_2 receptor (R) antagonist (Saifee and Edwards 2011) – the class of agents oft used for treating schizophrenia. The incidence rate of TD is approximately 5 % per year of treatment with a "classical" (first-generation) antipsychotic drug (e.g., haloperidol) (Kane et al. 1985) and two to threefold less so when an atypical antipsychotic agent (e.g., clozapine) is used (Tarsy et al. 2011). TD tends to be long lived, sometimes lifelong, and is usually refractory to treatment. TD remains a serious problem today. Tetrabenazine is the only approved drug for TD, but a variety of other drugs is often attempted.

2 Multineuronal Associations with Tardive Dyskinesia

Although TD arises from prolonged administration of a D_2R antagonist, it is recognized that many different neuronal phenotypes may have a role in the development or persistence of TD (Casey 1987; Egan et al. 1995; Jeste and Caligiuri 1993; Gong et al. 1992; Gunne and Häggström 1983; Knable et al. 1994; Kostrzewa 1995; Waddington 1990). Cholinergic (Kostrzewa and Neely 1993; Rupniak et al. 1983; 1985; Salamone et al. 1990), GABA-ergic (Gunne et al. 1988; Lloyd et al. 1985; Mithani et al. 1987; Tamminga et al. 1979), and serotoninergic (Gong and Kostrzewa 1992; Gong et al. 1992) involvements have been implicated in TD. Nevertheless, the focus in this chapter will remain on dopaminergic systems and the recently uncovered important role of serotoninergic systems in TD.

3 Oral Dyskinesia Arising in Rodents from Acute Dopamine Receptor Agonist or Antagonist Treatments

Acute administration of a DA D_2R antagonist to rats or mice results in the onset of oral dyskinesias, which are prominent over the following hour or more. The short-lived effect is related to the dose of the D_2R antagonist. Similarly, acute administration of a DA D_1R agonist is associated with a short-lived and dose-related induction of oral dyskinesia. These correlated findings give credence to the possibility that oral dyskinesia may be related to an imbalance in D_1R/D_2R activation by the endogenous neurotransmitter, DA (Rosengarten et al. 1983). Moreover, when a D_2R antagonist is administered chronically, as per animal modeling of TD, or when psychiatric patients are treated long term with an antipsychotic drug, one must consider the long-term effect of such treatment on the overall balance of D_1R/D_2R activation – and the effect this might have on the origin of TD.

3.1 Experimental Induction of Oral Dyskinesia in Rodents by Acute Dopamine D_1R Agonist Treatment or D_2R Antagonist Treatment

Antipsychotics and D_2R antagonists in general induce the occurrence of spontaneous oral activity – the so-called oral dyskinesias in humans and the so-called vacuous chewing movements in rodents (Clow et al. 1979; Iversen et al. 1980; Waddington and Gamble 1980). In contrast, DA D_1R agonists induce oral activity (Arnt et al. 1987; Koshikawa et al. 1987; Levin et al. 1989; Molloy and Waddington 1988; Murray and Waddington 1989; Rosengarten et al. 1983; 1986a, b; Rupniak et al. 1985), and it is the presumed balance in D_1R/D_2R activation that is considered to be important for this behavioral response (Rosengarten et al. 1983). For example, in rodents, there is an increase in oral activity when there is an increase in D_1R/D_2R activation, as occurs with D_2R antagonists, also after D_1R agonist treatment (Johansson et al. 1987; Molloy and Waddington 1987; 1988; Rosengarten et al. 1983), and in rat strains with reduced numbers of D_2R (Rosengarten et al. 1986a, b).

3.2 Effect of Neonatal Dopaminergic Denervation on Induction of Oral Dyskinesia in Rodents by Dopamine D_1R Agonists or D_2R Antagonists

When rats are lesioned perinatally with the neurotoxin 6-hydroxydopamine (6-OHDA; desipramine pretreatment), repeated D_1R agonist treatments "prime" DA D_1R. Initial D_1R agonist treatments have no marked effect, but repeated D_1R agonist treatments permanently sensitize D_1R, so that exaggerated behavioral responses are observed. This process is known as homologous priming of D_1R, and the resulting D_1R supersensitization is lifelong (Breese et al. (1985, 1987). Also, repeated D_2R agonist treatments of 6-OHDA-lesioned rats can induce the D_1R supersensitization, a process known as heterologous D_1R priming (Criswell et al. 1989).

When rats so-lesioned neonatally with 6-OHDA were challenged with a single dose of a D_2R antagonist, there was an increased in perioral movements when compared to the effect in non-lesioned control rats (Kostrzewa and Hamdi 1991). In an analogous manner the first challenge dose of a D_1R agonist to 6-OHDA-lesioned rats in adulthood surprisingly produced an increased number of perioral movements in comparison to non-lesioned control rats (Kostrzewa and Gong 1991). Thus, in contrast to the latent D_1R supersensitivity for locomotory and stereotyped activities that required unmasking by repeated D_1R agonist treatments (Breese et al. 1985; 1987), there was overt supersensitization of D_1R for D_1R agonist induction of perioral activity. Moreover, in the 6-OHDA-lesioned rats, perioral activity was induced by a D_1R agonist dose that was 100–1,000 times lower than was needed for induction of perioral activity in intact control rats (Kostrzewa and Gong 1991). The D_1R supersensitivity for this effect developed if the nigroneostriatal dopaminergic fiber lesioning by 6-OHDA occurred within the first week postbirth (Kostrzewa et al. 1993) and if neostriatal DA depletion was

98.5 % or more (Gong et al. 1993). This D_1R supersensitization was also lifelong. Moreover, the effect was unaccompanied by an increase in the B_{max} for D_1R (i.e., relative number of D_1R) or change in the K_d (i.e., relative affinity of the receptor for an agonist) (Gong et al. 1994).

Perinatal 6-OHDA treatment which produces near total destruction of nigroneostriatal dopaminergic neurons (Doucet et al. 1986) leaves the ventral tegmental nucleus largely intact and able to provide dopaminergic innervation to limbic regions (Snyder et al. 1986; Fernandes Xavier et al. 1994). Thus, the important element of the above studies is that a lesion of dopaminergic innervation to the neostriatum resulted in overt D_1R supersensitization for the induction of oral activity (Kostrzewa 1995; Kostrzewa et al. 1998, 2003, 2008).

4 Oral Dyskinesia Arising in Rodents from Acute Serotonin Agonist Treatment

When neonatally 6-OHDA-lesioned rats were challenged in adulthood with the serotonin (5-HT) 5-HT_2R agonist m-chlorophenylpiperazine (mCPP), the initial mCPP dose produced an enhanced increase in the number of perioral movements in comparison to that of intact control rats (Gong and Kostrzewa 1992). This finding and others indicate that 5-HT_2R is supersensitized by 6-OHDA lesioning (El Mansari et al. 1994). The effect was not replicated by 5-$HT_{1A}R$ agonist 8-OH-DPAT [(±)8-hydroxydipropylaminotetralin] or 5-$HT_{1B}R$ agonist CGS-12066B (7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo [1,2-alquinoxaline]); nor was the effect attenuated by 5-$HT_{1A/1B}R$ antagonist pindolol, nor by the predominate 5-$HT_{2A}R$ antagonist ketanserin, nor by 5-HT_3R antagonist MDL 72222 (3-tropanyl-3,4-dichlorobenzoate) (Gong et al. 1993). However, the selective 5-HT_2R antagonist mianserin blocked the effect of mCPP, indicating that 5-HT_2R, perhaps 5-$HT_{2C}R$ specifically, represents the subtype that is overtly supersensitized by 6-OHDA lesioning and associated with perioral movements (Gong et al. 1993).

In fact, the number of perioral movements after mCPP treatment was greater than that following D_1R agonist treatment (Gong et al. 1992). Mianserin pretreatment, in addition to blocking the effect of the 5-HT_2R agonist mCPP, blocked the effect of a D_1R agonist on perioral activity (Gong et al. 1992), and the dorsal striatum was one site for this interaction (Plech et al. 1995; Salamone et al. 1990). By contrast, a D_1R antagonist did not block the effect of mCPP (Gong et al. 1992). These findings indicate that DAR supersensitivity for perioral activity is mediated via a serotoninergic system and notably at the 5-HT_2R (Gong et al. 1993).

5-HT fiber hyperinnervation of neostriatum and the forebrain is prominent in rats lesioned perinatally with 6-OHDA (Berger et al. 1985; Snyder et al. 1986; Mrini et al. 1995; see Kostrzewa and Descarries 1998), and this is accompanied by an approximate 30–60 % increase in the number of 5-$HT_{1A}R$, 5-$HT_{1B}R$, 5-$HT_{1nonAB}R$, 5-$HT_{2A}R$, and 5-$HT_{2C}R$ (Radja et al. 1993). Therefore, to further explore the effect of 5-HT innervation on perioral activity, the neurotoxin 5,7-dihydroxytryptamine

(5,7-DHT) was coadministered with 6-OHDA to perinatal rats which were then studied as adults. In rats co-lesioned with 6-OHDA and 5,7-DHT, the supersensitized perioral response to a D_1R agonist was eliminated, indicating that 5-HT fibers indeed mediate the D_1R agonist effect on perioral movements (Brus et al. 1994).

5 Abatement of Oral Dyskinesia in a Rodent Model of Tardive Dyskinesia

Although acute effects of D_1R agonists on perioral activity are attenuated by acute D_1R antagonist treatment, a 5-HT_2R antagonist is just as effective (Gong et al. 1994). In the rodent model of TD, produced by chronic administration of a D_2R antagonist – over a period of months – acute treatment during the haloperidol-withdrawal phase with a D_1R antagonist does not reduce the incidence of perioral movements (Kostrzewa et al. 2007). Nor does a D_2R antagonist (spiperone), muscarinic receptor antagonist (scopolamine), histamine H_1R antagonist (cyproheptadine), opioid mu receptor agonist (morphine) or antagonist (naloxone), adenosine $A_{2A}R$ antagonist (theophylline), alpha-adrenoceptor antagonist (phentolamine, phenoxybenzamine), beta-adrenoceptor antagonist (propranolol); neither a 5-$HT_{1A}R$ agonist (pindolol) nor 5-$HT_{2A}R$ antagonist (ketanserin). However, agents with 5-$HT_{2C}R$ antagonist activity were all effective in acutely reducing the number of perioral movements in the rodent model of TD, produced by year-long administration of a D_2R antagonist. Thus, mianserin, mesulergine, ritanserin, and clozapine each acutely reduced spontaneous oral activity during 8 months of the haloperidol-withdrawn phase in the rodent model of TD (Kostrzewa et al. 2007).

Despite the fact that neural reorganization that occurs consequent to months-long continuous administration of a D_2R antagonist, it appears that it is an action mediated by serotoninergic 5-HT_2R that is most consequential (Gong et al. 1992). This is somewhat surprising, because 5-HT innervation in the brain is considered to be an afferent input, not an efferent output system. 5-HT innervation in the brain arises from the brainstem in the dorsal and medial raphe nuclei (see Kostrzewa et al. 1998). Also, from studies in rodents it is recognized that perinatal 6-OHDA destruction of dopaminergic nerves results in supersensitization of DA D_1R as well as 5-HT_2R (Gong et al. 1992; Brus et al. 1994). Therefore, in TD it may be that supersensitized 5-HT_2R has the major influence on the induction of spontaneous perioral activity.

6 Tardive Dyskinesia Arising in Rodents from Chronic Dopamine Receptor Antagonist Treatment

Waddington and colleagues (Waddington and Gamble, 1980; Waddington et al., 1983) produced the first rodent model of TD by administering haloperidol in

drinking water to rats for 6 consecutive months. An increase in spontaneous perioral movements developed only at 4 months, thus fulfilling the characteristic "tardive" element of TD (i.e., tardive signifies an onset of symptoms after chronic drug treatment). The increase in spontaneous oral activity persisted for as long as haloperidol continued to be administered, then waned and reverted to normal over a period of a month (see also Waddington 1990). Ultimately, Ellison and See (1989) showed that the form and periodicity of oral dyskinesias in haloperidol-treated rats was similar in form to those of humans with TD. A disadvantage to this model of TD was that the high level of oral activity in rats dissipated over a period of 1 month, back to control level, after haloperidol was withdrawn. This was unlike the persistence of perioral activity in people with TD.

Gunne et al. (1982) and Glassman and Glassman (1980) had observed that there is an increase in neuroleptic-evoked perioral activity in rats following damage to the brain. In the interest of developing a somewhat more novel model and possibly permanent model of TD, it occurred that DA denervation, as occurs with 6-OHDA lesioning, may be an important element in both the prominence and persistence of oral dyskinesia in rodents. Accordingly, we perinatally lesioned rats with 6-OHDA, then added haloperidol to drinking water when these rats were 2 months of age. In these largely DA-denervated rats, an increase in spontaneous oral activity occurred at 3 months, one full month earlier than in intact rats receiving haloperidol via drinking water. Also, the number of perioral movements induced in 6-OHDA-lesioned rats by chronic haloperidol was twice the level for chronic haloperidol-exposed intact rats. At 1 year the neostriatal Bmax for the D_2R antagonist [^{3}H] raclopride was increased ~25 % in 6-OHDA-lesioned rats and ~65 % in haloperidol-treated rats (both intact and 6-OHDA-lesioned). Notably, when 6-OHDA-lesioned rats were withdrawn from haloperidol at 1 year, increased perioral activity did not revert to control levels. The high level of perioral activity observed during the haloperidol phase continued to persist in the group during the withdrawal phase until the study was terminated 8 months later. Further, it is notable that there was no increase in the neostriatal D_2R (i.e., B_{max} for [^{3}H]raclopride binding to striatal homogenates) during the haloperidol-withdrawal phase in which there was a persistent high level of perioral activity (Huang et al. 1997). Andersson et al. (1990) have shown that humans with TD do not have an increase in D_2R in the brain.

7 Neural Mechanisms Attending Animal Modeling of Tardive Dyskinesia: Relevance to Human Tardive Dyskinesia

The multiple animal modeling studies relating to TD indicate the following neural mechanism relating to TD. The balance of DA D_1R/D_2R activation appears to be of critical importance. Both D_1R agonists and D_2R antagonists promote oral activity. However, acute effects do not represent a modeling of TD.

TD is, by definition, a "tardive" effect, one that develops over a period of months during prolonged or chronic treatments, specifically with a D_2R antagonist. TD appears to be induced by prolonged imbalance of D_1R/D_2R activation, consequent to a tip in the balance towards D_1R overactivation, possibly in neostriatum or other regions of the forebrain. The nigroneostriatal dopaminergic tract appears to relate most closely to TD. A remodeling of neural circuitry in the brain, over a period of months, represents an adaptive mechanism to the imbalance of D_1R/D_2R activation, which ultimately results in induction of spontaneous perioral movements. The neural adjustments and reorganization involved in the process are still a mystery.

Nevertheless, prolonged overactivation of DA D_1R appears to be the genesis for TD. Furthermore, it is apparent that damage to brain circuitry can accelerate the appearance of TD and impact on its permanence. From the most recent experimental studies, it is obvious that damage to dopaminergic innervation to neostriatum promotes earlier onset of TD and permanence. From studies by Gunne et al. (1988), it is possible that damage to other neural circuits in the brain can have similar influence on TD.

8 Implications for Treatment of Tardive Dyskinesia in Humans

Because chronic D_2R antagonist treatment of both humans and rodents produces TD, it is reasonable to assume that similar neural adaptive mechanisms occur in the brain of both rats and humans. Also, because nonspecific injury in the brain (see Gunne and Häggström 1983) as well as nigrostriatal dopaminergic damage confer permanence to the TD produced by prolonged D_2R antagonist treatment, it is possible that in those patients who develop lifelong TD, there is residual brain damage or dopaminergic fiber degeneration that impacts of the D_2R antagonist treatment. It is speculated that even a mild brain injury could have occurred earlier in life (i.e., head trauma) or perhaps dopaminergic innervation of the brain declines with aging. The latter event is thought to occur and thereby account for the origin of Parkinson's disease in aged humans (Kish et al. 1992).

Moreover, an increase in the number of neostriatal DA D_2R is not correlated with high levels of perioral movements, as noted by Huang et al. (1997). Studies by Breese et al. (1985) and Gong et al. (1994) established that DA D_1R supersensitivity is not necessarily accompanied by an increase in the number of D_1 receptors. Also, DA D_2R supersensitivity is not correlated with an increase in neostriatal DA D_2R number in rodents (Kostrzewa and Brus 1991) nor in humans with TD (Andersson et al. 1990). Therefore, the process of receptor supersensitization is not reliant on changes in DA receptor number or affinity. Regardless of underlying factors for development of TD, the animal experimental findings implicate 5-HT_2R antagonists as reasonable agents for the treatment of TD (Kostrzewa et al. 2007).

References

Andersson, U., Eckernas, S. A., Hartvig, P., Ulin, J., Langstrom, B., & Häggström, J. E. (1990). Striatal binding of ^{11}C-NMSP studied with positron emission tomography in patients with persistent tardive dyskinesia: No evidence for altered dopamine D_2 receptor binding. *Journal of Neural Transmission. General Section, 79*, 215–226.

Arnt, J., Hyttel, J., & Perregard, J. (1987). Dopamine D-1 receptor agonists combined with the selective D-2 agonist, quinpirole, facilitate the expression of oral stereotyped behaviour in rats. *European Journal of Pharmacology, 133*, 137–145.

Berger, T. W., Kaul, S., Stricker, E. M., & Zigmond, M. J. (1985). Hyperinnervation of the striatum by dorsal raphe afferents after dopamine-depleting brain lesions in neonatal rats. *Brain Research, 366*, 354–358.

Blanchet, P. F., Parent, M.-T., Rompré, P. H., & Lévesque, D. (2012). Relevance of animal models to human tardive dyskinesia. *Behavioral and Brain Functions, 8*(12), 1–9.

Breese, G. R., Baumeister, A., Napier, T. C., Frye, G. D., & Mueller, R. A. (1985). Evidence that D_1 dopamine receptors contribute to the supersensitive behavioral responses induced by L-dihydroxyphenylalanine in rats treated neonatally with 6-hydroxydopamine. *The Journal of Pharmacology and Experimental Therapeutics, 235*, 287–295.

Breese, G. R., Duncan, G. E., Napier, T. C., Bondy, S. C., Iorio, L. C., & Mueller, R. A. (1987). 6-Hydroxydopamine treatments enhance behavioral responses to intracerebral microinjection of D_1- and D_2-dopamine agonists into the nucleus accumbens and striatum without changing dopamine antagonist binding. *The Journal of Pharmacology and Experimental Therapeutics, 240*, 167–176.

Brus, R., Kostrzewa, R. M., Perry, K. W., & Fuller, R. W. (1994). Supersensitization of the oral response to SKF 38393 in neonatal 6-hydroxydopamine-lesioned rats is eliminated by neonatal 5,7-dihydroxytryptamine treatment. *The Journal of Pharmacology and Experimental Therapeutics, 268*, 231–237.

Casey, D. E. (1987). Tardive dyskinesia. In H. Y. Meltzer (Ed.), *Psychopharmacology: The third generation of progress* (pp. 1411–1419). New York, NY: Raven.

Clow, A., Jenner, P., & Marsden, C. D. (1979). Changes in dopamine-mediated behavior during one year's neuroleptic administration. *European Journal of Pharmacology, 57*, 365–375.

Criswell, H., Mueller, R. A., & Breese, G. R. (1989). Priming of D_1-dopamine receptor responses: Long-lasting behavioral supersensitivity to a D_1-dopamine agonist following repeated administration to neonatal 6-OHDA-lesioned rats. *The Journal of Neuroscience, 9*, 125–133.

Doucet, G., Descarries, L., & Garcia, S. (1986). Quantification of the dopamine innervation in adult rat neostriatum. *Neuroscience, 19*, 427–445.

Egan, M. F., Hyde, T. M., Kleinman, J. E., & Wyatt, R. J. (1995). Neuroleptic-induced vacuous chewing movements in rodents: Incidence and effects of long-term increases in haloperidol dose. *Psychopharmacology, 117*, 74–81.

El Mansari, M., Radja, F., Ferron, A., Reader, T. A., Molina-Holgado, E., & Descarries, L. (1994). Hypersensitivity to serotonin and its agonists in serotonin-hyperinnervated neostriatum after neonatal dopamine denervation. *European Journal of Pharmacology, 261*, 171–178.

Ellison, G. D., & See, R. E. (1989). Rats administered chronic neuroleptics develop oral movements which are similar in form to those in humans with tardive dyskinesia. *Psychopharmacology, 98*, 564–566.

Fernandes Xavier, F. G., Doucet, G., Geffard, M., & Descarries, L. (1994). Dopamine neoinnervation in the substantia nigra and hyperinnervation in the interpeduncular nucleus of adult rat following neonatal cerebroventricular administration of 6-hydroxydopamine. *Neuroscience, 59*, 77–87.

Glassman, R. B., & Glassman, H. N. (1980). Oral dyskinesia in brain-damaged rats withdrawn from a neuroleptic: Implications for models of tardive dyskinesia. *Psychopharmacology, 69*, 19–25.

Gong, L., & Kostrzewa, R. M. (1992). Supersensitized oral response to a serotonin agonist in neonatal 6-OHDA treated rats. *Pharmacology, Biochemistry, and Behavior, 41*, 621–623.

Gong, L., Kostrzewa, R. M., Fuller, R. W., & Perry, K. W. (1992). Supersensitization of the oral response to SKF 38393 in neonatal 6-OHDA-lesioned rats is mediated through a serotonin system. *The Journal of Pharmacology and Experimental Therapeutics, 261*, 1000–1007.

Gong, L., Kostrzewa, R. M., Perry, K. W., & Fuller, R. W. (1993). Dose-related effects of a neonatal 6-OHDA lesion on SKF-38393- and m-chlorophenylpiperazine-induced oral activity responses of rats. *Developmental Brain Research, 76*, 233–238.

Gong, L., Kostrzewa, R. M., & Li, C. (1994). Neonatal 6-hydroxydopamine and adult SKF 38393 treatments alter dopamine D_1 receptor mRNA levels: Absence of other neurochemical associations with the enhanced behavioral responses of lesioned rats. *Journal of Neurochemistry, 63*, 1282–1290.

Gunne, L. M., & Häggström, J. E. (1983). Reduction of nigral glutamic acid decarboxylase in rats with neuroleptic-induced oral dyskinesia. *Psychopharmacology, 81*, 191–194.

Gunne, L. M., Growdon, J., & Glaeser, B. (1982). Oral dyskinesia in rats following brain lesions and neuroleptic drug administration. *Psychopharmacology, 77*, 134–139.

Huang, N.-Y., Kostrzewa, R. M., Li, C., Perry, K. W., & Fuller, R. W. (1997). Persistent spontaneous oral dyskinesias in haloperidol-withdrawn rats neonatally lesioned with 6-hydroxydopamine: Absence of an association with the B_{max} for [^{3}H]raclopride binding to neostriatal homogenates. *The Journal of Pharmacology and Experimental Therapeutics, 280*, 268–276.

Iversen, S. D., Howells, R. B., & Hughes, R. P. (1980). Behavioral consequences of long-term treatment with neuroleptic drugs. *Advances in Biochemical Psychopharmacology, 24*, 305–313.

Jeste, D. V., & Caligiuri, M. P. (1993). Tardive dyskinesia. *Schizophrenia Bulletin, 19*, 303–315.

Johansson, P., Levin, E., Gunne, L., & Ellison, G. (1987). Opposite effects of a D_1 and D_2 agonist on oral movements in rats. *European Journal of Pharmacology, 134*, 83–88.

Kane, J. M., Woerner, M., & Lieberman, J. (1985). Tardive dyskinesia: Prevalence, incidence, and risk factors. *Psychopharmacology. Supplementum, 2*, 72–78.

Kish, S. J., Shannak, K., Rajput, A., Deck, J. H., & Hornykiewicz, O. (1992). Aging produces a specific pattern of striatal dopamine loss: Implications for the etiology of idiopathic Parkinson's disease. *Journal of Neurochemistry, 58*, 642–648.

Knable, M. B., Hyde, T. M., Egan, M. F., Tosayali, M., Wyatt, R. J., & Kleinman, J. E. (1994). Quantitative autoradiography of striatal dopamine D_1, D_2 and re-uptake sites in rats with vacuous chewing movements. *Brain Research, 646*, 217–222.

Koshikawa, N., Aoki, S., Tomiyama, M., Maruyama, Y., & Kobayashi, M. (1987). Sulpiride injection into the dorsal striatum increases methamphetamine-induced gnawing in rats. *European Journal of Pharmacology, 133*, 119–125.

Kostrzewa, R. M. (1995). Dopamine receptor supersensitivity. *Neuroscience and Biobehavioral Reviews, 19*, 1–17.

Kostrzewa, R. M., & Brus, R. (1991). Ontogenic homologous supersensitization of quinpirole-induced yawning in rats. *Pharmacology, Biochemistry, and Behavior, 39*, 517–519.

Kostrzewa, R. M., & Gong, L. (1991). Supersensitized D_1 receptors mediate enhanced oral activity after neonatal 6-OHDA. *Pharmacology, Biochemistry, and Behavior, 39*, 677–682.

Kostrzewa, R. M., & Hamdi, A. (1991). Potentiation of spiroperidol induced oral activity in rats after neonatal 6 hydroxydopamine. *Pharmacology, Biochemistry, and Behavior, 38*, 215–218.

Kostrzewa, R. M., & Neely, D. (1993). Enhanced pilocarpine-induced oral activity responses in neonatal 6-OHDA-treated rats. *Pharmacology, Biochemistry, and Behavior, 45*, 737–740.

Kostrzewa, R. M., Brus, R., Perry, K. W., & Fuller, R. W. (1993). Age-dependence of a 6-hydroxydopamine lesion on SKF 38393- and *m*-chlorophenylpiperazine-induced oral activity responses of rats. *Developmental Brain Research, 76*, 87–93.

Kostrzewa, R. M., Reader, T. A., & Descarries, L. (1998). Serotonin neural adaptations to ontogenetic loss of dopamine neurons in rat brain. *Journal of Neurochemistry, 70*, 889–898.

Kostrzewa, R. M., Kostrzewa, J. P., & Brus, R. (2003). Dopamine receptor supersensitivity: An outcome and index of neurotoxicity. *Neurotoxicity Research, 5*, 111–118.

Kostrzewa, R. M., Huang, N.-Y., Kostrzewa, J. P., Nowak, P., & Brus, R. (2007). Modeling tardive dyskinesia: Predictive 5-HT_{2C} receptor antagonist treatment. *Neurotoxicity Research, 11*, 41–50.

Kostrzewa, R. M., Kostrzewa, J. P., Brown, R., Nowak, P., & Brus, R. (2008). Dopamine receptor supersensitivity: Development, mechanisms, presentation, and clinical applicability. *Neurotoxicity Research, 14*, 121–128.

Levin, E. D., See, R. E., & South, D. (1989). Effects of dopamine D_1, D_2 receptor antagonists on oral activity in rats. *Pharmacology, Biochemistry, and Behavior, 34*, 43–48.

Lloyd, K. G., Willigens, M. T., & Goldstein, M. (1985). Induction and reversal of dopamine dyskinesia in rat, cat, and monkey. *Psychopharmacology. Supplementum, 2*, 200–210.

Mithani, S., Atmadja, S., Baimbridge, K. G., & Fibiger, H. C. (1987). Neuroleptic-induced oral dyskinesias: Effects of progabide and lack of correlation with regional changes in glutamic acid decarboxylase and choline acetyltransferase activities. *Psychopharmacology, 93*, 94–100.

Molloy, A. G., & Waddington, J. L. (1987). Assessment of grooming and other behavioural responses to the D_1 dopamine receptor agonist SKF38393 and its R- and S-enantiomers in the intact adult rat. *Psychopharmacology (Berlin), 92*, 164–168.

Molloy, A. G., & Waddington, J. L. (1988). Behavioural responses to the selective D_1-dopamine receptor agonist R-SK&F 38393 and the selective D_2-agonist RU 24213 in young compared with aged rat. *British Journal of Pharmacology, 95*, 335–342.

Mrini, A., Soucy, J.-P., Lafaille, F., Lemoine, P., & Descarries, L. (1995). Quantification of the serotonin hyperinnervation in adult rat neostriatum after neonatal 6-hydroxydopamine lesion of nigral dopamine neurons. *Brain Research, 669*, 303–308.

Murray, A. M., & Waddington, J. L. (1989). The induction of grooming and vacuous chewing by a series of selective D-1 dopamine receptor agonists: Two directions of D-1:D-2 interaction. *European Journal of Pharmacology, 160*, 377–387.

Plech, A., Brus, R., Kalbfleisch, J. H., & Kostrzewa, R. M. (1995). Enhanced oral activity responses to intrastriatal SKF 38393 and m-CPP are attenuated by intrastriatal mianserin in neonatal 6-OHDA-lesioned rats. *Psychopharmacology, 119*, 466–473.

Radja, F., Descarries, L., Dewar, K. M., & Reader, T. A. (1993). Serotonin 5-HT_1 and 5-HT_2 receptors in adult rat brain after neonatal destruction of nigrostriatal dopamine neurons: A quantitative autoradiographic study. *Brain Research, 606*, 273–285.

Rosengarten, H., Schweitzer, J. W., & Friedhoff, A. J. (1983). Induction of oral dyskinesias in naïve rats by D_1 stimulation. *Life Sciences, 33*, 2479–2482.

Rosengarten, H., Schweitzer, J. W., Egawa, J., & Friedhoff, A. J. (1986a). Diminished D_2 dopamine receptor function and the emergence of repetitive jaw movements. *Advances in Experimental Medicine and Biology, 235*, 159–167.

Rosengarten, H., Schweitzer, J. W., & Friedhoff, A. J. (1986b). Selective dopamine D_2 receptor reduction enhances a D_1 mediated oral dyskinesia. *Life Sciences, 39*, 29–35.

Rupniak, N. M., Jenner, P., & Marsden, C. D. (1983). Cholinergic manipulation of perioral behaviour induced by chronic neuroleptic administration to rats. *Psychopharmacology, 79* (2–3), 226–230.

Rupniak, N. M. J., Jenner, P., & Marsden, C. D. (1985). Pharmacological characterization of spontaneous or drug-associated purposeless chewing movements in rats. *Psychopharmacology, 85*, 71–79.

Saifee, T. A., & Edwards, M. J. (2011). Tardive movement disorders: A practical approach. *Practical Neurology, 11*, 341–348.

Salamone, J. D., Johnson, C. J., McCullough, L. D., & Steinpreis, R. E. (1990). Lateral striatal cholinergic mechanisms involved in oral motor activities in the rat. *Psychopharmacology, 102*, 529–534.

Snyder, A. M., Zigmond, M. J., & Lund, R. D. (1986). Sprouting of serotonergic afferents into striatum after dopamine depleting lesions in infant rats: A retrograde transport and immunocytochemical study. *The Journal of Comparative Neurology, 245*, 274–281.

Tamminga, C. A., Crayton, J. W., & Chase, T. N. (1979). Improvement in tardive dyskinesia after muscimol therapy. *Archives of General Psychiatry, 36*, 595–598.

Tarsy, D., Lungu, C., & Baldessarini, R. J. (2011). Epidemiology of tardive dyskinesia before and during the era of modern antipsychotic drugs. *Handbook of Clinical Neurology, 100*, 601–616.

Waddington, J. L. (1990). Spontaneous orofacial movements induced in rodents by very long-term neuroleptic drug administration: Phenomenology, pathophysiology and putative relationship to tardive dyskinesia. *Psychopharmacology (Berlin), 101*, 431–447.

Waddington, J. L., & Gamble, S. J. (1980). Neuroleptic treatment for a substantial proportion of adult life: Behavioral sequelae of 9 months haloperidol administration. *European Journal of Pharmacology, 67*, 363–369.

Waddington, J. L., Cross, A. J., Gamble, S. J., & Bourne, R. C. (1983). Spontaneous orofacial dyskinesia and dopaminergic function in rats after 6 months of neuroleptic treatment. *Science, 220*, 530–532.

Pathogenesis of Alzheimer's Disease

Rudy J. Castellani and George Perry

Contents

Abstract

Alzheimer disease (AD) is a clinically progressive decline in cortical function involving memory and executive function and pathologically defined by two hallmark lesions, the senile plaque and the neurofibrillary tangle. Nearly 30 years ago, these hallmark lesions were purified and their protein constituents identified by quantitative analysis, which led in turn to a substantial expansion of knowledge, as well as optimism about the ultimate success of targeted therapy. Unfortunately, despite copious facts of new knowledge of the biochemical cascades that produce these protein abnormalities, there has been no meaningful progress toward disease modification for AD, a disease only found in humans. The repeated failures in this regard have been attributed to tardiness in intervention rather than instead an overdue need for a paradigm shift. The ruling theories for AD pathogenesis have their root in lesion removal. We argue that the lack of progress may instead reflect the evolving concept that pathological

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lesions, be they plaques, tangles, or soluble low-n protein species, signify mechanisms of neuroprotection, in response to a decade-long adaptation to an aging-hostile environment. The lesions themselves may similarly be a manifestation of neuroprotection, and likewise the targeting of such lesions as the offending agents is done at the very real risk of disrupting homeostasis and the body's attempt at fighting disease. Rather than simply shifting the same ideas and interventions to an earlier age or stage of disease, a broadening of the scope of treatment efforts to working with rather than against the biological processes of the brain, and the realities of repeated negative data, should be accepted both in theory and in practice.

Keywords
Aβ • Alzheimer disease • Amyloid-beta • Tau

1 Introduction

Alzheimer disease (AD) is complex and heterogeneous, differing in many respects from case to case, including age at onset, clinical signs and symptoms, presence or absence of various and numerous risk factors, extent and distribution of neuropathology, and genetic alterations including germline mutation and susceptibility alleles. Numerous hypotheses and the treatment implications inherent in them are not unexpected, although the success in treatment has, to date, been absent. Whether or not this failure reflects the complexity of the disease, the now numerous failures in the face of almost limitless knowledge of biochemical facts suggest that prevailing paradigms are significantly, if not fatally, flawed and not salvageable by overlooking theoretical flaws (Castellani et al. 2008a, b, 2009; Castellani and Smith 2011).

Presently, standard thought equates protein constituents of insoluble pathological lesions or their assembly intermediates with toxicity (Hardy and Higgins 1992). AD brains, for example, accumulate neurofibrillary tangles (the major discovery made by Alzheimer in 1906), which are comprised of phosphorylated tau disposed in insoluble fibrils, and those fibrils are derived from soluble tau species which are toxic to neurons in vitro). Thus, the protein "cascade" that produces phosphorylated tau is deleterious (Spires-Jones et al. 2009). Alternatively, one could say that AD brains accumulate amyloid plaques, which contain amyloid beta-protein (AβP) in insoluble fibrils of "amyloid"; the amyloid is itself derived from soluble toxic intermediates of Aβ, which are toxic to neurons. The cascade that produces these soluble intermediates is therefore inherently deleterious (Selkoe 2008). In addition, often included in discussions of toxic soluble intermediates is a direct attack on the synapse, closing the loop so to speak on the process in that lesions are tied directly to cognition, although the degree of separation from the in vivo environment is unclear (Tanzi 2005). To be sure, much work needs to be done (Benilova et al. 2012).

2 Amyloidosis in the Brain

Aβ was initially identified through purification of insoluble pathological lesions – senile plaque cores and cerebral vasculature involved by amyloid angiopathy (Glenner and Wong 1984; Masters et al. 1985). Subsequent identification of Aβ as a metabolic product of amyloid β-protein precursor (AβPP), its localization to the long arm (q) of chromosome 21, the identification of familial AD kindreds with pathogenic AβPP mutations, and the increased AD pathology in Down's syndrome patients who carry an extra copy of chromosome 21 produced a compelling argument in favor of the so-called amyloid cascade as the pathogenic mediator of disease (Castellani et al. 2008a). The cascade was further substantiated by the characterization of presenilin proteins, part of the notch signaling complex with γ-secretase activity (see below) and therefore inherently amyloidogenic.

Aβ is derived from AβPP, an integral membrane protein on chromosome 21q21 (Vetrivel and Thinakaran 2006; Wilquet and De Strooper 2004; Ling et al. 2003). Full expression of AβPP produces a cytoplasmic tail, a transmembrane domain, and a large extracellular domain, although only a fraction of newly synthesized AβPP molecules reach the cell surface. AβPP further exists as multiple alternatively spliced isoforms, three of which predominate: two isoforms AβPP770/751 contain the Kunitz protease inhibitor domain (exon 7) within the extracellular portion and are the predominant forms in cells other than neurons; AβPP695 is devoid of the Kunitz protease inhibitor domain and is the predominant form in neurons.

Cleavage of AβPP by either α-secretase or β-secretase produces soluble N-terminal fragments AβPP, and C83 and C99 membrane-bound C-terminal fragments, respectively. Further cleavage by γ-secretase leads to the release and secretion of nonpathogenic p3 peptide (previous α-secretase cleavage) and Aβ (previous β-secretase cleavage). Moreover, depending on the precise site of γ-secretase cleavage, different lengths of Aβ are produced, varying from 38 to 43 amino acids. The 42-amino acid form, Aβ42, has a greater tendency to form fibrils in vitro compared to other forms, has a tendency to deposit in brain parenchyma in the form of plaques relative to other species, and has traditionally been regarded as the "pathogenic" Aβ species. Aβ40 is generally considered benign as well as the principle component of Aβ that is deposited in cerebral vessels in cerebral amyloid angiopathy, particularly cerebral amyloid angiopathy type 2, which tends to spare cortical capillaries. Aβ42 synthesis and deposition represents the basis for the amyloid cascade hypothesis and therefore the theoretical underpinning of most clinical trials attempted to date.

The constituents and cell biology of γ-secretase have proven a challenge, although these questions were answered in part with the appearance of familial early-onset AD linked not to AβPP mutations but to so-called presenilins 1 and 2, presenilin 1 mutations being much more common. Presenilin mutations markedly increase Aβ deposits as well as phospho-tau accumulation in affected patients (Citron et al. 1997; Duff et al. 1996; Scheuner et al. 1996) and have been linked to γ-secretase activity (Maiorini et al. 2002). On the other hand, despite the presumption of presenilin as an important component of the multimeric secretase

complex, the biochemical mechanism of presenilin action is largely unknown. During development, presenilins appear to cleave a transmembrane protein termed notch, which in turn is a transcriptional activator of gene involvement in cellular differentiation (De Strooper et al. 1999). PS1 and PS2 have also been found to be involved in a range of biological processes, including cell adhesion, G-protein-mediated signal transduction, and unfolded protein response (Baki et al. 2001; Smine et al. 1998; Niwa et al. 1999) (Baki et al. 2001; Smine et al. 1998; Katayama et al. 1999; Niwa et al. 1999). Nicastrin has also been shown to interact strongly with the presenilins and appears to be required for normal notch signaling in *Caenorhabditis elegans* (Yu et al. 2000).

APP cleavage with generation of Aβ fragments also differs as a function of cellular subcompartment. At the cell surface, AβPP is proteolytically processed, primarily by α-secretases, resulting in shedding of the majority of the extracellular domain. Rapid and efficient internalization is mediated by a "YENPTY" internalization motif (Vetrivel and Thinakaran 2006). Once endocytosed, AβPP may be recycled to the cell surface, degraded, or further processed. β-site AβPP cleaving enzyme-1 (BACE1) appears to act on AβPP in late Golgi/TGN and endosomes, as indicated by the acidic optimal pH of BACE1. γ-secretase complex activity on the other hand takes place in multiple cellular compartments including ER, Golgi, and plasma membrane; the last is thought to comprise only a small fraction of the γ-secretase activity.

A key question that has existed since the elucidation of AβPP is the normal cellular function of this molecule, which is unresolved. One candidate ligand, secreted neuronal protein F-spondin, is implicated in neuronal sprouting and development. F-spondin binds AβPP as well as APLP-1 and APLP-2, which may interfere with β-secretase cleavage and cell signaling effected by the cytoplasmic domain (Wilquet and De Strooper 2004). AβPP has been suggested to serve as a receptor for intracellular transport of synaptic vesicles through interaction with kinesin and microtubules (Kamal et al. 2001). Both AβPP and the low-density lipoprotein receptor-related protein have been shown to bind the adaptor protein Fe65 via their cytoplasmic domains which increases AβPP proteolytic processing (Pietrzik et al. 2004). Interestingly, both LRP and AβPP are also γ-secretase substrates after cleavage and removal of their extracellular domains. A role of AβPP in heavy metal binding and as an antioxidant may also be an important role with direct implications in the disease process when dysfunctional (Cho et al. 2010).

In brief, the fundamentally toxic Aβ42, otherwise a product of normal cellular metabolism, is thought to be overproduced in disease resulting in neurodegeneration, or so the amyloid cascade theory postulates. Support for this comes principally from Mendelian diseases with pathogenic AβPP mutations leading to extensive Aβ deposits and early-onset disease. In vitro toxicity of Aβ42 peptides is considered further evidence for the cascade, although toxicity lies within narrow conditions, irrelevant to brain physiology in terms of toxicity. Despite the commonly held notions, whether or not Aβ42 is toxic in vivo remains to be elucidated. On the other hand, a role of Aβ42 in neuroprotection, which is made intuitively difficult by prevailing ideas, has been demonstrated (Nunomura et al. 2006). The significance of

this cannot be understated, given again that the overwhelming majority of clinical trials accept Aβ42 toxicity as fact. The first major and somewhat infamous phase II active immunization approach (AN-1792) may have been the most informative of all trials to date given the now long-term follow-up. The evidence is now clear that removal of Aβ from the brain in mild to moderate AD has no major cognitive benefits. Moreover, the two individuals studied who had almost complete removal of Aβ plaques progressed to dementia at the same rate as placebo, and each expiring with a mini-mental status score of 0. This effectively answered the question of whether dementia progresses in the face of Aβ removal from the brain whether it be fibrillar amyloid or oligomers (Holmes et al. 2008) and objectively speaking leaves considerable doubt as to whether Aβ42 is toxic at all. Yet the enthusiasm for anti-Aβ42 therapy is seemingly undaunted and is now progressing toward earlier intervention, based on perhaps an equally flawed notion that failure to reverse mild to moderate dementia means that intervention was too late, something for which there is no evidence whatsoever.

2.1 Does Aβ Correlate with Clinical and Anatomic Indices of Disease?

While the above question data seems to have closed the case on whether Aβ causes dementia (the lag in acceptance of this evidence notwithstanding), the relationship between Aβ pathology and disease has been the subject of a number of studies prior to the AN-1792 trial, and indeed the data indicate unambiguously that the correlation between Aβ and clinical disease is imprecise at best (Castellani and Smith 2011). An early study in the 1960s showed an overall tendency toward increased disease severity with plaque burden (Blessed et al. 1968; Giannakopoulos et al. 2003), although numerous subsequent studies have refuted this concept (Giannakopoulos et al. 2003; Braak and Braak 1991; Arnold et al. 1991). At present, it is accepted that amyloid burden overall correlates poorly with disease severity, and the distribution of Aβ tends to be diffused throughout the neocortex with no meaningful region specificity. Diffuse deposits of amyloid also occur in the striatum and cerebellar cortex late in disease (Montine et al. 2012), with no discernible selectivity in terms of loss of function subserved by these regions. Relative to neocortical Aβ, it is of some interest that medial temporal allocortical tissue involved in memory processing shows decreased Aβ (Arnold et al. 1991), something that gets little attention in the literature. Given the role of ApoE in facilitating fibrillogenesis of Aβ, it is also interesting that the extent of neocortical Aβ deposits shows variable correlation in the literature with ApoE susceptibility alleles, including ε4 (Berg et al. 1998; Nunomura et al. 2001).

A relatively new paradigm that is more functional than structural has emerged, in part, in response to the growing realization of the imprecise relationship between Aβ and clinical disease and to the clinical trials that have effectively removed Aβ and have not altered the neurodegenerative process. It is now suggested that soluble low-n Aβ oligomers cause synaptic damage and functional neurologic deficits (Selkoe 2008).

Experimental studies involving injection of conditioned medium, derived from oligomer-secreting APP V717F Chinese hamster ovary cells, into rat lateral ventricle, demonstrated alterations in long-term potentiation (LTP) that was related to low-n oligomers per se and not monomers (Walsh et al. 2002). LTP alteration was also shown in vitro in hippocampal mouse slices, along with concomitant changes in cell cycle signaling cascades and behavioral abnormalities. Here again, however, there is a premature juxtaposition of nakedly artificial experimental data and human brain function, separated from each other by so many degrees of relevance that irrelevancy is the only logical conclusion. This is in addition to lack of insight into those soluble species that are the most toxic and to the overall lack of reproducibility of many of the studies (Benilova et al. 2012). The implication is nevertheless present that these soluble low-n species, which are in vitro elaborations that cannot be directly measured or even reproduced between laboratories, are attacking the synapse, which is also not directly assessed either clinically or pathologically, and, moreover, that this combination is the true substrate for cognitive decline, even with implications for so-called mild cognitive impairment. Again, with these data in mind, the failure of treatment efforts is expected and leaves us in urgent need of a reorganization of the way AD pathogenesis is viewed by the medical and scientific communities.

3 Phosphorylated Tau

When the subject of the findings by Alzheimer is discussed by way of introduction to pathogenesis, it is often stated that amyloid plaques were a key discovery. The fact of the matter is that plaques were discovered some 15 years prior to Alzheimer's description, which he knew at the time, and in fact plaques were a known accompaniment of senile dementia. The first description of the neurofibrillary tangle (NFT), however, can be attributed to Alzheimer (Castellani and Smith 2011; Alzheimer 1907). It is also interesting to note that Alzheimer devoted ten sentences and two paragraphs to his initial description of the NFT, compared to only two sentences to the senile plaque (Alzheimer 1907; Wilkins and Brody 1969), suggesting that the NFT was the more intriguing, or at least novel, lesion for him. Alzheimer and his contemporaries were nevertheless hesitant to draw conclusions regarding disease pathogenesis and were more consumed with the age of onset and clinical signs which differed from "senile dementia," a known condition at that time (Moller and Graeber 1998).

Neurofibrillary tangles received relatively little attention in subsequent years, until the advent of electron microscopy (Terry et al. 1964) and, more importantly, the advent of modern molecular techniques which allowed NFT purification and identification of tau protein as a major protein component (Grundke-Iqbal et al. 1986). The enthusiasm for tau phosphorylation as a primary process in AD, however, has always been blunted by the absence of genetic linkage and the association of TAU mutations with the frontotemporal dementia clinicopathological phenotype, rather than the AD phenotype (Cairns et al. 2007). For this reason,

more than any other, phospho-tau is regarded as downstream to Aβ in terms of the pathogenic process, despite greater fidelity in its association with clinical disease (see below).

Tau is the major protein component of neurofibrillary pathology. Similar to the situation with Aβ, knowledge of tau has expanded considerably since it was purified and molecular species elucidated from the insoluble lesions. We now know that the phosphorylated tau is a major protein component of neurofibrillary pathology, which in turn promoted the study of tau in copious detail.

The tau gene is comprised of over 100 kb and contains 16 exons (Hernandez and Avila 2007). Upstream of the first exon are consensus binding sites for transcription factors. Alternative splicing of tau nuclear RNA in the adult brain involving exons two, three, and ten results in six tau isoforms. These six isoforms in turn differ in the presence of either three of four repeats of 31 or 32 peptide residues in the C-terminal region (exon 10). This peptide repeat region also comprises the microtubule-binding domain and therefore has direct implications in tau pathophysiology. Moreover, tau isoforms differ in the expression of zero, one, or two inserts encoded on exons two and three. The relative amounts of these tau isoforms as well as their phosphorylation status change during development; 3-repeat tau with no inserts is expressed in the fetus and early postnatal infant, while heterogeneous isoforms are expressed in the adult brain. This switch in RNA splicing also corresponds to an overall reduction in tau phosphorylation. Tau is relatively abundant in neurons but is present in all nucleated cells given. Its major physiologic function appears to be in binding microtubules and in stabilizing microtubule assembly for polymerization.

In disease, tau is abnormally hyperphosphorylated at proline-directed serine/threonine phosphorylation sites, including Ser-202/Thr-205 (AT8 site), Ser-214 and/or Ser-212 (AT100 site), Thr-231 and/or Ser-235 (TG3 site), and Ser-396/Ser-404 (PHF-1 site). In addition, alternative tau splicing differs according to pathological phenotype, such that tau accumulation in AD is a mixture of 3R and 4R tau, Pick disease tends to be 3R tau, corticobasal degeneration and progressive supranuclear palsy tend to be 4R tau, and so-called argyrophilic grain disease accumulates small inclusions comprised of 3R tau.

Does phospho-tau correlate with clinical and anatomic indices of disease? In spite of the fact that tau tends to appear in the cortex subsequent to Aβ and is generally considered a secondary or downstream phenomenon (Nelson et al. 2009), it is interesting that neurofibrillary pathology correlates closely clinical signs (Braak and Braak 1991) and much more closely than Aβ deposits. Phosphorylated tau deposition, for example, has a striking tendency to involve memory circuitry early in disease as well as in the aging process (Giannakopoulos et al. 2003; Arnold et al. 1991). It is also remarkable that abundant neocortical neurofibrillary pathology is virtually always associated with clinical signs of AD (Nelson et al. 2009, 2012), whereas extensive neocortical Aβ deposits are often seen in aged individuals in the absence of significant cognitive impairment or evidence of neuronal loss. In other words, heavy tau "burden" is generally incompatible with preserved cerebral function, while heavy amyloid burden often is not.

The role of phosphorylated tau in functional disease is progressing in a manner very much similar to Aβ. Recent studies, for example, indicate that insoluble tau accumulation is somewhat benign, while oligomeric phospho-tau intermediates may be more toxic and may be toxic at the specific level of the synapse (Santacruz et al. 2005). Also similar to Aβ studies, support for this concept is limited to highly experimental models (Stokin et al. 2005; Yoshiyama et al. 2007). The results highlight the growing theme that insoluble pathological lesions, and in this case NFT formation is a late stage nontoxic event, and that attention might be better directed toward upstream soluble tau intermediates. That the entire process, from changes in synthesis of soluble species, to putative soluble assembly intermediates, to insoluble pathological lesions, has yet to be embraced as a response to an underlying pathogenic process is remarkable. With recent multiple failures of the cascade concept, testing of biological alternatives is long overdue.

4 Conclusion

Current hypotheses of AD pathogenesis encompass copious and sophisticated data, but nevertheless have their origin in hallmark pathological lesions described more than a century ago. The literature is now contradictory on whether hallmark lesions are best considered manifestations of neurotoxicity or instead insoluble epiphenomena to the more important events involving soluble, toxic intermediates and the synapse, events ironically that cannot be directly observed, and are based on in vitro elaborations which have proven challenging to reproduce between laboratories and have not passed the test of relevance to the human brain. More likely, and based on now considerable evidence, pathological lesions as well as their constituent proteins of whatever species, be they monomeric, oligomeric, or insoluble fibrils, can be tied to molecular pathogenesis on the basis of disease expression, or a host response. This response is likely fundamentally adaptive over a long period of time and more in line with Darwinian theory. Targeting of such lesions, or any individual protein as part of a putative pathogenic cascade for that matter, should be entertained only with considerable care, if not the sober realization that it will more likely do more harm than good, as has been demonstrated in abundance. As the pathogenesis of AD continues to be elucidated, a broadening of the focus, rather than the perseveration on a very narrow set of principles, appears to be needed.

References

Alzheimer, A. (1907). Uber eine eigenartige erkrankung der hirnrinde. *Allgemeine Zeits Psychiat PsychischYGerichtlich Med, 64*, 146–148.

Arnold, S. E., Hyman, B. T., Flory, J., Damasio, A. R., & Van Hoesen, G. W. (1991). The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cerebral Cortex, 1*(1), 103–116.

Baki, L., Marambaud, P., Efthimiopoulos, S., Georgakopoulos, A., Wen, P., Cui, W., et al. (2001). Presenilin-1 binds cytoplasmic epithelial cadherin, inhibits cadherin/p120 association, and regulates stability and function of the cadherin/catenin adhesion complex. *Proceedings of the National Academy of Sciences of the United States of America, 98*(5), 2381–2386.

Benilova, I., Karran, E., & De Strooper, B. (2012). The toxic Abeta oligomer and Alzheimer's disease: An emperor in need of clothes. *Nature Neuroscience, 15*(3), 349–357.

Berg, L., McKeel, D. W., Jr., Miller, J. P., Storandt, M., Rubin, E. H., Morris, J. C., et al. (1998). Clinicopathologic studies in cognitively healthy aging and Alzheimer's disease: Relation of Histologic markers to dementia severity, age, sex, and apolipoprotein E genotype. *Archives of Neurology, 55*(3), 326–335.

Blessed, G., Tomlinson, B. E., & Roth, M. (1968). The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *The British Journal of Psychiatry, 114*(512), 797–811.

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica, 82*(4), 239–259.

Cairns, N. J., Bigio, E. H., Mackenzie, I. R., Neumann, M., Lee, V. M., Hatanpaa, K. J., et al. (2007). Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: Consensus of the consortium for frontotemporal lobar degeneration. *Acta Neuropathologica, 114*(1), 5–22.

Castellani, R. J., & Smith, M. A. (2011). Compounding artefacts with uncertainty, and an amyloid cascade hypothesis that is 'too big to fail'. *Journal of Pathology*, *224*(2), 147–152.

Castellani, R. J., Rolston, R. K., & Smith, M. A. (2010). Alzheimer disease. *Disease-a Month, 56*(9), 484–546.

Castellani, R. J., Lee, H. G., Zhu, X., Perry, G., & Smith, M. A. (2008a). Alzheimer disease pathology as a host response. *Journal of Neuropathology and Experimental Neurology, 67*(6), 523–531.

Castellani, R. J., Nunomura, A., Lee, H. G., Perry, G., & Smith, M. A. (2008b). Phosphorylated tau: Toxic, protective, or none of the above. *Journal of Alzheimer's Disease, 14*(4), 377–383.

Castellani, R. J., Lee, H. G., Siedlak, S. L., Nunomura, A., Hayashi, T., Nakamura, M., et al. (2009). Reexamining Alzheimer's disease: Evidence for a protective role for amyloid-beta protein precursor and amyloid-beta. *Journal of Alzheimer's Disease, 18*(2), 447–452.

Cho, H. H., Cahill, C. M., Vanderburg, C. R., Scherzer, C. R., Wang, B., Huang, X., et al. (2010). Selective translational control of the Alzheimer amyloid precursor protein transcript by iron regulatory protein-1. *Journal of Biological Chemistry, 285*(41), 31217–31232.

Citron, M., Westaway, D., Xia, W., Carlson, G., Diehl, T., Levesque, G., et al. (1997). Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both Transfected cells and transgenic mice. *Nature Medicine, 3*(1), 67–72.

De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., et al. (1999). A presenilin-1-dependent gamma-secretase-like protease mediates release of notch intracellular domain. *Nature, 398*(6727), 518–522.

Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C. M., Perez-tur, J., et al. (1996). Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. *Nature, 383*(6602), 710–713.

Giannakopoulos, P., Herrmann, F. R., Bussiere, T., Bouras, C., Kovari, E., Perl, D. P., et al. (2003). Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology, 60*(9), 1495–1500.

Glenner, G. G., & Wong, C. W. (1984). Alzheimer's disease and down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. *Biochemical and Biophysical Research Communications, 122*(3), 1131–1135.

Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M., & Binder, L. I. (1986). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proceedings of the National Academy of Sciences of the United States of America, 83*(13), 4913–4917.

Hardy, J. A., & Higgins, G. A. (1992). Alzheimer's disease: The amyloid cascade hypothesis. *Science, 256*(5054), 184–185.

Hernandez, F., & Avila, J. (2007). Tauopathies. *Cellular and Molecular Life Sciences, 64*(17), 2219–2233.

Holmes, C., Boche, D., Wilkinson, D., Yadegarfar, G., Hopkins, V., Bayer, A., et al. (2008). Long-term effects of Abeta42 immunisation in Alzheimer's disease: Follow-up of a randomised, placebo-controlled phase I trial. *Lancet, 372*(9634), 216–223.

Kamal, A., Almenar-Queralt, A., LeBlanc, J. F., Roberts, E. A., & Goldstein, L. S. (2001). Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP. *Nature, 414*(6864), 643–648.

Katayama, T., Imaizumi, K., Sato, N., Miyoshi, K., Kudo, T., Hitomi, J., Morihara, T., Yoneda, T., Gomi, F., Mori, Y., Nakano, Y., Takeda, J., Tsuda, T., Itoyama, Y., Murayama, O., Takashima, A., St George-Hyslop, P., Takeda, M., Tohyama, M. (1999). Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. *Nat Cell Biol. 1*(8), 479–85.

Ling, Y., Morgan, K., & Kalsheker, N. (2003). Amyloid precursor protein (APP) and the biology of proteolytic processing: Relevance to Alzheimer's disease. *The International Journal of Biochemistry & Cell Biology, 35*(11), 1505–1535.

Maiorini, A. F., Gaunt, M. J., Jacobsen, T. M., McKay, A. E., Waldman, L. D., & Raffa, R. B. (2002). Potential novel targets for Alzheimer pharmacotherapy: I secretases. *Journal of Clinical Pharmacy and Therapeutics, 27*(3), 169–183.

Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L., & Beyreuther, K. (1985). Amyloid plaque core protein in Alzheimer disease and down syndrome. *Proceedings of the National Academy of Sciences of the United States of America, 82*(12), 4245–4249.

Moller, H. J., & Graeber, M. B. (1998). The case described by alois Alzheimer in 1911. Historical and conceptual perspectives based on the clinical record and neurohistological sections. *European Archives of Psychiatry and Clinical Neuroscience, 248*(3), 111–122.

Montine, T. J., Phelps, C. H., Beach, T. G., Bigio, E. H., Cairns, N. J., Dickson, D. W., et al. (2012). National institute on Aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease: A practical approach. *Acta Neuropathologica, 123*(1), 1–11.

Nelson, P. T., Alafuzoff, I., Bigio, E. H., Bouras, C., Braak, H., Cairns, N. J., et al. (2012). Correlation of Alzheimer disease neuropathologic changes with cognitive status: A review of the literature. *Journal of Neuropathology & Experimental Neurology, 71*(5), 362–381.

Nelson, P. T., Braak, H., & Markesbery, W. R. (2009). Neuropathology and cognitive impairment in Alzheimer disease: A complex but coherent relationship. *Journal of Neuropathology and Experimental Neurology, 68*(1), 1–14.

Niwa, M., Sidrauski, C., Kaufman, R. J., & Walter, P. (1999). A role for presenilin-1 in nuclear accumulation of Ire1 fragments and induction of the mammalian unfolded protein response. *Cell, 99*(7), 691–702.

Nunomura, A., Perry, G., Aliev, G., Hirai, K., Takeda, A., Balraj, E. K., et al. (2001). Oxidative damage is the earliest event in Alzheimer disease. *Journal of Neuropathology and Experimental Neurology, 60*(8), 759–767.

Nunomura, A., Castellani, R. J., Zhu, X., Moreira, P. I., Perry, G., & Smith, M. A. (2006). Involvement of oxidative stress in Alzheimer disease. *Journal of Neuropathology and Experimental Neurology, 65*(7), 631–641.

Pietrzik, C. U., Yoon, I. S., Jaeger, S., Busse, T., Weggen, S., & Koo, E. H. (2004). FE65 Constitutes the functional link between the low-density lipoprotein receptor-related protein and the amyloid precursor protein. *Journal of Neuroscience, 24*(17), 4259–4265.

Santacruz, K., Lewis, J., Spires, T., Paulson, J., Kotilinek, L., Ingelsson, M., et al. (2005). Tau suppression in a neurodegenerative mouse model improves memory function. *Science, 309*(5733), 476–481.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., et al. (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased

in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nature Medicine, 2*(8), 864–870.

Selkoe, D. J. (2008). Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. *Behavioural Brain Research, 192*(1), 106–113.

Smine, A., Xu, X., Nishiyama, K., Katada, T., Gambetti, P., Yadav, S. P., et al. (1998). Regulation of brain G-protein go by Alzheimer's disease gene presenilin-1. *Journal of Biological Chemistry, 273*(26), 16281–16288.

Spires-Jones, T. L., Stoothoff, W. H., de Calignon, A., Jones, P. B., & Hyman, B. T. (2009). Tau pathophysiology in neurodegeneration: A tangled issue. *Trends in Neurosciences, 32*(3), 150–159.

Stokin, G. B., Lillo, C., Falzone, T. L., Brusch, R. G., Rockenstein, E., Mount, S. L., et al. (2005). Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science, 307*(5713), 1282–1288.

Tanzi, R. E. (2005). The synaptic abeta hypothesis of Alzheimer disease. *Nature Neuroscience, 8*(8), 977–979.

Terry, R. D., Gonatas, N. K., & Weiss, M. (1964). Ultrastructural studies in Alzheimer's presenile dementia. *American Journal of Pathology, 44*, 269–297.

Vetrivel, K. S., & Thinakaran, G. (2006). Amyloidogenic processing of beta-amyloid precursor protein in intracellular compartments. *Neurology, 66*(2 Suppl. 1), S69–S73.

Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., et al. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature, 416*(6880), 535–539.

Wilkins, R. H., & Brody, I. A. (1969). Alzheimer's disease. *Archives of Neurology, 21*(1), 109–110.

Wilquet, V., & De Strooper, B. (2004). Amyloid-beta precursor protein processing in neurodegeneration. *Current Opinion in Neurobiology, 14*(5), 582–588.

Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S. M., Iwata, N., Saido, T. C., et al. (2007). Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron, 53*(3), 337–351.

Yu, G., Nishimura, M., Arawaka, S., Levitan, D., Zhang, L., Tandon, A., et al. (2000). Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and beta APP processing. *Nature, 407*(6800), 48–54.

Potential Therapeutic Effects of Statins in Alzheimer's Disease

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Abstract

Statins are a family of lipid-lowering agents, long known to be beneficial in conditions where dyslipidemia occurs, such as atherosclerosis. Very recently, statins also have been proposed for use in neurodegenerative conditions, including Alzheimer's disease (AD). However, it is clear that the purported effectiveness of statins in neurodegenerative disorders is not directly related to cholesterol-lowering effects of these agents but, rather, to their pleiotropic functions.

Moreover, evidence from randomized, double-blind clinical trials demonstrated that statins have only limited beneficial effects in improving cognitive function in AD patients with moderate dementia. There is also a suggestion that in nondemented elderly people, statin use can be associated with cognitive impairments. Possible mechanisms underlying these effects are discussed along with the pros and cons of the use of statins in neurodegenerative disorders.

Keywords

Alzheimer's disease • Cholesterol • Dementia • Statins

1 Basic Pharmacology of Statins

Statins are a family of drugs with pleiotropic functions. To this class belong eight drugs: mevastatin, lovastatin, pravastatin, and simvastatin, which are natural compounds derived from fungal products, whereas fluvastatin, atorvastatin, rosuvastatin, and pitavastatin are distinct synthetic compounds (Shitara and Sugiyama 2006). In addition, lovastatin and simvastatin have a lactone ring in the chemical structure which increases their liposolubility, whereas the remainder statins have open acid forms (Shitara and Sugiyama 2006). These differences in structure could modify the ability of individual statins to cross the blood-brain barrier.

From a pharmacodynamic viewpoint, statins inhibit hydroxy-methyl-glutaryl-CoA (HMG-CoA) reductase, which makes statins effective for the treatment of dyslipidemias (Shitara and Sugiyama 2006). By inhibiting HMG-CoA reductase, statins block the first step in cholesterol biosynthesis, namely, the conversion of HMG-CoA into mevalonate (Shitara and Sugiyama 2006; Bersot 2011). As a result of statin administration, low-density lipoprotein (LDL) cholesterol synthesis decreases in hepatocytes that in turn leads to reduced cholesterol blood level. In addition to this effect, statins reduce triglyceride and increase HDL cholesterol plasma levels. Taken together, statins are cardiovascular agents, due to their ability to counteract hyperlipidemias that are a major cause of atherosclerosis which, in turn, is a common pathogenic mechanism for coronary artery disease, ischemic cerebrovascular disease, and peripheral vascular disease (Shitara and Sugiyama 2006; Bersot 2011). In addition to this main pharmacological effect, statins are

endowed with other pleiotropic activities. Statins reduce primary cardiac risk in many types of cardiac and vascular surgeries, with greater benefits in high-risk patients (Gajendragadkar et al. 2009). Furthermore, due to the well-known immunomodulatory effect, statins prevent graft versus host disease and allogeneic transplantation (Shimabukuro-Vornhagen et al. 2009).

Although all statins share the same main mechanism of action, their pharmacokinetic profile is quite different. Statins are well absorbed by the intestine when given by oral route, even though they undergo marked first-pass effects by the liver, which reduces the systemic bioavailability (5–30 %) (Bellosta et al. 2004). With the exception of simvastatin and lovastatin, which are prodrugs and require hepatic activation, other statins are administered as β-hydroxy-acids. Upon administration, statins reach C_{max} (peak plasma concentration) ranging from 10 ng/ml (lovastatin and simvastatin) to 448 ng/ml (fluvastatin), with a T_{max} (time to reach C_{max}) from 0.5–2 h (fluvastatin and pravastatin) to 2–4 h (atorvastatin, lovastatin, rosuvastatin, simvastatin) (Bellosta et al. 2004). In the bloodstream, statins are bound to albumin (43–99 %), which accounts for their variable half-life. Atorvastatin and rosuvastatin are the statins with the longest half-life (15–30 h and 20.8 h, respectively), whereas fluvastatin, lovastatin, pravastatin, and simvastatin have half-lives around 0.5–3 h (Bellosta et al. 2004). All statins are metabolized by the liver through the isoforms 3A4 (atorvastatin, lovastatin, and simvastatin) and 2C9 (fluvastatin and rosuvastatin) of the cytochrome P-450 (CYP) system, with the exception of pravastatin which undergoes sulfation (Bellosta et al. 2004). The primary route of elimination is fecal, and only a minor fraction of statins is eliminated via urine (Bellosta et al. 2004; Shitara and Sugiyama 2006; Bersot 2011).

2 Statins' Toxicology

The main adverse effects of statins are hepatotoxicity and myopathy. A transient elevation of serum transaminases (up to three times the baseline value) is a common outcome of statin therapy (Bersot 2011). However, the incidence of this side effect is low and dose dependent and does not imply the contraindication of statins in individuals with concomitant liver diseases such as hepatitis C (Bersot 2011). Myalgia is often associated with statin use and is paralleled by a 10-fold increase in plasma creatine kinase (Bersot 2011). Rhabdomyolysis is quite rare, and the risk of developing this side effect is correlated with the statin dose and plasma concentration (Bersot 2011). About 30 cases of serious hepatic failure and 42 cases of death due to rhabdomyolysis associated with statin administration were reported to the FDA over the last 15 years (Bersot 2011; Law and Rudnicka 2006). Particularly serious was the occurrence of fatal rhabdomyolysis in 31 patients treated with cerivastatin in the USA; among these patients, 12 received cerivastatin and gemfibrozil, thus suggesting that the cerivastatin-induced miotoxicity was potentiated by gemfibrozil (Staffa et al. 2002; Shitara and Sugiyama 2006). Due to this severe adverse effect, Bayer voluntarily withdrew cerivastatin from the market.

Rare adverse effects due to statin therapy are tendinous disorders. Tendinitis and tendon rupture were mainly associated with atorvastatin, simvastatin, and pravastatin therapy (Marie et al. 2008).

The main determinants of statin toxicity are the following.

2.1 Transporters

The organic anion-transporting polypeptide (OATP1B1) is expressed in the sinusoidal membranes of hepatocytes and is the most important influx transporter of statins (Niemi 2010; Neuvonen 2010). The activity of OATP1B1, whose encoding gene *SLCO1B1* undergoes genetic variability among individuals, accounts for important differences in statin plasma levels and toxicity. Two common single nucleotide polymorphism (SNP) variants of the *SLCO1B1* gene were identified: c.388A>G (p.Asn130Asp; rs2306283) and c.521T>C (p.Val174Ala; rs4149056). Individuals who carry the c.388A>G (haplotype *SLCO1B1*1B*) have an increased OATP1B1 activity which is associated with a 35 % reduction in pravastatin bioavailability (measured as area under the concentration-time curve, AUC) but without significant clinical outcomes (Niemi 2010). Conversely, subjects harboring the haplotypes *SLCO1B1*5* (c.521T>C) and *SLCO1B1*15* (c.521T>C together with c.388A>G) have a reduced activity of the OATP1B1 transporter and an increased statin plasma concentration (Niemi 2010). Both the latter haplotypes are differentially distributed in the population, with a combined allele frequency of 15–20 % in Caucasians, 10–15 % in Asians, and 2 % in sub-Saharan Africans and African Americans (Niemi 2010). Individuals with the c.521CC genotype have an increased bioavailability of simvastatin, pitavastatin, atorvastatin, pravastatin, and rosuvastatin of 221 %, 173 %, 144 %, 90 %, and 87 %, respectively, with respect to subjects carrying the c.521TT genotype (Niemi 2010; Neuvonen 2010). These genetic polymorphisms have indeed important clinical outcomes. The administration of 80 mg/day simvastatin to individuals with myocardial infarction was associated with myopathy, and the odds ratios were 4.5 per copy of the C allele and 16.9 in c.521CC as compared with the c.521TT homozygotes (Link et al. 2008; Niemi 2010). More than 60 % of these cases of myopathy were attributable to the C variant (Link et al. 2008). Similar results were obtained when patients were treated with 40 mg simvastatin/day with a relative risk of 2.6 per copy of the c.521C allele (Link et al. 2008; Niemi 2010). It is also important to emphasize the c.521T>C polymorphism could be responsible for the onset of myopathy even when other statins are administered. In order to reduce the risk of adverse effects, Caucasian adults harboring the *SLCO1B1* c.521TC genotype should be treated with a half dose of simvastatin, atorvastatin, pravastatin, rosuvastatin, and pitavastatin, whereas those with the c.521CC genotype should receive a quarter dose of simvastatin, atorvastatin, and pitavastatin (Niemi 2010).

ABCG2 encodes the ATP-binding cassette G2 (ABCG2), an efflux transporter located on the apical membranes of several cell types including intestinal epithelial cells and hepatocytes. The *ABCG2* gene undergoes genetic variability, and the more

common SNP is the c.421C>A (p.Gln141Lys; rs 2231142) which reduces the transport function of ABCG2. Individuals who carry the c.421AA genotype have an increased AUC of simvastatin lactone (111 %) as well as atorvastatin and fluvastatin (72 %) with respect to those with the c.421CC genotype (Niemi 2010). Individuals with a c.421AA genotype have a greater risk to develop myopathy if treated with rosuvastatin, atorvastatin, and fluvastatin, even if no clinical trial definitely proved this possibility (Niemi 2010).

2.2 Drug Interactions

In order to reduce the incidence of hepatotoxicity and myopathy, statins should not be used in association with inhibitors of CYP3A4, including grapefruit juice (see below). Several cases of rhabdomyolysis were associated with the concomitant treatment of simvastatin, lovastatin, or atorvastatin with ritonavir, cyclosporine, azole antifungals, macrolides antibiotics, and calcium channel blockers (Neuvonen et al. 2006; Neuvonen 2010). Also the combination of statins and fibrates should be avoided, in particular gemfibrozil (Bersot 2011). For further information about the potential harmful effects deriving from the interaction of statins with other drugs or nutraceuticals, see below.

3 Rationale for the Use of Statins in Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive dysfunction, difficulties in performing the activities of daily living, and mood changes. At autopsy, the AD brain is characterized by the accumulation of amyloid-β peptide (Aβ) and hyperphosphorylated *tau* protein (Sultana and Butterfield 2010; Citron 2010; Querfurth and LaFerla 2010). Amyloid-β peptide is formed by the secretase-mediated cleavage of the amyloid precursor protein (APP). β-Secretase cleaves APP at the N-terminus, generating an extracellular soluble fragment named sAPPβ and leaves an intramembrane fragment called C99 (Mancuso et al. 2011; Querfurth and LaFerla 2010). The latter is cleaved at the C-terminus by γ-secretase and Aβ is released (Mancuso et al. 2011; Querfurth and LaFerla 2010). Once formed, Aβ aggregates as oligomers and fibrils, which form the central core of senile plaques (Sultana et al. 2009; Citron 2010; Querfurth and LaFerla 2010). Current research suggests that Aβ oligomers are more toxic than aggregates (Shankar et al. 2008; Butterfield et al. 2007; Querfurth and LaFerla 2010), and this evidence provides a plausible explanation of the reason why drugs targeted to facilitate the disaggregation of Aβ fibrils into oligomers, e.g., bapineuzumab and solanezumab, failed to improve cognitive function in AD patients (Ayrolles-Torro et al. 2011; Mancuso et al. 2011). Interestingly, the gene encoding APP is located on the chromosome 21; therefore, individuals with down syndrome (DS), who have 3 copies of this chromosome, have an increased risk of developing AD (Bush and Beail 2004). Autopsy findings in individuals with DS

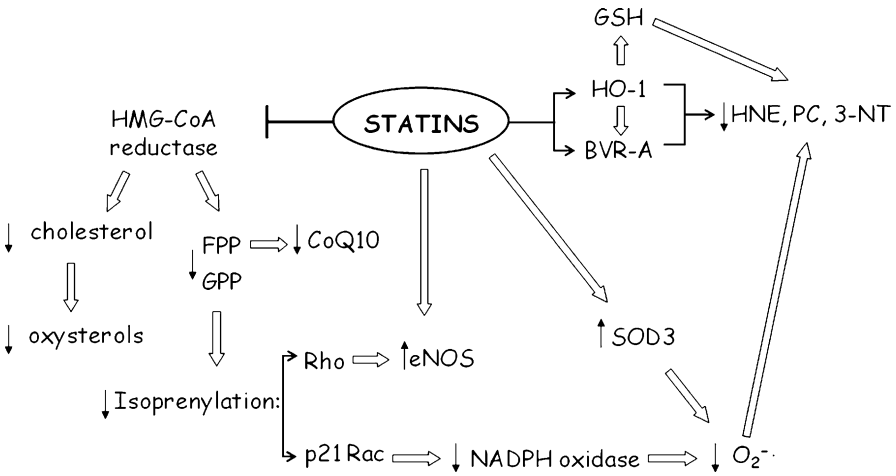

Fig. 1 Cholesterol-dependent and **c**holesterol-independent effects of statins and repercussion on the redox status of the cell. For further information, see text. Abbreviations: *3-NT* 3-nitrotyrosine, *BVR-A* biliverdin reductase-A, *CoQ10* coenzyme Q10, *eNOS* endothelial nitric oxide synthase, *FPP* farnesyl pyrophosphate, *GPP* geranyl pyrophosphate, *GSH* reduced glutathione, *HMG-CoA* hydroxyl-methyl-glutaryl-CoA, *HNE* 4-hydroxynonenals, *HO-1* heme oxygenase-1, *PC* protein carbonyls, *SOD3* extracellular superoxide dismutase

revealed senile plaques and neurofibrillary tangles in the brain, which are secondary to the formation and deposition of Aβ and tau protein, respectively (Gyure et al. 2001; Itoh and Yagishita 1998).

Amyloid-β peptide and tau protein synergize and exert their neurotoxic effects through several mechanisms: (a) generation of superoxide anion and nitric oxide through the activation of NADPH oxidase and inducible nitric oxide synthase (iNOS), respectively (Butterfield et al. 2007; Querfurth and LaFerla 2010), which react each other and form peroxynitrite ($ONOO^-$); (b) mitochondrial impairment secondary to the inhibition of key enzymes involved in the respiratory chain and Krebs cycle (Querfurth and LaFerla 2010); and (c) stimulation of the ionotropic glutamate receptor NMDA and increase of Ca^{2+} overload thus leading to excitotoxic cell death (Querfurth and LaFerla 2010). Taking into consideration these multiple toxic pathways, statins may be particularly useful for AD. Statins can inhibit endothelial $O_2^{-\bullet}$ formation by preventing the isoprenylation of p21 Rac, which is critical for the assembly of NADPH oxidase after activation of protein kinase C (Wallerath et al. 2003) (Fig. 1). In addition, the cellular superoxide clearance activity is increased given that SOD3 activity is more than doubled by simvastatin. Further, simvastatin treatment also increases the number of functionally active endothelial progenitor cells (Landmesser et al. 2005). Moreover, statins increase the expression of endothelial nitric oxide synthase (eNOS) through the inhibition of Rho isoprenylation (Laufs et al. 1998), and statins can also directly activate eNOS via posttranslational mechanisms involving activation of the PI3K/Akt pathway (Kureishi et al. 2000). Statins showed positive effects

against Aβ-induced oxidative stress in mouse models of AD (Kurinami et al. 2008; Tong et al. 2009) as well as a reduction in the phosphorylation of tau protein in human cerebrospinal fluid (CSF) (Riekse et al. 2006). However, although statins' treatment appears to provide benefits, it is difficult to determine whether the benefits are due to lower cholesterol levels or to statin pleiotropy. Atorvastatin treatment was neuroprotective against cell degeneration induced by Aβ(1-40), reducing inflammatory and oxidative responses and increasing the expression of glutamatergic transporters (Piermartiri et al. 2010). Murphy et al. showed that long-term atorvastatin did not reduce Aβ levels, despite a significant reduction in β-secretase 1 (BACE1) protein levels and activity in the brains of aged beagles (Murphy et al. 2010). Subsequently, Barone et al. found that although no changes in Aβ levels occur, long-term atorvastatin significantly reduced lipid peroxidation, protein oxidation and nitration, and increased GSH levels in the parietal cortex of treated dogs (Barone et al. 2011). This effect was cholesterol and Aβ independent and specific for brain (Barone et al. 2011). Another possible mechanism by which statins exert neuroprotective effects is the enhancement of the cell stress response. The administration of atorvastatin 80 mg/day for 14.5 months to aged beagles increased the expression of the heme oxygenase-1/biliverdin reductase-A (HO-1/BVR-A) system, a main effector of the adaptive stress response (Mancuso and Barone 2009), and this correlated with a significant reduction in both oxidative and nitrosative stress biomarkers in the parietal cortex of treated beagles that showed improved cognition (Barone et al. 2012; Butterfield et al. 2011). Conversely, side effects of long-term statin treatment include a decrease in serum and tissue CoQ10 levels, which could result in an energy metabolism impairment in heart, skeletal muscle, and liver (Martin et al. 2011; Bliznakov and Wilkins 1998). The importance of statin-induced energy metabolism impairment and its role in the development of their side effects was demonstrated also in humans. The administration of 240 mg/day CoQ10 reverted fatigue, myalgia, and peripheral neuropathy in 50 patients chronically treated with statins (Langsjoen et al. 2005).

More detailed research into the pharmacology of statins is necessary, particularly the concentrations achieved in the central nervous system and the level at which they block the production of cholesterol and they modulate all the above pathways, in order to prove their beneficial effect and support the potential use of statins in neurodegenerative disorders.

4 Statins and Dementia: The Support Given by the Evidence-Based Medicine

Although the majority of the preclinical evidence shows neuroprotective effects of statins in ameliorating cognitive dysfunction, clinical data largely do not show similar benefits. In addition, some clinical studies show the opposite results depending on whether they are observational studies or randomized clinical trials (RCT).

Early cohort and case-control studies demonstrated that statins reduced the risk of developing dementia, including AD, and this protective effect was maintained

over a 6-year follow-up period (Beydoun et al. 2010; Li et al. 2010; Smeeth et al. 2009; Haag et al. 2009; Horsdal et al. 2009; Rosenberg and Allard 2008; Sparks et al. 2008; Cramer et al. 2008). These findings were recently challenged by Benito-Leon et al., who demonstrated that statins did not improve cognitive performance in nondemented elderly subjects with a median age of 72 years (Benito-Leon et al. 2010). Similar results were obtained in a large cohort study, which involved more than two million participants aged 30–84 years of whom 10.7 % received statins (Hippisley-Cox and Coupland 2010). A cross-sectional research study, with a follow-up of 5 years, demonstrated that in 123 Caucasian subjects with DS (41–78 years), total cholesterol levels $>$ 200 mg/dl positively correlated with the development of AD (hazard rate 2.59), whereas individuals taking statins had less than half the risk of developing AD with respect to nonusers (hazard rate 0.402) (Zigman et al. 2007).

In order to confirm these epidemiological studies, some RCT were performed with comparable results. The PROSPER study, which enrolled approximately 6,000 people aged 70–82 years, demonstrated that pravastatin (40 mg/day) did not have any effect on cognitive function over a follow-up of 3 years (Trompet et al. 2010; Shepherd et al. 1999). The LEADe study tested the hypothesis that atorvastatin (80 mg/day) over 72 weeks delayed cognitive decline in patients with mild-to-moderate AD. The results of this study did not support any significant positive effect of atorvastatin on cognitive or global function in patients receiving the statin compared to those with placebo (Feldman et al. 2010). In August 2011, Sano et al. published the results of an RCT of simvastatin (20 mg/day for 6 weeks and 40 mg/day for the remainder 18 months) in participants with mild-to-moderate AD that also demonstrated the lack of efficacy (Sano et al. 2011). On the other hand, the ADCLT trial demonstrated that atorvastatin (80 mg/day) for 1 year exhibited a significant positive effect on cognitive performance after 6 months of therapy compared with placebo. However, this beneficial effect was selective for individuals who matched restricted criteria, such as a higher MMSE score at baseline, total cholesterol levels higher than 200 mg/dl, and the presence of an apolipoprotein E-4 allele (APOE-4) (Sparks et al. 2006a, b). When all the RCT study results are combined, a meta-analysis suggests that there is insufficient evidence to recommend statins for the treatment of dementia and AD (McGuinness et al. 2010). This statement matches the guidelines of the British Association for Psychopharmacology who also do not recommend statins for the prevention or treatment of AD (O'Brien and Burns 2010). However, in a pilot clinical trial, a modest improvement in cognition was detected in individuals with mild cognitive impairment (MCI), arguably the earliest form of AD, treated with statins (Sparks et al. 2010).

5 Unresolved Issues

In order to understand the reasons for the inconsistent beneficial effects of statins in aged or demented individuals, several issues need to be addressed and carefully evaluated.

Ability to Cross the Blood-Brain Barrier. As mentioned earlier, the presence of a lactone ring in the chemical structures of simvastatin and lovastatin makes these latter very liposoluble, whereas the open acids atorvastatin, fluvastatin, and pitavastatin have an intermediate liposolubility, and pravastatin has the lowest one (Shitara and Sugiyama 2006). Such different degree of liposolubility might suggest the use of lipophilic statins in demented patients to increase the bioavailability in the brain. However, both the LEADe and CLASP trials failed to demonstrate a beneficial effect of lipophilic atorvastatin and simvastatin on cognitive function in AD patients, similar to the results obtained in the PROSPER study involving the lipophobic pravastatin. Thus, the different degrees of liposolubility are unlikely to be a key determinant of the limited effectiveness of statins in these clinical trials.

Age. Epidemiological data demonstrate that the incidence of AD increases with age and doubles every 5 years after 65 years of age with 1,275 new cases/1,00,000 persons/year (Querfurth and LaFerla 2010). In the Western hemisphere, the prevalence of AD was calculated at approximately 1% in subjects aged 60–64 but increases to between 33 % and 50 % in people aged 85 or older (Mayeux 2010). Much of the epidemiological and clinical studies designed to examine the role of statins in AD enrolled included individuals aged 65–84 years. Although the clinical studies and the meta-analysis discussed above did not support an overall beneficial effect for statins in dementia and AD, it is noteworthy to mention that those studies in which statins had a major effect on cognitive functions included individuals aged 68–74 years (Simons et al. 2002; Li et al. 2010; Haag et al. 2009). Consequently, it is possible that 68–74 years of age should be considered an optimal age for statin efficacy in preventing dementia. On the other hand, both the LEADe and PROSPER studies, which recruited individuals within the same range of age, failed to demonstrate any beneficial effect of atorvastatin and pravastatin in people with AD. Subjects aged 80 or older also did not have any beneficial effects from statins (Li et al. 2010).

Cholesterol Blood Levels Before and After Treatment with Statins. An important question to address when studying the HMG-CoA reductase-independent effects of statins is the concomitant reduction of LDL cholesterol plasma levels. This issue is quite important when the pleiotropic effects of statins are studied in the nervous system because cholesterol is a main component of cell membranes, in particular myelin (Saher et al. 2005), and if cholesterol blood levels fall due to uncontrolled therapy with lipid lowering agents, nervous function would also decrease. The majority of AD patients recruited for clinical trials had serum LDL cholesterol at baseline around 131–147 mg/dL, which was significantly reduced by 50–54 % after the administration of atorvastatin (80 mg for 52 weeks) or simvastatin (40 mg for 26 weeks) (McGuinness et al. 2009; McGuinness et al. 2010). These values of LDL cholesterol, before and after statin treatment, are acceptable and do not imply any possible adverse effects. However, although there is a marked effect of statins on LDL cholesterol plasma levels, no beneficial effects on cognitive function are observed in normocholesterolemic patients (McGuinness et al. 2009; McGuinness et al. 2010). Indeed, even in hypercholesterolemic patients, the administration of statins did not have any disease-modifying effect on AD (Hoglund et al. 2004;

Riekse et al. 2006). Evans et al. showed that in AD patients heterozygous for APOE-4 allele or carriers of PS1 mutations, the administration of simvastatin or atorvastatin only slightly reduced the concentration of CSF cholesterol at 6–7 months followed by a peak at 2 years and a return to baseline levels after 3 years (Evans et al. 2009). This finding suggests that despite changes in plasma cholesterol levels, statins cause only minimal changes in brain cholesterol, and, therefore, the effect on cognitive functions may be independent of systemic cholesterol metabolism.

Interaction with Xenobiotics. Patients with AD, as well as other types of dementia, usually take additional drugs for other age-related disorders or comorbidities. As mentioned above, all statins, with the exception of pravastatin, are metabolized by CYP3A4 or CYP2C9, and their plasma levels could be reduced or increased with concomitant administration of drugs that induce or inhibit these CYP isoforms. Donepezil and galantamine, two acetylcholinesterase inhibitors provided to AD patients, are metabolized by CYP3A4 (Jann et al. 2002) and, therefore, could compete with statins. As a consequence of this competition, statin plasma levels could increase as well as the risk of side effects. Also, AD patients may also be supplemented with nutraceuticals including curcumin, green tea extracts, or grapefruit juice as these natural substances are widely considered to be free radical scavengers and therefore neuroprotective. Unfortunately, these natural substances are inhibitors of CYP3A4 and, therefore, increase plasma concentrations of statins (Kiani and Imam 2007; Stump et al. 2006; Hare and Elliott 2003). Interestingly, it was reported that the concomitant administration of simvastatin and consumption of grapefruit or green tea causes rhabdomyolysis (Dreier and Endres 2004; Werba et al. 2008).

Taking into consideration the results from evidence-based medicine, it is possible to suggest that select individuals with AD may benefit more from statins: aged 65–74 years, carrier of an ApoE-4 allele (Sparks et al. 2006a) but without the haplotype *SLCO1B1*5* or *SLCO1B1*15* or *SLCO1B1*1B*/**1B* (Niemi 2010), normocholesterolemic and sparing user of drugs which inhibit CYP3A4.

6 Conclusion

Although preclinical data suggest beneficial effects of statins in the treatment of dementia and AD, results derived from epidemiological studies and RCT are contradictory. Several critical issues may need to be considered (1) the genetic profile of individuals with regard to specific genes involved in statin absorption, (2) the rate of inhibition of cholesterol synthesis in normocholesterolemic patients, (3) age of the individual, and (4) concomitant administration of drugs or nutraceuticals. Thus, future RCT using statins may consider from recruiting AD patients aged 65–75 years, which is an age range that appears to benefit from statin therapy, with primary endpoint, the analysis of cognitive function, and a significant longer follow-up. Another suggestion is to study the effect of statins in individuals with MCI in order to understand whether or not the neuroprotective effect of statins could block or slow the transition to AD. This recommendation could be especially

important since preclinical studies with atorvastatin decreased oxidative stress in aged dogs, and this benefit was correlated with levels of Aβ(1–42), which is same sequence as that of humans (Barone et al. 2011). Oxidative stress is strongly associated with amnestic MCI and AD (Perluigi et al. 2009; Sultana et al. 2010; Sultana et al. 2009), and Aβ is hypothesized to contribute to this oxidative stress (Butterfield et al. 2001).

In conclusion, the current clinical evidence is not strong enough to support the widespread use of statins to treat dementia and AD. However, it is critical that researchers and clinicians in the near future investigate whether or not statin therapy should be restricted to selected populations of demented individuals with the best chance of efficacy derived from evidence-based medicine is recommended.

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References

Ayrolles-Torro, A., Imberdis, T., Torrent, J., Toupet, K., Baskakov, I. V., Poncet-Montange, G., Gregoire, C., Roquet-Baneres, F., Lehmann, S., Rognan, D., Pugniere, M., Verdier, J. M., & Perrier, V. (2011). Oligomeric-induced activity by thienyl pyrimidine compounds traps prion infectivity. *Journal of Neuroscience, 31*(42), 14882–14892. doi:31/42/14882 [pii] 10.1523/JNEUROSCI.0547-11.2011.

Barone, E., Cenini, G., Di Domenico, F., Martin, S., Sultana, R., Mancuso, C., Murphy, M. P., Head, E., & Butterfield, D. A. (2011). Long-term high-dose atorvastatin decreases brain oxidative and nitrosative stress in a preclinical model of Alzheimer disease: A novel mechanism of action. *Pharmacological Research, 63*(3), 172–180. doi:S1043-6618(10)00232-X [pii] 10.1016/j.phrs.2010.12.007.

Barone, E., Mancuso, C., Di Domenico, F., Sultana, R., Murphy, M. P., Head, E., & Butterfield, D. A. (2012). Biliverdin reductase-A: A novel drug target for atorvastatin in a dog pre-clinical model of Alzheimer disease. *Journal of Neurochemistry, 120*(1), 135–146. doi:10.1111/j.1471-4159.2011.07538.x.

Bellosta, S., Paoletti, R., & Corsini, A. (2004). Safety of statins: Focus on clinical pharmacokinetics and drug interactions. *Circulation, 109*(23 Suppl 1), III50–III57. doi:10.1161/01.CIR.0000131519.15067.1f 109/23_suppl_1/III-50 [pii].

Benito-Leon, J., Louis, E. D., Vega, S., & Bermejo-Pareja, F. (2010). Statins and cognitive functioning in the elderly: A population-based study. *Journal of Alzheimer's Disease, 21*(1), 95–102. doi:T60L52283451G644 [pii] 10.3233/JAD-2010-100180.

Bersot, T. P. (2011). Drug therapy for hypercholesterolemia and dyslipidemia. In L. B. Laurence (Ed.), *Goodman and Gilman's, The pharmacological basis of therapeutics* (12th ed., pp. 877–908). New York: McGraw-Hill.

Beydoun, M. A., Beason-Held, L. L, Kitner-Triolo, M. H., Beydoun, H. A., Ferrucci, L., Resnick, S. M., Zonderman, A. B. (2010). Statins and serum cholesterol's associations with incident dementia and mild cognitive impairment. *Journal of Epidemiology and Community Health*. doi:jech.2009.100826 [pii]. 10.1136/jech.2009.100826.

Bliznakov, E. G., & Wilkins, D. J. (1998). Biochemical and clinical consequences of inhibiting coenzyme Q(10) biosynthesis by lipid-lowering HMG-CoA reductase inhibitors (statins): A critical overview. *Advances in Therapy, 15*(4), 218–228.

Bush, A., & Beail, N. (2004). Risk factors for dementia in people with down syndrome: issues in assessment and diagnosis. *American Journal of Mental Retardation, 109*(2), 83–97. doi:10.1352/0895-8017(2004)109<83:RFFDIP>2.0.CO;2.

Butterfield, D. A., Drake, J., Pocernich, C., & Castegna, A. (2001). Evidence of oxidative damage in Alzheimer's disease brain: Central role for amyloid beta-peptide. *Trends in Molecular Medicine, 7*(12), 548–554. doi:S1471-4914(01)02173-6 [pii].

Butterfield, D. A., Reed, T., Newman, S. F., & Sultana, R. (2007). Roles of amyloid beta-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radical Biology & Medicine, 43*(5), 658–677. doi:S0891-5849(07)00392-9 [pii] 10.1016/j.freeradbiomed.2007.05.037.

Butterfield, D. A., Barone, E., Di Domenico, F., Cenini, G., Sultana, R., Murphy, M. P., Mancuso, C., Head, E. (2011). Atorvastatin treatment in a dog preclinical model of Alzheimer's disease leads to up-regulation of haem oxygenase-1 and is associated with reduced oxidative stress in brain. *International Journal of Neuropsychopharmacol*:1–7. doi:S1461145711001118 [pii]. 10.1017/S1461145711001118.

Citron, M. (2010). Alzheimer's disease: Strategies for disease modification. *Nature Reviews Drug Discovery, 9*(5), 387–398. doi:nrd2896 [pii]. 10.1038/nrd2896.

Cramer, C., Haan, M. N., Galea, S., Langa, K. M., & Kalbfleisch, J. D. (2008). Use of statins and incidence of dementia and cognitive impairment without dementia in a cohort study. *Neurology, 71*(5), 344–350. doi:71/5/344 [pii]. 10.1212/01.wnl.0000319647.15752.7b.

Dreier, J. P., & Endres, M. (2004). Statin-associated rhabdomyolysis triggered by grapefruit consumption. *Neurology, 62*(4), 670.

Evans, B. A., Evans, J. E., Baker, S. P., Kane, K., Swearer, J., Hinerfeld, D., Caselli, R., Rogaeva, E., St George-Hyslop, P., Moonis, M., & Pollen, D. A. (2009). Long-term statin therapy and CSF cholesterol levels: implications for Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders, 27*(6), 519–524. doi:000221835 [pii]. 10.1159/000221835.

Feldman, H. H., Doody, R. S., Kivipelto, M., Sparks, D. L., Waters, D. D., Jones, R. W., Schwam, E., Schindler, R., Hey-Hadavi, J., DeMicco, D. A., & Breazna, A. (2010). Randomized controlled trial of atorvastatin in mild to moderate Alzheimer disease: LEADe. *Neurology, 74*(12), 956–964. doi:WNL.0b013e3181d6476a [pii]. 10.1212/WNL.0b013e3181d6476a.

Gajendragadkar, P. R., Cooper, D. G., Walsh, S. R., Tang, T. Y., Boyle, J. R., & Hayes, P. D. (2009). Novel uses for statins in surgical patients. *International Journal of Surgery, 7*(4), 285–290. doi:S1743-9191(09)00066-1 [pii]. 10.1016/j.ijsu.2009.04.016.

Gyure, K. A., Durham, R., Stewart, W. F., Smialek, J. E., & Troncoso, J. C. (2001). Intraneuronal abeta-amyloid precedes development of amyloid plaques in Down syndrome. *Archives of Pathology & Laboratory Medicine, 125*(4), 489–492. doi:10.1043/0003-9985(2001)125<0489:IAAPDO>2.0.CO;2.

Haag, M. D., Hofman, A., Koudstaal, P. J., Stricker, B. H., & Breteler, M. M. (2009). Statins are associated with a reduced risk of Alzheimer disease regardless of lipophilicity The Rotterdam Study. *Journal of Neurology, Neurosurgery and Psychiatry, 80*(1), 13–17. doi:jnnp.2008.150433 [pii]. 10.1136/jnnp.2008.150433.

Hare, J. T., & Elliott, D. P. (2003). Grapefruit juice and potential drug interactions. *The Consultant Pharmacist, 18*(5), 466–472.

Hippisley-Cox, J., & Coupland, C. (2010). Unintended effects of statins in men and women in England and Wales: Population based cohort study using the QResearch database. *BMJ, 340*, c2197.

Hoglund, K., Wiklund, O., Vanderstichele, H., Eikenberg, O., Vanmechelen, E., & Blennow, K. (2004). Plasma levels of beta-amyloid(1-40), beta-amyloid(1-42), and total beta-amyloid remain unaffected in adult patients with hypercholesterolemia after treatment with statins. *Archives of Neurology, 61*(3), 333–337. doi:10.1001/archneur.61.3.333. 61/3/333 [pii].

Horsdal, H. T., Olesen, A. V., Gasse, C., Sorensen, H. T., Green, R. C., & Johnsen, S. P. (2009). Use of statins and risk of hospitalization with dementia: A Danish population-based case-control study. *Alzheimer Disease and Associated Disorders, 23*(1), 18–22. doi:10.1097/WAD.0b013e318180f55b.

Itoh, Y., & Yagishita, S. (1998). Scanning electron microscopical study of neurofibrillary tangles in a presenile patient with Down's syndrome. *Acta Neuropathologica, 96*(2), 179–184.

Jann, M. W., Shirley, K. L., & Small, G. W. (2002). Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. *Clinical Pharmacokinetics, 41*(10), 719–739. doi:411003 [pii].

Kiani, J., & Imam, S. Z. (2007). Medicinal importance of grapefruit juice and its interaction with various drugs. *Nutrition Journal, 6*, 33. doi:1475-2891-6-33 [pii]. 10.1186/1475-2891-6-33.

Kureishi, Y., Luo, Z., Shiojima, I., Bialik, A., Fulton, D., Lefer, D. J., Sessa, W. C., & Walsh, K. (2000). The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nature Medicine, 6*(9), 1004–1010. doi:10.1038/79510.

Kurinami, H., Sato, N., Shinohara, M., Takeuchi, D., Takeda, S., Shimamura, M., Ogihara, T., & Morishita, R. (2008). Prevention of amyloid beta-induced memory impairment by fluvastatin, associated with the decrease in amyloid beta accumulation and oxidative stress in amyloid beta injection mouse model. *International Journal of Molecular Medicine, 21*(5), 531–537.

Landmesser, U., Bahlmann, F., Mueller, M., Spiekermann, S., Kirchhoff, N., Schulz, S., Manes, C., Fischer, D., de Groot, K., Fliser, D., Fauler, G., Marz, W., & Drexler, H. (2005). Simvastatin versus ezetimibe: pleiotropic and lipid-lowering effects on endothelial function in humans. *Circulation, 111*(18), 2356–2363. doi:01.CIR.0000164260.82417.3F [pii]. 10.1161/01.CIR.0000164260.82417.3F.

Langsjoen, P. H., Langsjoen, J. O., Langsjoen, A. M., & Lucas, L. A. (2005). Treatment of statin adverse effects with supplemental Coenzyme Q10 and statin drug discontinuation. *Biofactors, 25*(1–4), 147–152.

Laufs, U., La Fata, V., Plutzky, J., & Liao, J. K. (1998). Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation, 97*(12), 1129–1135.

Law, M., & Rudnicka, A. R. (2006). Statin safety: A systematic review. *The American Journal of Cardiology, 97*(8A), 52C–60C. doi:S0002-9149(05)02143-0 [pii]. 10.1016/j.amjcard.2005.12.010.

Li, G., Shofer, J. B., Rhew, I. C., Kukull, W. A., Peskind, E. R., McCormick, W., Bowen, J. D., Schellenberg, G. D., Crane, P. K., Breitner, J. C., & Larson, E. B. (2010). Age-varying association between statin use and incident Alzheimer's disease. *Journal of American Geriatrics Society, 58*(7), 1311–1317. doi:JGS2906 [pii]. 10.1111/j.1532-5415.2010.02906.x.

Link, E., Parish, S., Armitage, J., Bowman, L., Heath, S., Matsuda, F., Gut, I., Lathrop, M., & Collins, R. (2008). SLCO1B1 variants and statin-induced myopathy–a genomewide study. *The New England Journal of Medicine, 359*(8), 789–799. doi:NEJMoa0801936 [pii]. 10.1056/NEJMoa0801936.

Mancuso, C., & Barone, E. (2009). The heme oxygenase/biliverdin reductase pathway in drug research and development. *Current Drug Metabolism, 10*(6), 579–594. doi:Abstract no. 0015 [pii].

Mancuso, C., Siciliano, R., Barone, E., Butterfield, D. A., & Preziosi, P. (2011). Pharmacologists and Alzheimer disease therapy: To boldly go where no scientist has gone before. *Expert Opinion on Investigational Drugs, 20*(9), 1243–1261. doi:10.1517/13543784.2011.601740.

Marie, I., Delafenetre, H., Massy, N., Thuillez, C., & Noblet, C. (2008). Tendinous disorders attributed to statins: A study on ninety-six spontaneous reports in the period 1990-2005 and review of the literature. *Arthritis and Rheumatism, 59*(3), 367–372. doi:10.1002/art.23309.

Martin, S. B., Cenini, G., Barone, E., Dowling, A. L., Mancuso, C., Butterfield, D. A., Murphy, M. P., & Head, E. (2011). Coenzyme Q10 and cognition in atorvastatin treated dogs. *Neuroscience Letters, 501*(2), 92–95. doi:S0304-3940(11)01009-3 [pii]. 10.1016/j.neulet.2011.06.054.

Mayeux, R. (2010). Clinical practice. Early Alzheimer's disease. *The New England Journal of Medicine, 362*(23), 2194–2201. doi:362/23/2194 [pii]. 10.1056/NEJMcp0910236.

McGuinness, B., Craig, D., Bullock, R., Passmore, P. (2009). Statins for the prevention of dementia. Cochrane Database System Review (2):CD003160. doi:10.1002/14651858.CD003160.pub2.

McGuinness, B., O'Hare, J., Craig, D., Bullock, R., Malouf, R., Passmore, P. (2010). Statins for the treatment of dementia. Cochrane Database System Review (8):CD007514. doi:10.1002/14651858.CD007514.pub2.

Murphy, M. P., Morales, J., Beckett, T. L., Astarita, G., Piomelli, D., Weidner, A., Studzinski, C. M., Dowling, A. L., Wang, X., Levine, H., 3rd, Kryscio, R. J., Lin, Y., Barrett, E., & Head, E. (2010). Changes in cognition and amyloid-beta processing with long term cholesterol reduction using atorvastatin in aged dogs. *Journal of Alzheimer's Disease, 22*(1), 135–150. doi:R81617377L4X3511 [pii]. 10.3233/JAD-2010-100639.

Neuvonen, P. J. (2010). Drug interactions with HMG-CoA reductase inhibitors (statins): The importance of CYP enzymes, transporters and pharmacogenetics. *Current Opinion in Investigational Drugs, 11*(3), 323–332.

Neuvonen, P. J., Niemi, M., & Backman, J. T. (2006). Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clinical Pharmacology and Therapeutics, 80*(6), 565–581. doi:S0009-9236(06)00360-2 [pii].10.1016/j.clpt.2006.09.003.

Niemi, M. (2010). Transporter pharmacogenetics and statin toxicity. *Clinical Pharmacology and Therapeutics, 87*(1), 130–133. doi:clpt2009197 [pii]. 10.1038/clpt.2009.197.

O'Brien, J. T., Burns A. (2010) Clinical practice with anti-dementia drugs: a revised (second) consensus statement from the British Association for Psychopharmacology. *J Psychopharmacol*. doi:0269881110387547 [pii]. 10.1177/0269881110387547.

Perluigi, M., Sultana, R., Cenini, G., Di Domenico, F., Memo, M., Pierce, W. M., Coccia, R., & Butterfield, D. A. (2009). Redox proteomics identification of 4-hydroxynonenal-modified brain proteins in Alzheimer's disease: Role of lipid peroxidation in Alzheimer's disease pathogenesis. *Proteomics. Clinical Applications, 3*(6), 682–693. doi:10.1002/prca.200800161.

Piermartiri, T. C., Figueiredo, C. P., Rial, D., Duarte, F. S., Bezerra, S. C., Mancini, G., de Bem, A. F., Prediger, R. D., & Tasca, C. I. (2010). Atorvastatin prevents hippocampal cell death, neuroinflammation and oxidative stress following amyloid-beta(1-40) administration in mice: evidence for dissociation between cognitive deficits and neuronal damage. *Experimental Neurology, 226*(2), 274–284. doi:S0014-4886(10)00329-8 [pii]. 10.1016/j.expneurol.2010.08.030.

Querfurth, H. W., & LaFerla, F. M. (2010). Alzheimer's disease. *The New England Journal of Medicine, 362*(4), 329–344. doi:362/4/329 [pii].

Riekse, R. G., Li, G., Petrie, E. C., Leverenz, J. B., Vavrek, D., Vuletic, S., Albers, J. J., Montine, T. J., Lee, V. M., Lee, M., Seubert, P., Galasko, D., Schellenberg, G. D., Hazzard, W. R., & Peskind, E. R. (2006). Effect of statins on Alzheimer's disease biomarkers in cerebrospinal fluid. *Journal of Alzheimer's Disease, 10*(4), 399–406.

Rosenberg, H., & Allard, D. (2008). Women and statin use: A women's health advocacy perspective. *Scandinavian Cardiovascular Journal, 42*(4), 268–273. doi:791850044 [pii]. 10.1080/14017430801993180.

Saher, G., Brugger, B., Lappe-Siefke, C., Mobius, W., Tozawa, R., Wehr, M. C., Wieland, F., Ishibashi, S., & Nave, K. A. (2005). High cholesterol level is essential for myelin membrane growth. *Nature Neuroscience, 8*(4), 468–475. doi:nn1426 [pii]. 10.1038/nn1426.

Sano, M., Bell, K. L., Galasko, D., Galvin, J. E., Thomas, R. G., van Dyck, C. H., & Aisen, P. S. (2011). A randomized, double-blind, placebo-controlled trial of simvastatin to treat Alzheimer disease. *Neurology, 77*(6), 556–563. doi:WNL.0b013e318228bf11 [pii]. 10.1212/WNL.0b013e318228bf11.

Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., Brett, F. M., Farrell, M. A., Rowan, M. J., Lemere, C. A., Regan, C. M., Walsh, D. M., Sabatini, B. L., & Selkoe, D. J. (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nature Medicine, 14*(8), 837–842. doi:nm1782 [pii]. 10.1038/nm1782.

Shepherd, J., Blauw, G. J., Murphy, M. B., Cobbe, S. M., Bollen, E. L., Buckley, B. M., Ford, I., Jukema, J. W., Hyland, M., Gaw, A., Lagaay, A. M., Perry, I. J., Macfarlane, P. W., Meinders, A. E., Sweeney, B. J., Packard, C. J., Westendorp, R. G., Twomey, C., & Stott, D. J. (1999).

The design of a prospective study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER Study Group. PROspective Study of Pravastatin in the Elderly at Risk. *The American Journal of Cardiology, 84*(10), 1192–1197. doi:S0002914999005330 [pii].

Shimabukuro-Vornhagen, A., Glossmann, J., Liebig, T., Scheid, C., & von Bergwelt-Baildon, M. (2009). The use of statins in hematopoietic stem cell transplantation. *Current Stem Cell Research & Therapy, 4*(4), 260–265. doi:ABSTRACT # 12.

Shitara, Y., & Sugiyama, Y. (2006). Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug-drug interactions and interindividual differences in transporter and metabolic enzyme functions. *Pharmacology and Therapeutics, 112*(1), 71–105. doi:S0163-7258(06)00040-4 [pii]. 10.1016/j.pharmthera.2006.03.003.

Simons, M., Schwarzler, F., Lutjohann, D., von Bergmann, K., Beyreuther, K., Dichgans, J., Wormstall, H., Hartmann, T., & Schulz, J. B. (2002). Treatment with simvastatin in normocholesterolemic patients with Alzheimer's disease: A 26-week randomized, placebo-controlled, double-blind trial. *Annals of Neurology, 52*(3), 346–350. doi:10.1002/ana.10292.

Smeeth, L., Douglas, I., Hall, A. J., Hubbard, R., & Evans, S. (2009). Effect of statins on a wide range of health outcomes: A cohort study validated by comparison with randomized trials. *British Journal of Clinical Pharmacology, 67*(1), 99–109. doi:BCP3308 [pii]. 10.1111/j.1365-2125.2008.03308.x.

Sparks, D. L., Connor, D. J., Sabbagh, M. N., Petersen, R. B., Lopez, J., & Browne, P. (2006a). Circulating cholesterol levels, apolipoprotein E genotype and dementia severity influence the benefit of atorvastatin treatment in Alzheimer's disease: Results of the Alzheimer's Disease Cholesterol-Lowering Treatment (ADCLT) trial. *Acta Neurologica Scandinavica. Supplementum, 185*, 3–7. doi:ANE690 [pii]. 10.1111/j.1600-0404.2006.00690.x.

Sparks, D. L., Sabbagh, M., Connor, D., Soares, H., Lopez, J., Stankovic, G., Johnson-Traver, S., Ziolkowski, C., & Browne, P. (2006b). Statin therapy in Alzheimer's disease. *Acta Neurologica Scandinavica. Supplementum, 185*, 78–86. doi:ANE689 [pii]. 10.1111/j.1600-0404.2006.00689.x.

Sparks, D. L., Kryscio, R. J., Sabbagh, M. N., Connor, D. J., Sparks, L. M., & Liebsack, C. (2008). Reduced risk of incident AD with elective statin use in a clinical trial cohort. *Current Alzheimer Research, 5*(4), 416–421.

Sparks, D. L., Kryscio, R. J., Connor, D. J., Sabbagh, M. N., Sparks, L. M., Lin, Y., & Liebsack, C. (2010). Cholesterol and cognitive performance in normal controls and the influence of elective statin use after conversion to mild cognitive impairment: results in a clinical trial cohort. *Neurodegenerative Diseases, 7*(1–3), 183–186. doi:000295660 [pii] 10.1159/000295660.

Staffa, J. A., Chang, J., & Green, L. (2002). Cerivastatin and reports of fatal rhabdomyolysis. *The New England Journal of Medicine, 346*(7), 539–540. doi:10.1056/NEJM200202143460720. 346/7/539-a [pii].

Stump, A. L., Mayo, T., & Blum, A. (2006). Management of grapefruit-drug interactions. *American Family Physician, 74*(4), 605–608.

Sultana, R., & Butterfield, D. A. (2010). Role of oxidative stress in the progression of Alzheimer's disease. *Journal of Alzheimer's Disease, 19*(1), 341–353. doi:0U88727833343HV6 [pii]. 10.3233/JAD-2010-1222.

Sultana, R., Perluigi, M., & Butterfield, D. A. (2009). Oxidatively modified proteins in Alzheimer's disease (AD), mild cognitive impairment and animal models of AD: Role of Abeta in pathogenesis. *Acta Neuropathologica, 118*(1), 131–150. doi:10.1007/s00401-009-0517-0.

Sultana, R., Perluigi, M., Newman, S. F., Pierce, W. M., Cini, C., Coccia, R., & Butterfield, D. A. (2010). Redox proteomic analysis of carbonylated brain proteins in mild cognitive impairment and early Alzheimer's disease. *Antioxidants & Redox Signaling, 12*(3), 327–336. doi:10.1089/ars.2009.2810.

Tong, X. K., Nicolakakis, N., Fernandes, P., Ongali, B., Brouillette, J., Quirion, R., & Hamel, E. (2009). Simvastatin improves cerebrovascular function and counters soluble amyloid-beta,

inflammation and oxidative stress in aged APP mice. *Neurobiology of Disease, 35*(3), 406–414. doi:DOI 10.1016/j.nbd.2009.06.003.

Trompet, S., van Vliet, P., de Craen, A. J., Jolles, J., Buckley, B. M., Murphy, M. B., Ford, I., Macfarlane, P. W., Sattar, N., Packard, C. J., Stott, D. J., Shepherd, J., Bollen, E. L., Blauw, G. J., Jukema, J. W., & Westendorp, R. G. (2010). Pravastatin and cognitive function in the elderly. Results of the PROSPER study. *Journal of Neurology, 257*(1), 85–90. doi:10.1007/s00415-009-5271-7.

Wallerath, T., Poleo, D., Li, H., & Forstermann, U. (2003). Red wine increases the expression of human endothelial nitric oxide synthase: a mechanism that may contribute to its beneficial cardiovascular effects. *Journal of the American College of Cardiology, 41*(3), 471–478. doi:S0735109702028267 [pii].

Werba, J. P., Giroli, M., Cavalca, V., Nava, M. C., Tremoli, E., & Dal Bo, L. (2008). The effect of green tea on simvastatin tolerability. *Annals of Internal Medicine, 149*(4), 286–287. doi:149/4/286 [pii].

Zigman, W. B., Schupf, N., Jenkins, E. C., Urv, T. K., Tycko, B., & Silverman, W. (2007). Cholesterol level, statin use and Alzheimer's disease in adults with Down syndrome. *Neuroscience Letters, 416*(3), 279–284. doi:S0304-3940(07)00176-0 [pii] 10.1016/j.neulet.2007.02.023.

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